Supplemental Material

The supplemental material provides the details of the calculations underlying Materials & Methods and Results in the main text.

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1. **Energy requirements of non-signalling related activities in the resting glomerulus**

Non-signalling related, or vegetative, cellular activities add to the basal energy requirements of neural tissue. They include the metabolism of proteins, lipids and oligonucleotides, cellular transport mechanisms, and proton leak in mitochondria.

The rate of $0.17 \, \mu\text{mol ATP g}^{-1}\text{s}^{-1}$ proposed by Attwell and Laughlin (2001) might be an overestimation for the glomerulus since only a few cell somata are located within the glomerulus, and somatic activities therefore do but little contribute to overall costs. We assume a rate of $0.05$ to $0.1 \, \mu\text{mol ATP g}^{-1}\text{s}^{-1}$.

2. **Energy requirements of signalling related activities in the resting glomerulus**

Signalling related cellular activities in the resting glomerulus refer to the maintenance of the resting membrane potential.

2.1. *Mitral cell tufts.*

From Mori *et al.* (1984) and as used by Migliore *et al.* (2005) we base our calculations on an input resistance of the tuft of $R_{in} = 100\,\text{M}\Omega$. Further we assume a resting potential of $V_{RP} = -70 \, \text{mV}$ (Puopulo *et al.* 2001), and reversal potentials of $V_{Na} = +60\,\text{mV}$ and $V_{K} = -90\,\text{mV}$. Using the relevant equation

$$\text{ATP}_{\text{needs}} / \text{sec} = (V_{Na} - V_{RP}) \times (V_{RP} - V_{K}) / \{ c \times R_{in} \times (V_{RP} + 2V_{Na} - 3V_{K}) \}$$

(1)

from Attwell and Laughlin (2001), where $c$ is the Coulomb constant, this gives $7.37 \times 10^8$ ATPs per second per tuft to run the $\text{Na}^+/\text{K}^+$ pump at rest.

2.2. *Tufted cell tufts.*
Since tufted cells are smaller and electronically more compact than mitral cells we assume a higher input resistance of the tuft with $R_{in} = 200 \text{ M}\Omega$, and we set $V_{RP} = -70 \text{ mV}$. Using equation (1), this gives $3.87 \times 10^8 \text{ ATP per second per tuft to run the Na}^+$/K$^+$ pump. The lower cost in energy compared to the mitral cell reflects the higher input resistance.

2.3. *PG cell tufts.*

From Hayar *et al.* (2004) we base our calculations on an input resistance of the tuft of $R_{in} = 1 \text{ G}\Omega$ and a resting potential of $V_{RP} = -65 \text{ mV}$. Using equation (1), this gives $0.65 \times 10^8 \text{ ATP per second per tuft to run the Na}^+$/K$^+$ pump.

Note how the compactness of this interneuron is associated with a low cost of maintaining the resting potential.

2.4. *ORN axons and axonal terminals.*

The ORN axon has a long and slender structure, with a diameter of approximately 0.2 microns and an average length of 2mm from the cell body in the epithelium to the target glomerulus. At the distal end it branches into a tree structure with usually more than 10 synaptic terminals (Klenoff *et al.* 1998). We deduce its resting membrane properties from the similarly built axon of the retinal horizontal cell in goldfish studied by Yagi *et al.* (1988). The study shows that the unit membrane conductance of axonal terminals can be as low as 1/20 of the somatic conductance. Given a diameter of 10µm and a somatic input resistance of 3 GΩ in rodent olfactory nerve cells (Liman *et al.* 1996), we calculate a somatic conductance of 100 µS/cm$^2$ in ORNs, and we estimate the axonal conductance in the range of 5 µS/cm$^2$. Thus, with the axonal dimensions given above, the axonal input resistance is in the range of 8 GΩ. Using equation (1), this gives $1.75 \times 10^7 \text{ ATP per}$
second per axon to run the Na\(^+\)/K\(^+\) pump. Considering that the axonal length within the
glomerulus of the rat is approximately 10% of its total length, or 195 µm (Klenoff \textit{et al.}
1998), we conclude that only ten percent of the pumping costs are created within the
glomerulus. Thus, we deduce energy demands of 1.75 x 10\(^6\) ATP per second per axonal
segment within the glomerulus.

2.5. \textit{Astrocytes.}

In the absence of electrophysiological data on input resistance of glial processes, we base
our calculations on studies of astrocyte somata. As shown by De Saint Jan and
Westbrook (2005), glomerular astrocytes in rat olfactory bulb exhibit a linear current-
voltage dependency, have a resting potential of \(V_{RP} = -80\) mV and a low input resistance
of \(R_{in} = 20\) MΩ. It has also been found that in the mature olfactory bulb, glomerular glia
cells are GFAP positive (eg. Bailey \textit{et al.} 1999). Given these properties, glomerular
astrocytes can be classified “passive astrocytes” (Walz, 2000).

Using equation (1), we derive 1.4 x 10\(^9\) ATP per second run the Na\(^+\)/K\(^+\) pump.
However, the same study by De Saint Jan and Westbrook (2005) confirmed that
glomerular astrocytes are electrically coupled such that the calculated resting demands
refer to a group of coupled astrocytes, rather than to a single cell. The degree of coupling
within the glomerulus has not been determined, but we can resort to a study on passive
astrocytes in the hippocampus where the relation between input resistance and degree of
coupling has been investigated (Schools \textit{et al.} 2006). From the results of this study we
estimate that all 20 astrocytes associated with a single glomerulus are connected by gap
junctions, resulting in resting demands of 1.4 x 10\(^9\) ATP s\(^{-1}\) for the total population of
glomerular astrocytes. Note that this an order of magnitude higher than the resting values for neurons.

2.6. Summary.

Total signalling-related resting demands for all neural and glial elements in the glomerulus amount to $4.3 \times 10^{10}$ ATP s$^{-1}$ (see chapter13 for cell numbers).

3. Energy requirements of an action potential in the olfactory axon

The amount of electric charge entering the cell during an action potential can be estimated by the following formula (Attwell and Laughlin (2001)):

$$\pi \times d \times l \times C_m \times \Delta V = \text{charge influx in Coulombs},$$

where $d$ is the diameter of the axon, $l$ is the length, $C_m$ is the membrane capacitance, and $\Delta V$ is the voltage change.

Olfactory sensory cell axons are believed to account for all afferent axons entering the olfactory bulb. They are unmyelinated and have a unimodal diameter of approximately 0.2 $\mu$m. Membrane capacitance is a uniform 1 $\mu$F/cm$^2$. The voltage excursion of the action potential is set to 125 mV, referring to the maximal voltage change $\max \{V_m - V_{Na}\}$, where $V_m$ is the membrane potential of the axon and $V_{Na}$ is the sodium reversal potential. With $V_{Na} = +60$ mV as documented for cortical neurons and assuming resting potential between -50 and -80 mV in vertebrate olfactory receptor neurons (e.g. -56 mV in the bullfrog: Tomaru et al. 2005; -54 mV in the salamander: Firestein and Werblin, 1987; -70 mV in the rat: Lynch and Barry, -80 mV in the rat:}
Trombley and Westbrook, 1991), the physiological voltage change thus amounts to approximately 110-140 mV, and we take an average of 125 mV for the calculations.

Using equation (2), an action potential in an ORN axonal segment of 1 µm length requires an influx of

\[ \pi \times 0.2 \mu m \times 1 \mu m \times 1 \mu F/cm^2 \times 0.125V = 7.85 \times 10^{-16} \text{ Coulombs} \quad (3) \]

which is equivalent to the entry of 4,900 Na\(^+\) ions per action potential per µm axonal length. This corresponds to approximately 7,800 Na\(^+\) ions per µm\(^2\), requiring consumption of 2,600 ATP molecules per action potential per µm\(^2\) membrane. Since for a cylindrical neural element the number of ions entering per µm\(^2\) is independent of diameter (the diameter cancels out), the calculated values per µm\(^2\) are the same for regions of the axon of greater diameter, such as the preterminals. For our calculations of energy requirements we therefore need only take into account the total surface area of the relevant compartment.

Considering that during action potentials the outflow of potassium ions overlaps with the effect of incoming sodium ions on the membrane potential, the actual number of sodium ions that are needed to raise the voltage by 125 mV is higher than 7,800 ions per µm\(^2\). Calculations show that this effect can be closely approximated (Attwell and Laughlin, 2001) by quadrupling the number of sodium ions to 31,200 per µm\(^2\), and both for the cortical and the olfactory neuron this results in quadrupled energy demand of approximately 10,400 ATP molecules per action potential per µm\(^2\).

These are fundamental values that can be used to estimate the energy demands of action potentials in different lengths and diameters of pre- and postsynaptic processes.
For an elementary compartment length of 1µm, the number of ions flowing (less than 4000) seems small and finite. The actual density of channels and pumps has also been estimated to be relatively low (Ritchie et al. 1976). There is a possibility that ion channels and pumps may be distributed non-uniformly along the axon, clustered near mitochondria. We will be forced to ignore these for our initial calculations. We also do not address in this study the role of the olfactory receptor cell body in dealing with the total energy load for pumping all ions along its axon, nor the mechanism by which it is able to respond to energy demands of AP propagation and synaptic transmission.

4. **Presynaptic energy demands at axo-dendritic synapses**

The action potential in an olfactory axon invades the preterminal segment and brings about the release of the neurotransmitter glutamate to act on glutamate receptors in the postsynaptic dendrites in the glomerulus. A high release probability (p > 0.8) has recently been demonstrated. The olfactory receptor cells release glutamate (GLU) as their transmitter. Following Attwell and Laughlin (2001), we assume that the release of 1 vesicle liberates some 4,000 molecules of glutamate.

4.1. **Presynaptic Ca\(^{2+}\) entry and restoration.**

At the synapse, the depolarization due to the action potential invading the terminal opens Ca\(^{2+}\) channels. Since significant signalling-related costs in the presynaptic terminals arise from this ion influx, we need to estimate the total presynaptic calcium influx \(Ca_{in}\) using

\[
Ca_{in} = \Delta [Ca^{2+}] \ast V \ast N_A \ast (1+ k_B), \quad (4)
\]
where $\Delta [Ca^{2+}]$ is the presynaptic increase in $Ca^{2+}$ concentration, $V$ is the bouton volume, $N_A$ is the Avogadro constant, and $k_B$ is the endogenous $Ca^{2+}$ absorption rate.

We further assume a volume of ORN synaptic boutons of approximately $0.5 - 2 \mu m^3$, based on diameters of $0.5 - 8 \mu m$ derived from electron-microscopic evidence (e.g. Kasowski et al. 1999). This is within the range spanned by hippocampal CA3-CA1 synapses ($\sim 0.13 \mu m^3$; Shepherd and Harris, 1998), and the volume of the calyx of Held ($\sim 400 \mu m^3$; Helmchen et al. 1997).

On the grounds that for bouton volumes at both extremes of this range the increase in free $Ca^{2+}$ concentration following an action potential (AP) has been estimated $300 - 500 nm$ (calyx of Held, Helmchen et al. 1997; pyramidal cell, Koester and Sakmann, 2000), we assume a similar rise in free $Ca^{2+}$ concentration after the arrival of an AP in the olfactory nerve synaptic bouton.

The estimated endogenous $Ca^{2+}$ absorption rate $k_B$ ranges from 40 to 140 - corresponding to 2.5% and <1% free $Ca^{2+}$ ions - in cortical neurons (Helmchen et al. 1997; Koester and Sakmann, 2000), where the higher absorption rate was proposed to be partly due to the employed indicator. We therefore prefer using the lower absorption rate in our calculations.

Bringing it all together, we base our estimation on an average volume of $1.2 \mu m^3$, an average increase in $Ca^{2+}$ concentration $\Delta [Ca^{2+}]$ of $400 \text{nm}$, and an endogenous $Ca^{2+}$ absorption rate $k_B$ of 40. By plugging these parameters into the equation above, we derive an influx of $Ca^{2+}$ ions per AP of $Ca_{in} \approx 12,000$. This number corresponds to the approximate ion influx per synaptic vesicle released since vesicle release probability in ORN terminals is very high (>80%) whereas multivesicular release has not been observed.
(Murphy et al. 2004). With 12,000 ions per vesicle, we are within the range of values estimated in the calyx or Held (14,000; Helmchen et al. 1997) and pyramidal cell boutons (5,500; Koester and Sakmann, 2000).

If we increased $k_B$ to 140, we would derive an influx of 40,000 ions per vesicle. In our calculations, this would increase total presynaptic costs per AP (including action potential costs in the axon, calcium influx and vesicle release) by 0.003 %. We therefore consider the exactness of $k_B$ as a negligible factor for our calculations.

The Ca$^{2+}$ ions are extruded by the Na$^+$/Ca$^{2+}$ exchanger and/or the Ca$^{2+}$ pump, both mechanisms consuming 1 ATP per Ca$^{2+}$ ion, i.e. a total of 12,000 ATP per vesicle (cf. above and Attwell and Laughlin, 2001).

4.2. Vesicle release and recycling.

The Ca$^{2+}$ ions interact with the components of the synaptic vesicle to bring about vesicle mobilization, fusion and exocytosis, followed by endocytosis and clathrin coating. Although many protein components of these actions have been identified, the enzymatic and mechanical steps are not well understood. In general they are believed to have only modest energy requirements.

Phosphorylation of the approximately 400 molecules/vesicle involved in vesicle fusion and exocytosis requires 1 ATP per molecule, i.e. a total of 400 ATP. It can be estimated that mechanical fusion and exocytosis requires 10 ATP, and endocytosis and clathrin coating another 10 ATP per vesicle. Thus, vesicle release and recycling can be estimated to consume some 420 ATP/vesicle (cf. Attwell and Laughlin, 2001).
In sum, the total presynaptic energy requirements associated with Ca\(^{2+}\) entry and pumping and the release of one synaptic vesicle are dominated by the Ca\(^{2+}\) pump, amounting to approximately 12,400 ATP/vesicle.

5. Postsynaptic energy demands at axo-dendritic synapses

At the afferent synapse in the glomerulus, the released neurotransmitters act at different types of receptors on the postsynaptic dendrites of mitral, tufted and glomerular cells such that receptor-mediated ionic channels are opened. Following Attwell and Laughlin (2001), we can estimate the numbers \(n_{\text{ion}}\) of a certain ion type entering the cell following receptor activation by the following formula:

\[
    n_{\text{ion}} = \Delta V (mV) \times t_{\text{open}} (\text{sec}) \times g_{\text{channel}} (pS) \times r_{\text{ion}} \times n_{\text{channels}} \times c
\]  

(5)

where \(r_{\text{ion}}\) is the fraction of channel conductance due to the ion in question, \(\Delta V\) is the driving force for the ion in question, \(t_{\text{open}}\) is the average opening time of the channel, \(g_{\text{channel}}\) is the average conductance of a single channel, \(n_{\text{channel}}\) is the approximate number of channels activated by one vesicle neurotransmitter, and \(c\) is the Coulomb constant.

In the following we consider three types of glutamergic receptors.

5.1. Non-NMDA receptors.

Release of a single vesicle containing glutamate causes opening of postsynaptic non-NMDA channels. We assume that approximately 35 non-NMDA channels are activated, based on an immunogold labeling essay in the glomerular layer of the rat olfactory bulb (Sassoé-Pognetto et al. 2003) where we estimated the total number of channels per synapse from the average number of channels per section profile. We further assume a mean channel opening time of 1 msec, a channel conductance of approximately 12 pS,
and that 2/3 of the conductance is due to \( \text{Na}^+ \) with a driving force of 120mV. Due to the lack of data from the olfactory bulb, these values are based on data for non-NMDA receptors in the cortex as summarized in Attwell and Laughlin, 2001.

With equation (5) above, we estimate that this will allow approximately 200,000 \( \text{Na}^+ \) ions to enter, requiring 1/3 as many ATP (67,000) for the \( \text{Na}^+ / \text{K}^+ \) pump to extrude.

5.2. NMDA receptors.

For the NMDA receptors, we assume a single-channel conductance of ~50 pS as documented in the rat accessory olfactory bulb (Charlesworth et al. 1995). From the immunogold labeling study (Sassoé-Pognetto et al. 2003) we take that the number of NMDA receptors per axodendritic synapse of the glomerular layer is about 18 (i.e. half the number of non-NMDA receptors) but we consider a smaller number of activated channels per vesicle (as low as 2 in the hippocampus; Spruston et al. 1995). Similarly, the effect of longer open times of NMDA channels is counterbalanced through a partial blockage in physiological \( \text{Mg}^{2+} \), such that the total charge transfer through NMDA channels is estimated equivalent to that of the non-NMDA channels.

NMDA channels also are permeable to \( \text{Ca}^{2+} \); we assume that 10% of the charge is carried by \( \text{Ca}^{2+} \). This suggests that the NMDA receptors bring in 180,000 \( \text{Na}^+ \) and 10,000 \( \text{Ca}^{2+} \). Extruding the \( \text{Ca}^{2+} \) by the \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger or the \( \text{Ca}^{2+} \) pump brings in an extra 30,000 \( \text{Na}^+ \) ions. Extrusion of the total of 210,000 \( \text{Na}^+ \) ions requires 70,000 ATP.

5.3. Metabotropic glutamate receptors.

There is evidence for mGluRs at afferent synapses (Kinzie et al. 1997). They may act to release intracellular \( \text{Ca}^{2+} \) through phospholipase C and IP3. Attwell and Laughlin (2001) suggest that approximately 3,000 ATPs are required to pump the \( \text{Ca}^{2+} \) back into the
endoplasmic reticulum of the dendritic spine of a pyramidal cell, including a small amount to resynthesize IP3. In the absence of data on the change if intracellular free Ca concentration, buffering capacity and compartment volume, we adopt the same value.

5.4. Summary.

In sum, the total Na\(^+\) ion flows at a single postsynaptic site in response to the action of a single vesicle of glutamate on both non-NMDA and NMDA is 200,000 plus 210,000 = 410,000 Na\(^+\) ions. The total postsynaptic energy requirements - approximately a third of the Na\(^+\) load plus the costs for Ca\(^{2+}\) extrusion - amount to a total of 140,000 ATP per vesicle.

6. Glutamate recycling energy demands

6.1. Astrocytic glutamate uptake.

Following Attwell and Laughlin, after acting on the postsynaptic receptors glutamate is taken up from the extracellular space by astrocytes by means of co-transport of 3Na\(^+\)/1H\(^+\)/1glutamate and countertransport of 1 K\(^+\). The Na\(^+\)/K\(^+\) pump extrudes the Na\(^+\) and K\(^+\), requiring 1 ATP, and a Na\(^+\)/H\(^+\) exchanger pumps back the Na\(^+\) and H\(^+\), including an extra Na\(^+\) requiring 1/3 ATP, resulting in a total of 1.3 ATP per glutamate molecule.

6.2. Astrocytic glutamate metabolism.

The metabolic pathways include maintenance as glutamate, conversion to glutamine, conversion to \(\alpha\)-ketoglutarate, and transamination to aspartate as outlined by Attwell and Laughlin. A total consumption of 1 ATP is assumed.

6.3. Glutamate export to neurons.
Evidence suggests that export of glutamate to neurons is driven by sodium and proton-dependent glutamine transporters SN1 in the astroglial membrane (Umapathy et al. 2005), requiring 1/3 ATP per molecule glutamine. Uptake in neurons takes place through co-transport with one Na\(^+\) through system A transporters (Chaudhry et al. 2002) requiring 1/3 ATP.

6.4. Packaging into vesicles.

Approximately 1/3 ATP/glutamate is required (see Attwell and Laughlin, 2001).

6.5. Summary.

In sum, a total of 3.33 ATP is needed to recycle one glutamate molecule. For 4,000 glutamate molecules in a vesicle released and repackaged, a total of approximately 13,000 ATP is needed. This is an order of magnitude less than the energy required by the postsynaptic response itself.

7. Costs of presynaptic inhibition of glutamate release in ORN terminals

Glutamate release at ORN terminals is reduced by 30-50% through GABA release from postsynaptic PG cells (Aroniadou-Anderlaska et al. 2000, McGrann et al. 1, 2005). Rodent ORN terminals express GABA\(_B\) receptors (Priest and Puche, 2003), which are seven-transmembrane domain proteins with close homology to the metabotropic glutamate receptors. The activation of the GABA\(_B\) receptors leads to a suppression of calcium influx in the presynaptic terminal (Wachowiak et al. 2005). Attwell and Laughlin (2001) estimate that the population of metabotropic receptors on the presynaptic terminal requires 3,000 ATPs per vesicle GABA. On the postsynaptic release site, we assume that Ca\(^{2+}\) entry and restoration, vesicle release and recycling have demands of 12,400 ATP, as
in the case of presynaptic neurotransmitter release sites. Total costs (without GABA recycling) are 15,400 ATP per vesicle of GABA released.

8. **Energy requirements in glomerular dendrites**

The active properties of the dendrites have their own energy demands.

It is known that the mitral cell primary dendrite contains Na channels giving a conductance of 100 pS µm$^{-2}$ (Bischoffberger and Jonas, 1997). We assume a similar density of 100 pS µm$^{-2}$ in mitral, tufted and PG cell tufts since recent experiments have provided evidence that the branches of the glomerular tuft are also active (Chen et al. 1999, 2002). It was demonstrated that synaptic input in the primary dendrite of mitral cells can elicit action potentials in the soma that then back-propagate into the dendrites. On the other hand, strong synaptic input in the dendrites initiates a forward-propagating AP that travels to the soma. Either way we can assume that synaptic input through sensory neuron axons leads to an activation of the dendritic tuft.

The ionic properties and the extent of the activation determine its costs. In the mitral cell, a single back-propagating action potential (bAP) fully and reliably activates distal dendrites, and causes an action potential associated calcium influx in the mitral dendritic tuft (Debarbieux et al. 2003; Ma and Lowe, 2004). Calcium imaging in combination with paired soma–dendritic patch-clamp recordings have revealed that bAPs reliably evoke Ca$^{2+}$ transients even in distal mitral cell dendrites. Accompanying computational modeling confirms that those Ca$^{2+}$ currents are consistent with active, not passive, backpropagation (Ma and Lowe, 2004).
In PG cells, calcium influx and calcium mediated EPSPs, produced by synaptic input from principal cell dendrites and from sensory nerve axons have been demonstrated as well (Murphy et al. 2005; Zhou et al. 2006).

We therefore assume that the cost of dendritic bAPs is determined by the influx of both sodium and calcium ions. Na\(^+\) current causes the initial spike for which we assume an average voltage excursion of \(\Delta V = 115\) mV, given a sodium reversal potential of 60 mV as documented for principal cortical neurons, and a resting potential of mitral cells of circa \(-55\) mV (Margrie et al. 2000). Ca\(^{2+}\) currents lead to an after-depolarization. The strength of the calcium mediated after-depolarization is difficult to estimate but from voltage traces of calcium spikes from Yuan et al. (2006) and Murphy et al. (2005) we take that the voltage excursion in glomerular dendritic tufts amounts to maximal 20% of the sodium mediated depolarization, and we assume \(\Delta V = 20\) mV. The smaller voltage excursion could be caused by the relatively lower density and partly higher activation thresholds of the various voltage-dependent calcium channel types found in dendrites compared to sodium channels (Johnston, 1996).

Using equation (2) again, the ATP demand in bulbar dendrites for the extrusion of Na\(^+\) ions following an action potential amounts to 9,600 ATP per \(\mu m^2\). For Ca\(^{2+}\) we calculate an influx of approximately 625 ions per \(\mu m^2\) from equation (2). Since Ca\(^{2+}\) is divalent, we double this number to 1250 ions per \(\mu m^2\) (instead of quadrupling) in order to take into account the temporarily overlapping potassium outflow. As described before, we have a removal cost of one ATP molecule per Ca\(^{2+}\). Thus, the energy demand for calcium is 1,250 ATP per \(\mu m^2\). Together, the energy costs for dendritic bAP propagation are approximately 10,850 ATP per AP per \(\mu m^2\).
We assume that bAPs in mitral cell, tufted cell and PG cell tufts are governed by the same active properties. As a consequence, differences in the energetic costs will only depend on the surface areas assumed for each cell type.

9. Presynaptic energy demands at dendro-dendritic synapses
Astrocyte recordings of glutamate transporter currents show that glutamate release from axonal terminals is followed by asynchronous glutamate release from dendritic terminals (De Saint Jan and Westbrook, 2005). Thus, either through local EPSPs or through bAPs we have activation of dendro-dendritic synapses as a direct consequence of axonal input.

As for afferent synapses, we estimate the costs for the release of one synaptic vesicle to be approximately 12,400 ATP molecules.

10. Postsynaptic energy demands at dendro-dendritic synapses
Dendro-dendritic synapses onto PG cells are assumed to be excitatory. As outlined before, we assume postsynaptic demands of 13,000 molecules ATP per vesicle glutamate. Dendro-dendritic synapses onto mitral/tufted cells are assumed to be GABAergic. The assumptions regarding postsynaptic energy demands for glutamate also apply to γ-aminobutyric acid (GABA) receptors (see equation 5). GABA receptors on mitral and tufted cells receive synaptic input from PG cells that opens chloride channels. From electronmicroscopic staining evidence (Panzanelli et al. 2004) we can assume a density of at least 15 activated chloride channels per reciprocal synapse between M/T and PG cell dendrites. In embryonic hippocampal neurons in mice a predominant chloride channel conductance of 28 pS was found, along with an average opening time of 2 ms (Lorez et
al. 2000). If we assume a resting potential of $< 70$ mV in mitral/tufted cells and a chloride reversal potential of -70 mV, the driving force for chloride ions will be close to zero. If we however consider membrane oscillation and spontaneous firing among M/T cells (Chen et al. 1997), the effective average membrane potential should be slightly depolarized. We assume an effective resting potential of -55 mV (Margrie et al. 2000; Cang et al. 2003), and therefore an average driving force for Cl$^-$ of 15 mV. Based on these assumptions the number of Cl$^-$ entering the postsynaptic mitral/tufted cell per release of one vesicle GABA$_A$ amounts to approximately 80,000 chloride ions (cf. equation 3). We assume an active chloride transport mechanism for restoring ionic concentrations that is ATP dependent, a Cl$^-$-ATPase (Shiroya et al. 1998). The total costs for chloride ion extrusion then amounts to 80,000 ATP per vesicle.

Note that subpopulations of dendritic tufts and PG cells in the glomerulus of adult rats have been shown to accumulate chloride, effectively reversing or canceling the driving force for chloride ions (Siklos et al. 1995). Under such conditions our calculations are not valid, but we consider the majority of GABAergic synapses inhibitory.

11. GABA recycling energy demands

11.1. Astrocytic and neuronal GABA uptake.

After release into the synaptic cleft, GABA is taken up both by neuronal and astrocytic transporters which co-transport one molecule of GABA with 2 Na$^+$ and 1Cl$^-$ (Kanner, 1994). The cost of extruding the sodium ions is 2/3 ATP, and for chloride it is 1 ATP,
assuming an ATP-depending Cl⁻-ATPase, such that the total transportation cost per GABA molecule is 1.66 ATP.

**11.2. GABA metabolism.**

In neurons, GABA is directly recycled and packaged into vesicles. In astrocytes, GABA enters the Krebs cycle, exits as glutamate, and is amidated to glutamine by glutamine synthetase which costs 1 ATP per molecule.

**11.3. GABA export to neurons.**

From the astrocytes, glutamine is exported to neurons, where we assume transport through SN1 and system A transporters with total energy demands of 2/3 ATP. In neurons, the glutamine is transformed into glutamate by neuronal glutaminase. It then can be converted into GABA through glutamic acid decarboxylase.

**11.4. GABA packaging.**

Analogous to glutamate, we assume 1/3 ATP per molecule GABA is required (see Attwell and Laughlin, 2001).

**11.5. Summary.**

In sum, if we assume an equal uptake by neurons and glia cells, recycling of one GABA molecule requires approximately 3 ATP per molecule. With an estimated 4,000 GABA molecules per vesicle we obtain 12,000 molecules ATP per vesicle. This is approximately 1/5 of the postsynaptic response itself.

**12. Energy demands in postsynaptic PG cell tufts**

The activation of a principal cell tuft leads to glutamergic release onto PG cells. In the PG cell, the excitatory input causes an EPSP with ΔV = 10mV (Murphy et al. 2005) and
a subsequent calcium spike that we estimate with $\Delta V = 30$ mV (Hayar et al. 2004; Zhou et al. 2006).

From Ohm’s law and given a PG cell input resistance of at least $1 \, \text{G}\Omega$, we calculate a sodium current of $0.01\text{nA}$ during the EPSP. We assume an average duration of the input current of $5\text{ms}$, and from this we derive a $\text{Na}^+$ influx of $3 \times 10^5$ ions per EPSP, equaling a cost of $10^5 \text{ATP}$ per EPSP.

From equation (2) we derive a $\text{Ca}^{2+}$ influx of $950$ ions per $\mu\text{m}^2$ during the calcium spike. As described before, we have a removal cost of one ATP molecule per $\text{Ca}^{2+}$, amounting to $950 \text{ATP}$ per $\mu\text{m}^2$.

Thus, total energy demands for ion removal following an EPSP and a $\text{Ca}^{2+}$ spike are $10^5 \text{ATP}$ per PG cell plus $950 \text{ATP}$ per $\mu\text{m}^2$ of PG cell membrane surface area.

13. **Calculations of population volumes**

Applying the estimates of energy demands per unit membrane area or synaptic site requires making quantitative estimates of the populations of active elements. We imposed an important constraint on these estimates: that the calculated volumes of the populations of participating elements had to combine to fit into the total volume of a glomerulus. This in turn required estimates of the total populations of the participating elements. These constraints turned out to be considerably demanding. We consider first average values, and then ranges.

A glomerulus contains the incoming ORN axons, and the dendrites of mitral, tufted and PG cells. Since the time of Allison and Warwick (1949) quantitative data for these elements have been available for various species. Based on recent data for the rat,
there are approximately 15,000,000 receptor cells for 3,500 glomeruli on one side for a convergence ratio of nearly 4,500:1 (see Table S2 for references). We also assume 25 mitral cells, 60 tufted cells (Meisami et al. 1981) and 100 PG cells (cf. Woo et al. 1991) per glomerulus.

13.1. Volume of presynaptic axonal elements

We have derived a model of the ORN axonal tree based on camera lucida reconstructions and exhaustive work on axon morphology by Klenoff et al. (1998). From this we can estimate the volumes of ORN axonal branches, preterminal segments and synaptic terminals.

We assume that upon entering a glomerulus an olfactory axon gives rise on average to 17 terminal branches. The axon maintains a diameter of 0.2 µm over its total length (including branches) of 195 µm, except for the preterminals and synaptic terminals. Preterminals have diameters of 0.4 µm over a length of 5 µm, and synaptic terminals have a diameter of 0.6 µm over a length of 0.6 µm. The average number of en-passant boutons and varicosities per axon is 26.

With these dimensions the volume of an axon and its branches is 3.5 µm$^3$, the preterminal segments have a volume of 10.7 µm$^3$, and the synaptic terminals (varicosities and boutons) have a volume of 4.1 µm$^3$, for a total of approximately 18.5 µm$^3$.

Given a total number of 4,500 ORNs per glomerulus, the volume of all axonal segments amounts to 15,500 µm$^3$. Preterminals and synaptic terminals take up 48,000 µm$^3$ and 20,000 µm$^3$, respectively.
The total volume of axonal elements in a single glomerulus is thus approximately 83,500 µm$^3$. This is approximately 31 % of the total glomerular volume, which is close to the estimate of 29% by Kasowski et al. (1999), based on electronmicroscopical observations.

13.2. *Volume of dendritic elements*

Three types of neuron contribute dendrites to a glomerulus: the large mitral (M) cells, medium size tufted (T) cells, and small periglomerular (PG) cells. It is known that M and T cells have a primary dendrite which, at its terminus at the glomerular border, sends off multiple branches, which then branch in a bifurcating manner. The simplifying approach taken here is to represent the origin of the tree in a canonical form, and adjust the size of the branching tree to the three cell types.

For the mitral cell we first estimate the volume of a unit length of dendritic branches of different diameters, corresponding to successive branching levels. Based on observations using two-photon microscopy (Zhou et al. 2003), we assume a canonical model in which each primary dendrite with an average diameter of 3.5 µm gives rise to three dendritic trees (initial diameter 3.2 µm), each of these giving rise to six branch levels (5 bifurcations). It would be convenient to have the diameters decrease to satisfy the $d^{3/2}$ rule, Rall’s criteria for equivalent cylinders (Rall, 1962), such that

$$d_{\text{parent}}^{2/3} = \sum d_{\text{daughter}}^{2/3} \quad (5)$$
(where $d_{\text{parent}}$ denotes the diameter of the parent branch, and $d_{\text{daughter}}$ denotes the diameter of the diverging branches). Our observations suggest a bifurcation rule closer to $d^2$.

Consensus observations suggest that a mitral cell dendritic tree extends mostly across the glomerulus. The calculations for the diameters, lengths, volumes and surface areas for the successive levels of branching of the canonical trees are shown in table 2.

For the **mitral cell** these calculations give a total volume of 1700 $\mu m^3$. For 25 mitral cells per glomerulus this gives a total volume of approximately 42,000 $\mu m^3$, in other words approximately 15% of the glomerular volume.

For the **tufted cells**, we assume the same canonical model, scaled for the smaller size of the tufted cell. We used a radius of \( \frac{3}{4} \) of the radius of a mitral cell dendritic tree, such that it extends approximately 54 microns. The primary dendrites being 3 $\mu m$ in diameter, each of the three first level dendritic branch has an average diameter of 2.7 $\mu m$ such that a single dendritic tuft has a total volume of 930 $\mu m^3$. Assuming 60 tufted cells per glomerulus this gives a total volume of approximately 55,700 $\mu m^3$, in other words approximately 21% of the glomerular volume.

For the **PG cells**, we scale the radius of the tuft to $\frac{1}{2}$ the radius of a mitral cell dendritic tree, such that it extends approximately 36 microns. The primary dendrite gives rise to three trees with 3 branch levels (2 bifurcations). The primary dendrite is 3 $\mu m$ in diameter which reduces with subsequent branching levels to 0.4 $\mu m$ in the dendritic tips. The two most distal branching levels are covered with dendritic spines (cf. Pinching and Powell, 1971; McQuiston and Katz, 2001). From imaging data we estimate a density of one spike per $\mu m$ length, a spine shaft of 0.2 $\mu m$ in diameter and 1 $\mu m$ in length, and a spine dead of 1 $\mu m$ in diameter and 0.5 $\mu m$ in length.
We derive a total volume of a dendritic tuft of approximately 280 µm$^3$. Assuming 100 PG cells per glomerulus this gives a total volume of approximately 28000 µm$^3$, in other words approximately 10 % of the glomerular volume.

It follows that the total volume of all three types of dendritic tuft within a 80 µm diameter glomerulus is approximately 125,000 µm$^3$, in other words approximately 46 % of the glomerular volume.

13.3. Astroglial volumes

The volume and distribution of astrocytes and their processes in our model of the glomerulus are based on imaging data, suggesting a formal network of processes within the glomerulus whereas all the cell bodies are located superficially in a nearly geometric arrangement. The processes reaching into the glomerulus are thin, sheet-like structure as described by Valverde et al. (1991) in velate astrocytes, and as indicated in electronmicroscopic images of glomerular sections (Kasowski et al. 1999).

In this model there is a compartmentalization of the glomerulus by astroglial processes separating neuronal elements, and enclosing dense clusters of synaptic connections. This complies with the compartmental organization of the glomerulus suggested through immunocytochemistry (Klenoff et al. 1999), and with electronmicroscopic data showing glial elements forming a border around patches of high synaptic density (Chao et al. 1997). From the latter we derive a simplified cubic compartment demarcated by glial processes with dimensions of approximately 4 µm x 4 µm x 4 µm = 64 µm$^3$. 
The astrocytic processes are electrically coupled by gap junctions (Sul et al. 2004). There is a high sensitivity of astrocyte glutamate transporter currents to glutamate release within the glomerulus (De Saint Jan and Westbrook, 2005). This indicates a quasi-continuous network of astroglial processes.

An intimate interdigitation of glial and neural elements is also indicated by studies of the developing glomerulus (Bailey et al. 1999). The results show that proto-glomeruli are formed through the arrival of axons from the olfactory nerve layer (ONL), and of glial processes from the extracellular plexiform layer (EPL), followed later by dendritic elements, which also come from the EPL. This temporal order suggests that dendritic elements use the framework provided by glial cells to invade the proto-glomerulus and contact the axonal synapses.

For our calculations, we assume a compartment size of 64 $\mu$m$^3$ and an average section area of astroglial sheets of 0.4 $\mu$m$^2$ which is equivalent to a cylinder of 0.7 $\mu$m in diameter. Given a glomerular volume of 270,000 $\mu$m$^3$, we have approximately 4200 compartments per glomerulus, demarcated by glial processes that take up approximately 20,000 $\mu$m$^3$, or 7.5% of the glomerular volume.

14. Calculations of population surface areas

Given that the morphologies of the participating elements account for the volume of a glomerulus, we can next calculate the surface areas of all elements (see table 2), which will be the basis for the calculations of energy requirements related to axon potential generation.

14.1. Surface area of axon and axonal branches.
We derive a surface area (SA) of a single axon and its branches of approximately 70 µm$^2$.

Given 4,500 ORNs per glomerulus, the total SA of axons and axon branches equals approximately 310,000 µm$^2$.

14.2. Surface area of preterminal axonal segments.  
We assume a diameter of 0.4 and a length of 5 µm. With an average of 17 terminal branches per axon, we can derive a total surface area (SA) of approximately 106 µm$^2$ for all preterminal segments of a single axon.

With 4,500 ORNs per glomerulus, the total SA of preterminals equals approximately 480,000 µm$^2$, or 1.5 times the surface area that all axons and axonal branches contribute.

14.3. Surface area of presynaptic terminals.  
We obtain a surface area per synaptic terminal of 1.1 µm$^2$, and a total surface area of all synaptic terminals per single ORN axon of approximately 30 µm$^2$.

With 4,500 ORNs per glomerulus, the total SA of synaptic terminals equals approximately 132,000 µm$^2$. Note that although the presynaptic terminals make up a third of the presynaptic axonal volume, they constitute only a sixth of the presynaptic surface area.

The total surface area of axons, axonal branches, preterminal segments and synaptic terminals combined have is approximately 920,000 µm$^2$. This membrane area contains the ion channels for action potential propagation.

14.4. Surface area of the tuft of a mitral cell primary dendrite.
As in the volume calculations, we assume a tuft consisting of 3 binary branching trees with 6 branch levels (5 bifurcations), where the diameter decreases according to a bifurcation rule of $d^2$.

The surface areas for the branches each level of a single binary branching tree are given in table 2. The total dendritic surface area of a single binary branching tree is 6000 $\mu m^2$. There are approximately 25 mitral cells per glomerulus, which gives a total mitral cell tuft surface area of 150,000 $\mu m^2$ per glomerulus.

Similarly, we estimate the surface areas of the dendritic branching tree of the other two cell types.

14.5. **Surface area of the tuft of a tufted cell primary dendrite.**

A single tufted cell tuft gives a surface area of 3900 $\mu m^2$; the total surface area for 60 tufted cells is therefore approximately 230,000 $\mu m^2$.

14.6. **Surface area of the tuft of a PG cell primary dendrite.**

A single PG cell tuft with spines has a surface area of 880 $\mu m^2$. The total surface area for 100 PG cells is therefore approximately 88,000 $\mu m^2$.

**15. Upper limit of ON activation through electrophysiological stimulation**

15.1. **Energy requirements of action potential propagation in presynaptic axons.**

For an upper limit of energy demands, we assume that all 4,500 ORNs axons converging onto the glomerulus fire once and simultaneously.

As detailed in chapter 1, the sodium ion influx during action potential propagation amounts to 32,200 Na$^+$ per square micron of membrane surface. For the population of
axons, with a total surface area of approximately 930,000 µm², this gives an influx of 7.2 x 10⁹ Na⁺, corresponding to a demand of 9.6 x 10⁹ ATP molecules per action potential.

15.2.  **Presynaptic energy demands at axo-dendritic synapses.**

As detailed in chapter 2, presynaptic energy demands amount to 12,400 ATP/vesicle. Given a transmitter release probability of 1, all 117,000 axo-dendritic synaptic terminals respond to an AP with the release of a vesicle, requiring 1.45 x 10⁹ ATP at the presynaptic terminals.

The energy demands of an action potential in presynaptic axons thus includes 9.6 x 10⁹ ATP for the action potential in the axon and 1.5 x 10⁹ ATP per vesicle at the synapses, giving a total cost of 1.1 x 10¹⁰ ATP per action potential for all 4,500 axons.

15.3.  **Postsynaptic energy demands at axo-dendritic synapses.**

As shown in chapter 3, the postsynaptic energy demands in response to glutamate are 140,000 ATP per vesicle. For a total of 117,000 postsynaptic sites on all dendritic tuft types (mitral, tufted, PG), the total postsynaptic energy requirements are 1.6 x 10¹⁰ ATP per vesicle.

15.4.  **Energy demands of bAPs in dendritic elements.**

We assume that all mitral and tufted cells are being activated and respond with one AP to a single axonal input (Chen et al. 1997), and we assume one subsequent bAP. From the energy demands of one bAP per surface unit derived in chapter 6, and the surface areas, we calculate dendritic energy demands of 1.66 x 10⁹ ATP per bAP in mitral cells, and 2.9 x 10⁹ ATP per bAP in tufted cells, i.e. 4.6 x 10⁹ ATP per bAP in M/T cells.
For simplification, we assume that PG cells also respond with one bAP per strong depolarizing input (from receptor cell neurons), and we derive dendritic energy demands of \(1.7 \times 10^9\) ATP.

15.5. **Energy demands at dendro-dendritic synapses.**

There are approximately one-sixth as many dendro-dendritic synapses as axo-dendritic synapses in the glomerulus, i.e. 20,500 synapses (Kasowski *et al.* 1999). Approximately 2/3 of these dendro-dendritic synapses project from PG onto mitral/tufted cells and are considered GABAergic. Their activation has been shown to be mediated by EPSP-associated calcium influx following glutamatergic activation through sensory nerve axons and principal cell dendrites (Murphy *et al.* 2005). We assume that calcium influx associated with bAPs also leads to synaptic activity and release of GABA.

The remaining 6,800 synapses are thought to be excitatory connections from mitral/tufted cells onto PG dendrites.

15.6. **Presynaptic demands at dendro-dendritic synapses.**

Assuming a transmitter release probability of 1 in dendro-dendritic synaptic terminals, 6,900 excitatory synaptic terminals, and 13,700 inhibitory synapses are activated following each bAP, and will release one vesicle each. As outlined in chapter 2, we estimate the costs for the release of one synaptic vesicle close to 12,400 ATP molecules. Thus, the presynaptic energy demands for the excitatory synapses are \(8.5 \times 10^7\) ATP per bAP, and those for inhibitory synapses are \(1.7 \times 10^8\) ATP per bAP, giving a total cost of \(2.6 \times 10^8\) ATP per bAP.

15.7. **Postsynaptic demands at dendro-dendritic synapses.**
Dendro-dendritic synapses onto mitral/tufted cells are assumed to be inhibitory. As outlined in chapter 8, we base our calculations on postsynaptic demands of 80,000 molecules ATP per vesicle GABA$_A$. With 13,700 synaptic inputs from PG onto M/T cells per bAP, we obtain postsynaptic energy demands in M/T cells of $1.1 \times 10^9$ ATP per bAP.

Dendro-dendritic synapses onto PG cells are assumed to be excitatory. Based on chapter 3 we estimate postsynaptic demands of 140,000 molecules ATP per vesicle glutamate. Given 6,700 synaptic inputs onto PG cells, we obtain energy demands in PG cells of $9.6 \times 10^8$ ATP per bAP. The EPSP caused by glutamergic input and the subsequent calcium spike add respective requirements of $10^7$ ATP and $8.3 \times 10^7$ ATP per bAP in the total PG cell population. Thus, total postsynaptic energy demands in PG cells are $1.6 \times 10^9$ ATP per bAP.

All dendro-dendritic synapses together have postsynaptic energy demands of $2 \times 10^9$ ATP per axonal input volley.

**15.8. Total energy demands of processing by the dendro-dendritic circuits.**

In sum, the response to one maximal axonal input in mitral and tufted cells requires approximately $4.6 \times 10^9$ ATP molecules for bAP propagation and dendo-dendritic presynaptic events, and $1.1 \times 10^9$ ATP for dendro-dendritic postsynaptic events. The corresponding values for PG cells are $1.7 \times 10^9$ ATP and $1.6 \times 10^9$ ATP respectively. Thus, a single maximal activation of the dendro-dendritic circuit has total energy requirements of approximately $10^{10}$ ATP molecules.

**15.9. Glutamate recycling at axo-dendritic synapses.**
A total of 13,000 ATP are needed for the recycling of one vesicle of glutamate released. The energy demands for glutamate recycling at 117,600 afferent synapses amount to $1.5 \times 10^9$ ATP molecules. This is an order of magnitude less than the postsynaptic response.

15.10. **Glutamate recycling at excitatory dendro-dendritic synapses.**

Approximately 13,000 molecules ATP are needed for the recycling of one vesicle glutamate. The energy demands for glutamate release at all 6,800 excitatory dendro-dendritic synapses amount to $9.0 \times 10^7$ ATP molecules.

**GABA recycling at inhibitory dendro-dendritic synapses.**

As shown in Methods, approximately 12,000 molecules ATP are needed for the recycling of one vesicle GABA. The energy demands for GABA release at all 13,700 inhibitory dendro-dendritic synapses amount to $1.7 \times 10^8$ ATP molecules.

15.11. **Total costs.**

Total costs of neurotransmitter recycling are therefore approximately $2.6 \times 10^8$ ATP. Total signalling costs in response to activation of all 4,500 axons amount to $4 \times 10^{10}$ ATP. One fourth can be attributed to dendro-dendritic processing, the other three quarters to axonal and axo-dendritic processing.

16. **Lower limit of ON activation through electrophysiological stimulation**

Again, we will outline the energy demands of the glomerulus in the ideal case (transmitter release probability of one, a single synchronous input volley from the ORN axons). Since the calculation follows the same principles as above, we will give details where additional assumptions are made, but in general present only the main results.
We consider a single input volley from 45 axons, or 1% of the axon population. The responses of 1200 axo-dendritic synaptic terminals lead to costs of $1.5 \times 10^7$ at the presynaptic terminals, to costs of $1.6 \times 10^8$ ATP at the postsynaptic terminals, and to glutamate recycling costs of $1.5 \times 10^7$ ATP per AP.

There are no data available about the connectivity rules within the glomerulus but considering the ratios of surface areas of the different cell types, as well as the proportional distribution of the different synapse types (Kasowski et al. 1999) we estimate that of the 26 presynaptic terminals of each ORN axon, 8 will project onto PG cells, 11 onto tufted cells and 7 onto mitral cells. This of course suggests that all ORNs axons are equal in their preferences for the different cell types they target.

The area that is spanned by the branches of a single axon covers only 15% of the glomerular width (Kasowski et al. 1999), suggesting that a given axon converges its input onto few dendritic tufts. Since we are interested in a lower limit of activation, we require all axons to target only one single cell of each type. For example, 495 (45 x 11) synapses from the axons will converge onto a single tufted cell. This number is consistent with our model since in our calculations each tufted cell receives approximately 850 axonal synapses. The initial maximal response to 1% axonal input will therefore consist in the activation of one mitral, one tufted and one PG cell.

In the mitral and the tufted cell, we assume a response of one spike. The associated bAP will require $6.3 \times 10^7$ ATP in the mitral cell, and $4.8 \times 10^7$ ATP in the tufted cell. The PG cell also responds with one spike. The associated bAP requires $1.1 \times 10^7$ ATP.
According to the olfactory circuit model, the bAPs in M/T cells activate excitatory dendro-dendritic synapses onto PG cells. From our numbers for cell populations and synapses we estimate there are 110 dendro-dendritic presynaptic terminals per mitral cell, and 70 dendro-dendritic presynaptic terminals per tufted cell. Given a synaptic release probability of 1, maximally 180 terminals will be activated. The costs at the presynaptic terminals amount to $2.2 \times 10^6$ ATP, the costs at the postsynaptic terminals (on PG cells) correspond to $2.5 \times 10^7$ ATP, and the recycling of glutamate requires $2.3 \times 10^6$ ATP. The activation of a tufted cell reproducibly evokes excitatory synaptic input to at least 5 PG cells (Murphy et al. 2005). For a lower limit, we assume that the activated mitral cell projects to the same 5 PG cells. We assume the 5 PG cells are fully activated through dendro-dendritic input. The resulting calcium spikes and EPSPs in the PG cells lead to energy demands of $4.1 \times 10^6$ and $5.2 \times 10^5$ ATP, respectively.

On the other hand, the bAP in the PG cell activates excitatory dendro-dendritic synapses onto M/T cells. With a release probability of 1, all 230 dendro-dendritic presynaptic terminals of the PG cell will respond with release of GABA, leading to energy requirements of $2.8 \times 10^6$ ATP at the presynaptic terminals, $1.8 \times 10^7$ ATP at the postsynaptic terminals (on M/T cells), and $2.8 \times 10^6$ ATP for the recycling of GABA.

Total signalling costs at a lower limit of activation are $4.7 \times 10^8$ ATP.

17. Concentration-response model of the glomerulus
In order to predict the cellular energy demands in response to varying odor concentrations, we need to estimate the degree of glomerular activation in dependence on odor concentration. We employ a simple mathematical model that describes the response of each cell type depending on the mean firing rate of the receptor cells (cf. van Droengelen et al. 1978).

17.1. Model of ORN response.

In a simple approach, we assume that the mean firing frequency of olfactory receptor neurons expressing the same olfactory receptor is a direct measure of relative odor concentration and can be approximated with Michaelis-Menten kinetics (Meister et al. 2001):

\[ R = R_{\text{max}} \frac{C}{C + K_i} \]  

(6)

Here \( R \) is the mean firing rate, \( R_{\text{max}} \) the maximal firing rate (saturation), \( C \) the odor concentration, and \( K_i \) is the half-saturating concentration of that odor. The resulting sigmoid concentration-response curve describes ligand binding to olfactory receptors, or a dominant binding interaction in the signal transduction cascade.

While \( R \) describes the average response of the ORN population, we assume that firing thresholds and excitability are varying between individual receptors (Grosmaitre et al. 2006). Therefore the mean firing frequency is not sufficient to describe the range of responses. We assume a Poisson distribution of response frequencies (van Drongelen et al. 1978) such that higher odor concentrations correspond to both higher mean firing frequencies and a greater proportion of responding receptor cells. From the Poisson distribution, we derive the proportion of receptor neurons that respond with at least one spike:
where $R$ is mean firing rate of the population and $T$ is the time interval of response.

The time interval we allow for ORN response is 60 ms (Duchamp-Viret et al. 2000), since simple odors can be distinguished in less than 200 ms (Abraham et al. 2004). In our model, the mean frequency varies between $R = 0$ Hz for a relative odor concentration of zero, and $R_{\text{max}} = 150$ Hz for a relative odor concentration of one (saturation).

17.2. Model of target cell response.

The target cells are mitral, tufted and PG cells. In a simple approach, the response of a target cell to odor depends on the number of active receptor cells that converge onto it, and on the number of axonal inputs that are necessary to elicit an action potential. As in the receptor cells, higher odor concentrations correspond to both higher mean firing frequencies and a greater proportion of responding target cells.

We describe the proportion of target cells that respond with at least one spike following OR stimulation with

$$P_{\text{OR}}(N_S > 0) = 1 - e^{-R \cdot T}$$

where $R$ is mean firing rate of the population, $T$ is the time interval of receptor cell response, $K$ is the convergence rate of receptor cell axons onto a single cell, and $m$ is the number of axonal inputs necessary to evoke an action potential.
Based on our calculations, we suggest different axonal convergence rates for each cell type. For mitral, tufted and PG cells, we use a convergence rate of $K = 45, 180, \text{ and } 75$ respectively. The parameter $m$ assumes a minimal number of axonal inputs that are necessary to evoke an action potential in a given cell. We have found that the response of glomerular target cells to the stimulation of the ORN fascicle is indeed a step function which seems to depend on the number of axons recruited. For a single tufted or PG cell, the rise in membrane potential per axonal input has been estimated 1 mV (Arjun). Given a resting potential of -55 mV, the input of 15 axons would be needed to elicit an AP. We know that the neuronal elements of glomerulus vary in their electrotonic compactness and should therefore be considered heterogeneous in their response to synaptic currents. For a qualitative representation of those differences, we choose $m=25$ and $m=15$ for the large mitral and the smaller tufted cells, respectively. For the even smaller PG cells we choose a value of $m=8$.

17.3. Model of dendro-dendritic connectivity and GABAergic inhibition.

We try to estimate the effect that intraglomerular inhibition has on the glomerular response to a second odor stimulus directly succeeding a first odor stimulus (eg. in the case of two consequent sniffs). In a simple approach, we assume that the inhibitory response of the PG cell population to sensory input decreases the excitability of M/T cell dendrites, and decreases the release probability of glutamate at ORN presynapses. Both processes can be included in the model by raising the parameter $m$ in (6). A higher value of $m$ corresponds to a lower impact of a single axonal AP on the target cell. We assume that the strength of inhibition is proportional to the number of activated PG cells times
their mean firing rate, such that a higher odor concentration results in a greater level of inhibition:

\[
P_{M/T/PG}(N_S > 0) = 1 - e^{-R \cdot T \cdot K \cdot \tilde{m}}
\]  

where \( \tilde{m} = \text{impactfactor} \cdot P_{PG}(N_S > 0) \cdot R \). The impact factor of the inhibition can be varied to adjust for the different cell types. We assume that both M/T and PG cell responses are affected by presynaptic inhibition of glutamate release at the ORN terminals through PG cells (Aroniadou-Anderlaska et al. 2000, McGrann et al. 1, 2005). In addition, M/T cells receive inhibitory input through dendro-dendritic synapses. Our calculations suggest that a single PG cell sends inhibitory input to 5-10 mitral and tufted cells. We therefore assume that M/T cell responses are relatively more affected by PG cell activation than PG cells.

18. Sensitivity of the results


The sensitivity of the results to changes in the relationship between ORN and target cell activity, and to changes of electrophysiological parameters is limited.

As our results show, dominant costs result from maintaining the resting potential, from the presynaptic action potentials, and from postsynaptic responses. All these factors are independent of the minimum number of ORN inputs that activates a target cell. Thus, the effect of changes in the relationship between ORN and target cell activity on the glomerular budget is limited. However, the output of principal cells is influenced
significantly through these changes as can be seen in figures S1 and S2. Figure S1 compares the response curve of three mitral cell populations with different thresholds; figure S2 does the same under the assumption of presynaptic and dendro-dendritic inhibition. The threshold of 25 inputs corresponds to the assumption employed in our simulation which was based on electrophysiological findings.

In sum, changing the relationship between ORN and target cell activity has a large impact on principal cell output for a given odor concentration but does not qualitatively change our results on overall costs. In the case that within one glomerulus different activation thresholds are used, a wide span of target cell sensitivity to odor could result. Because of the limited impact of target cell activation on total costs, it seems that a variation in thresholds is rather of functional than of energetic importance.

18.2. Changes in main electrophysiological parameters.

In olfactory receptor axons and glomerular dendrites, and in glomerular postsynapses we assume a voltage excursion of respectively 125mV and 115mV during activation. These are rough approximations of the psychological values which bears the risk of under- or overestimating costs, or altering the distribution of energy consumption between the different elements. However, comparing a voltage excursion of 100mV to one of 140mV during an axonal action potential, axonal costs differ by less than 10%. This difference is proportionally small and does not affect the qualitative result of this study stating that the demands of postsynaptic receptor responses exceed the energy requirements of presynaptic events by a factor of up to 2.

If we compare total costs using overall low voltage changes during AP’s (100mV in olfactory axons, MCs, TCs and PG cells) with total costs using overall high voltage
changes during AP’s (140mV in olfactory axons, MCs, TCs and PG cells), total glucose consumption per second of maximal electrophysiological stimulation rises by less than 5% from 21 µMol to 22 µMol.

Thus, our main results are not sensitive to reasonable adjustments of the assumed physiological properties.

19. Additional references for Supplemental Material


20. Supplemental Table Legends

**Table S1.** Quantitative data on the olfactory glomerulus in several mammalian species.

**Table S2.** Summarized results of volume and surface calculations of glomerular elements.

21. Supplemental Figure Legends

**Figure S1.** Mitral cell response curves with different activation thresholds (defined as minimal number of ORN inputs within one integration window)

**Figure S2.** Mitral cell response curves with different activation thresholds (defined as minimal number of ORN inputs within one integration window) under consideration of presynaptic and dendro-dendritic inhibition.
<table>
<thead>
<tr>
<th>Species</th>
<th>No of ORs</th>
<th>Refs.</th>
<th>No of ORNs per bulb</th>
<th>Refs.</th>
<th>No of Glom. per bulb</th>
<th>Refs.</th>
<th>Diam of Glom.</th>
<th>Refs.</th>
<th>No of Mitral cells per bulb</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td></td>
<td></td>
<td>8,000,000 - 20,000,000</td>
<td>Schoenfeld et al, 2004</td>
<td>3.100</td>
<td>Schoenfeld et al, 2004</td>
<td>110</td>
<td>Schoenfeld et al, 2004</td>
<td>51,000</td>
<td>Schoenfeld et al, 2004</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>50,000,000</td>
<td>Allison et al, 1949</td>
<td>1.900</td>
<td>Allison et al, 1949</td>
<td>80</td>
<td>Bailey et al, 1999</td>
<td>45,000</td>
<td>Allison et al, 1949</td>
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<tr>
<td>Rabbit</td>
<td>870</td>
<td>Quignon et al, 2005</td>
<td></td>
<td></td>
<td>450-700</td>
<td>Nezlin and Schild, 1999</td>
<td></td>
<td></td>
<td>3000 - 3700</td>
<td>Nezlin and Schild, 1999</td>
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<tr>
<td>Dog</td>
<td>110</td>
<td>Niimura and Nei, 2005</td>
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## ORN Axons

<table>
<thead>
<tr>
<th>Single Axon</th>
<th>Astroglia</th>
<th>Capillaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total branch length [µm]</td>
<td>195.0</td>
<td>length of edge [µm]</td>
</tr>
<tr>
<td>Branch Ø [µm]</td>
<td>0.2</td>
<td>glial process section area [µm²]</td>
</tr>
<tr>
<td>Preterminal length [µm]</td>
<td>5.0</td>
<td>single edge Vol [µm³]</td>
</tr>
<tr>
<td>Preterminal Ø [µm]</td>
<td>0.4</td>
<td>edges Vol /compartment [µm³]</td>
</tr>
<tr>
<td>No. of preterminals per axon</td>
<td>17.0</td>
<td>compartment Vol [µm³]</td>
</tr>
<tr>
<td>Synaptic terminal length [µm]</td>
<td>0.6</td>
<td>compartments /glomer</td>
</tr>
<tr>
<td>Synaptic terminal Ø [µm]</td>
<td>0.6</td>
<td>Total glial process Vol [µm³]</td>
</tr>
<tr>
<td>No. of synaptic terminals per axon</td>
<td>26.0</td>
<td>% of Glom Vol</td>
</tr>
<tr>
<td>Total axonal Vol [µm³]</td>
<td>18.5</td>
<td>Total axonal SA [µm²]</td>
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</tbody>
</table>

### Axon population in one glomerulus

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of axons per glomerulus</td>
<td>4500.0</td>
<td></td>
</tr>
<tr>
<td>Total Vol of axons [µm³]</td>
<td>83465.8</td>
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<tr>
<td>% of Glom Vol</td>
<td>31.1</td>
<td></td>
</tr>
<tr>
<td>Total SA of axons [µm²]</td>
<td>924005.2</td>
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</tbody>
</table>

## Mitral cells

### Single mitral cell

<table>
<thead>
<tr>
<th></th>
<th>Single tufted cell</th>
<th>PG cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of single trees per tuft</td>
<td>3.0</td>
<td>No. of single trees per tuft</td>
</tr>
<tr>
<td>No. of single tree branch levels</td>
<td>6.0</td>
<td>No. of PG tuft branching levels</td>
</tr>
<tr>
<td>Primary dendrite Ø [µm]</td>
<td>3.5</td>
<td>Primary dendrite Ø [µm]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Single tree Vol [µm³]</td>
<td>561.1</td>
<td>309.2</td>
<td>277.9</td>
</tr>
<tr>
<td>Single tree SA [µm²]</td>
<td>2006.9</td>
<td>1290.1</td>
<td>883.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Single tuft Vol [µm³]</td>
<td>1683.3</td>
<td>927.5</td>
<td>277.9</td>
</tr>
<tr>
<td>Single tuft SA [µm²]</td>
<td>6020.6</td>
<td>3870.4</td>
<td>883.7</td>
</tr>
</tbody>
</table>

### Mitral cell population in one glomerulus

<table>
<thead>
<tr>
<th></th>
<th>Tufted cell population in one glomerulus</th>
<th>PG cell population in one glomerulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mitral cells</td>
<td>25.0</td>
<td>No. of tufted cells</td>
</tr>
<tr>
<td>Total mitral tuft Vol [µm³]</td>
<td>42082.8</td>
<td>Total Tufted cell tuft Vol [µm³]</td>
</tr>
<tr>
<td>% of Glom Vol</td>
<td>15.7</td>
<td>% of Glom Vol</td>
</tr>
<tr>
<td>Total mitral tuft SA [µm²]</td>
<td>150513.9</td>
<td>Total Tufted cell tuft SA [µm²]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total PG tuft Vol [µm³]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total PG tuft SA [µm²]</td>
</tr>
</tbody>
</table>

**Sum of all volumes [µm³]** 244170

**% of Glom vol** 91
Effect of different activation thresholds of Mitral cell response.

Response probability of Mitral cell

Relative odor concentration

Activation threshold 5
Activation threshold 25
Activation threshold 125
Effect of different activation thresholds of Mitral cell response with inhibition