Cerebellar glomeruli: Does limited extracellular calcium implement a sparse encoding strategy?

David M. Eagleman, Olivier J-M. D. Coenen, Vladimir Mitsner, Thomas M. Bartol, Anthony J. Bell, Terrence J. Sejnowski

Computational Neurobiology Laboratory, The Salk Institute, 10010 N. Torrey Pines Rd, La Jolla, CA 92037. {eagleman, olivier, vlad, bartol, tony, terry}@salk.edu

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Abstract

A class of synaptic learning models – in which presynaptic terminals have access to a weighted sum of the postsynaptic activity – has traditionally been dismissed as biologically unfeasible. This rejection is not surprising under traditional notions of synaptic connectivity, since postsynaptic cell bodies may be far apart, and there are no backwards signals known to sum activity in a terminal-specific manner. However, many synapses in the CNS become specialized by glial cell ensheathment. We suggest that such ensheathment may force neighboring cellular elements to share a limited resource: extracellular calcium. We propose the novel theory that certain glomeruli are configured so that the instantaneous external calcium concentration will encode the level of spike activity in postsynaptic cells. We concentrate on the specialized glomeruli that exist in the cerebellum at the interface of the mossy fiber and granule cell layers. Here, dendrites from scores of granule cells swirl around a mossy fiber terminal, and the whole structure is tightly ensheathed in an astrocyte. Simulations demonstrate that the calcium concentration is indeed proportional to a sum of postsynaptic activity in the granule cells. We demonstrate that these extracellular calcium changes are interpretable from an information-processing point of view, generating a novel learning rule for control of plasticity at the mossy fiber/granule cell synapse. This learning rule implements a sparsely distributed and statistically independent representation in the parallel fibers. Both of these coding properties reduce the complexity of the credit assignment problem

between active parallel fibers and climbing fiber at a Purkinje cell. Although traditional models of neural function only emphasize neurotransmitters and point-to-point connections, our results highlight the need to quantitatively address the extracellular context in which axon terminals and dendrites are found.

Introduction

One fifth of the mammalian brain is comprised of extracellular space (ECS). The extracellular space is not empty, but instead comprises a complex network of proteins and a variety of molecular species. One extracellular species, calcium, holds a prominent position as one of the most important messengers known in the brain. However, calcium exists in low concentrations in the ECS, and its diffusion is slowed by the restricted volumes of the extracellular space – therefore, normal neural activity may cause calcium to move out of the ECS faster than diffusion can fill it in. As opposed to the traditional view that extracellular calcium exists at a stable concentration, theoretical analyses suggest that calcium concentrations may rapidly change as a reflection of local neural activity (1-6). The theory finds experimental support from microelectrode recordings (7-15), and more recently, from assays of neurotransmitter release in rat brainstem (16), measurement of tail current in chick ciliary ganglia (17), calcium-dependent binding of antibodies (18), effects on neighboring cells (19), and direct measurements with extracellular calcium dyes (20). Such changes in extracellular calcium are likely to carry significant functional impact, due to the many signaling roles calcium plays.

Glial ensheathment: the Mossy fiber – granule cell glomerulus

The association of neurons and nerve fibers with glial cells is nearly ubiquitous throughout phylogeny, and seems to provide many mechanical functions. For example, myelinating sheaths around an axon enable salutatory conduction. In more specialized cases, many synapses in the CNS are ensheathed by glia (21); such synapses can be found in the retina, hippocampus, and cerebellum.

As we will outline in this study, such ensheathment may serve a computational role. We concentrate on the intriguing example of the mossy fiber – granule cell glomerulus in the

cerebellum. This glomerulus is a strategic site for neuronal connectivity in the cerebellar cortex. Within this structure, mossy fiber axons originating from various regions of the CNS form synapses with the dendrites of granule cells. These claw-shaped dendrites, as well as axons of Golgi cells, swirl around the mossy fiber terminals, forming characteristic rosettes (22-24). A glial cell, known as the velate astrocyte, develops a tight sheath around each glomerulus (24, 25). The role of the glial ensheathment has been speculative and controversial, receiving essentially no attention from a computational point of view. Roles that have been postulated for the neuroglial sheaths are structural support, electrophysiological insulation of individual glomeruli, and the maintenance of chemical equilibrium in the interstitial fluid (26), (23), (27), (28), as well as chemical barriers to the further outgrowth of granule cell dendrites and Golgi cell axons (29). Our findings described in this paper suggest a different, novel role for the neuroglial sheath: this anatomical substrate limits the supply of extracellular calcium, such that the extracellular calcium concentration comes to represent a sum of total postsynaptic activity. This sum can then be used by the neural elements within the glomerulus to navigate plasticity.

But is there a principled reason to think that it might be desirable for the cerebellum to need the knowledge of such a sum? From a theoretical point of view, there is a class of learning rules that describe the evolution of connection strengths within the glomerulus in terms of the coding strategy of the granule cells, and these rules require just such a sum over postsynaptic activity. Let us look at the motivation behind those rules now.

How does the granule cell layer encode its input?

A knowledge of the firing pattern of GCs is essential to any theory of the function of the cerebellar cortex. The only output neurons of the cerebellum, the Purkinje cells, receive synapses from up to 200,000 GCs (30). It is theorized that the activity of too many GCs would carry no information and abolish response selectivity (31). It is therefore reasonable to assume that the granule cells may be most effective by encoding their input sparsely. This would mean that for any given context (as carried by the mossy fibers), only a small subset of the granule cells would become active, while the rest would remain

silent. Sparse encoding has two advantages: (1) lowering of the total amount of action potential generation greatly lowers metabolic cost, and (2) it simplifies the credit assignment problem at the Purkinje cell. That is, if only a very small number of granule cells drives a Purkinje cell, their responsibility is more easily assessed (they are given 'credit'), and plasticity can be appropriately distributed.

If we assume sparseness, and desire to maximize the mutual information between the mossy fiber inputs and the granule cell outputs, then a learning rule for synaptic weights can be derived. The details of the rules and their derivation are relegated to the Appendix, but the point that will become important is quite simple: under the sparseness constraint, mutual information is maximized when the weight change is *a function of a sum of postsynaptic activity*. Given the neural arrangements in most parts of the brain, such a weight rule can usually be dismissed as biologically unfeasible. However, in the special case of the cerebellar glomerulus (and perhaps glomeruli in other brain regions), the configuration of parts suggests that such a learning rule might be feasible after all. By examining and simulating the relevant structures, we will now construct the logic that leads us to the suggestion that the glomerulus may be built to implement such a 'backward-sum' learning rule.

Totality of ensheathment

Ensheathment means that diffusion of extracellular calcium from neighboring regions of the ECS is cut off, or at best greatly restricted. In this situation, the competition for calcium is more fierce, since calcium taken from the shared pool in the glomerular extracellular space (GECS) immediately becomes absent from the point of view of the other dendrites. We have been speaking as though the ensheathment of a glomerulus is total, i.e., the extracellular calcium is a limited pool of fixed size, in which case the amount of calcium that fluxes into a dendrite equals the amount that is missing from the extracellular space. Of course, the glomerulus cannot be without communication to outside extracellular space, or else action potentials would not be able to propagate into the glomerulus along the mossy fiber axon and the granule cell dendrites, since such propagation requires an open circuit of ion flow. On this subject, it should be noted that lamellar processes of the velate astrocytes separate one glomerulus from another in the

islet and interweave with the dendrites and Golgi cell axons at the periphery of the glomerulus (23). This means that although the GECS may have limited communication with surrounding ECS, the space outside the glomerulus is also quite restricted. In general, the extent to which the glomerular ECS communicates with outside ECS will not qualitatively change the results presented here. Even with a communicating GECS, the extracellular calcium concentration will still reflect a sum of the postsynaptic activity. The extent of outside communication will modulate the size of the calcium fluctuations and the speed of recovery to ambient levels.

Action potentials in granule cell dendrites

Granule cells are electrotonically compact, and both experiments and modeling indicate that the soma and dendrites of the granule cell will behave as a single electrical compartment (32-35); in other words, action potentials in the soma will appear almost immediately throughout the short dendrites. Granule cell dendrites contain voltage-gated calcium channels (VGCCs) (36, 37), NMDA receptors (35, 38), ATP-ase calcium pumps (39) and Na+/Ca++ transporters (40), all of which exist not only on the granule cell body but are clearly seen with immunohistochemistry to express heavily on the dendritic endings inside the glomeruli. The action potentials along the GC dendrites are expected to cause large fluxes of calcium into the dendrite through the calcium-fluxing channels. This influx of calcium is mirrored by an efflux of calcium from the extracellular space outside the dendritic membrane. Hence, the strobing of the GC dendrite by a spike is encoded as a change in calcium in the extracellular space (1, 2). This effect provides a natural computational mechanism for integrating the activity of several disparate post-synaptic cells.

There is a very large imbalance between the time scales of influx and extrusion (2 orders of magnitude), which means that quickly heightening postsynaptic activity in a glomerulus can lower the available calcium, especially as a remarkable 58% of the glomerular volume is comprised of GC dendrites (41, 42). This would generally be true in situations where the total volume of the ECS is limited, as when some numbers of consumptive elements are ensheathed by glial cells, for example (3). In this way,

consumption by a set of elements can lower the available calcium to other elements. This leads to a rapid bi-directional transfer of information -- in this case, external calcium fluctuations encode information about post-synaptic (granule cell) activity as effectively as pre-synaptic (mossy fiber) activity.

This leads to the hypothesis that the extracellular calcium will encode an average level of granule cell activity. This hypothesis is explored both at the biophysical and theoretical levels. At the biophysical level, we explore the dynamics of external calcium changes in a Monte Carlo simulation of the mossy fiber/granule cell glomerulus. At the theoretical level, we attempt to interpret how such calcium changes serve as information-bearing signals, allowing the pre-synaptic mossy fiber terminal to have a measure of the post-synaptic spike activity of several granule cells.

This dual approach allows us to address several questions:

Are cerebellar glomeruli configured to magnify changes in extracellular calcium concentration?

What information would such calcium changes carry?

What are the computations carried out by that flow of information?

Details of the model

We have simulated the glomerular structure in such a way that the statistics would match those that were determined in (41, 43). In our Neural Growth Simulator (programmed in C by D.M.E., Fig 2A), dendritic tips determine their paths by local avoidance rules. Each tip attempts to take a step under the constraint that it cannot come within a fixed distance of any other dendrite. After 80 steps of the simulation, growth is stopped, and the dendrites ‘inflate’ to fill any available voxels. When the voxels have all been committed, a Marching Cubes algorithm constructs each dendrite into a 3D polygon mesh. The coordinates of the polygon mesh are read into MCell, where calcium ions, channels, and pumps are distributed appropriately

In the studies presented here, we have used the fact that in the mammalian hippocampus, dendritic calcium channel densities are estimated between 1-15 channels/μm^2 (44). We estimate each channel to have a 7 pS calcium conductance, and we choose a density of 7 channels/μm^2. For these Ca++ channels, we used a Neuron mod file (based on Friedman et al, 1993). We constructed pumps for first order extrusion with a decay constant of ~300 ms. In order to control for a mis-estimation of the total integrated current, we will be exploring consumption over a range of calcium channel density estimates in the near future. The diffusion coefficient was 2.2e-6 cm^2/s.

How much calcium does a BPAP consume?

To estimate the extracellular volume of a glomerulus, we take the average of 4 serially-reconstructed glomeruli from Jakab and Hamori (1988). The average glomerular volume is 151.37 um^3, and the average mossy fiber terminal volume is 50.9 um^3. The difference, 100.4 um^3, reflects the volume occupied by the GC dendrites and Golgi axons. Taking the extracellular volume fraction (EVF) to be between 13 – 20% (ref Sykova), we estimate that the extracellular volume is approximately between 13 – 20 um^3. At an assumed resting $[Ca^{++}]_o$ = 2 mM, this translates from 15.7 x 10^6 to 24 x 10^6 calcium atoms available in the extracellular space.

Would a single BPAP engender a large enough signal to be distinguishable from statistical fluctuations in the resting calcium concentration? Statistical mechanics predicts the expected density fluctuations in a volume of particles to be $\sigma = \sqrt{N}$. Thus, in a typically sized glomerular cleft at 2 mM resting concentration, we expect at any time to find 20 x 10^6 atoms plus or minus a standard deviation of 4472 atoms. This is approximately a 0.02% fluctuation, which for a 2 mM resting concentration translates to a first standard deviation at 1.9996 mM. By comparison, a dendrite is likely to consume much more calcium. It is therefore apparent that even a single decrement of 14,000 atoms would be clearly distinguishable as a signal from the normal background fluctuations.

Throughout this work, we assume passive diffusion. The extent to which calcium changes may be magnified or dampened by active mechanisms *in vivo* is unknown and remains for the future.

Extracellular calcium as a function of postsynaptic activity

To build our argument, we will begin with the simplest possible equation to describe how extracellular calcium as a function of post-synaptic activity in the granule cells:

$$C \propto -\sum_j p_j s_j \qquad (1)$$

where C is a time-averaged deviation from a concentration set-point, θ, i.e., $C=[Ca^{++}]_o-\theta$, and [Ca++]o is the extracellular concentration in the glomerulus. s_j is the firing rate in a granule cell j, and p_j is the amount of calcium consumed by the dendrites of that cell during a BPAP. The contribution each granule cell dendrite makes to the sum will be weighted by several factors: to name a few, the density and distribution of calcium channels on the dendrite, the amplitude of the back-propagating spike, and the total surface area of dendrite. Thus, a granule cell with a high p will consume more calcium from the shared resource in the glomerulus each time it generates a spike than a GC with a low p. Since each GC thus can make a different contribution, the total calcium concentration will reflect a weighted average of GC activities. We will refer to the quantity p_j as the *backward weight* of granule cell j (conversely, we will denote the efficacy of synaptic transmission, traditionally called a weight, as the *forward weight*, below).

The assumption here is that the extracellular calcium concentration will (on average) reflect a weighted sum of the postsynaptic GC activity. The time average over which we consider the calcium concentration is around 200 ms.

The simplicity of equation 1 ignores several other contributors to the total calcium concentration – the mossy fiber terminal, the Golgi cell axons, and the velate astrocyte – on the grounds that those contributions will be small compared to that of GC dendrites.

To justify equation 1, we now briefly discuss consumption by these other elements, and then the issue of replenishment, below.

Consumption

The glomeruli contain at least 2 calcium consuming elements besides the GC dendrites: the mossy fiber axon terminal and the golgi cell axon terminals. However, theoretical analysis indicates that the amount of calcium consumption by axon terminals is much smaller than that by dendrites (1, 2, 5). The simple reason is because VGCCs are clustered at hotspots on axon terminals in order to concentrate calcium influx at the neurotransmitter release sites, whereas VGCCs on dendrites occupy a much larger surface area. When a spike appears in dendrites, the surface area over which calcium fluxes in means the total decrement in calcium can be quite substantial (1, 2, 5).

In further support of equation 1, note that of the volume of the glomerulus, a striking 58% of the volume is taken up by the granule cell dendrites (41, 43).

It is possible that the velate astrocyte that provides the ensheathment may participate in determining the extracellular calcium concentration, since astrocytes are known to have both voltage-gated calcium channels (45) and ligand-gated calcium-permeable channels (46), and there is also the suggestion that Na^+/Ca^{++} transporter can reverse under conditions of lowered $[Na^+]_o$, taking calcium in from the ECS (45, 47, 48). While we do not further consider the role of the glial cell in this paper, we suggest it may be regulative on longer time scales.

As a caveat, ignoring the contribution of the MF terminal depends in part on the relative firing rates of the MF and the GCs, for the MF could potentially makes a larger contribution to the [Ca++]o if its firing rate goes up and the GC firing rate goes down. In the limit, if the MF alone were firing, with no activity in the GCs, then equation 1 would be entirely incorrect – however, we consider this limit quite unlikely, and will here consider the reasonable range wherein the GC dendrites are contributing most the calcium changes.

Replenishment

Calcium extrusion via exchangers and pumps operates on a time scale approximately 2 orders of magnitude slower than the rapid calcium transient due to APs (49-52). As a result of the imbalance between the depletion and extrusion times, regional background activity may be expected to regulate a background Ca^{++} level; such a level may set important parameters in attention, learning, and/or plasticity

Plasticity as a function of extracellular calcium

The dominant experimental model for use-dependent synaptic learning is long-term potentiation and depression (LTP/LTD), a property observed at glutamatergic synapses throughout the CNS. Calcium influx is an essential trigger for plasticity (53), while the *magnitude* of the calcium influx tips the balance between the outcomes of LTP vs LTD (54-57). LTP occurs when there is a high level of postsynaptic calcium, and LTD results with moderately elevated postsynaptic calcium. The mechanics underlying this phenomenon appears to be the biochemical balance of phosphorylation and dephosphorylation, which can tipped in either direction by the amount of available intracellular calcium, and can change the phosphorylation state of various downstream targets. Specifically, a large rise in intracellular calcium can activate protein kinase C and/or calcium/calmodulin-dependent kinase II (CamKII), which can lead to LTP. On the other hand, a lesser amount of calcium influx can activate protein phosphatase 1, causing LTD (58, 59). In other words, postsynaptic enzyme cascades measure intracellular calcium near the postsynaptic density and translate it into changes in synaptic strength (54).

This is important in the present study, since calcium influx is a function of extracellular calcium: lowering extracellular calcium lowers the amount of available ions for influx. If plasticity at the MF-GC synapses follows the same pattern of LTP and LTD in other brain areas – specifically, based on calcium influx – it follows that the plasticity will be a function of the shared extracellular calcium.

We therefore ask the question: what would a learning rule look like in which each weight change of the MF-GC synapses depended on the global $[Ca^{++}]_o$? We begin with the simplest possible rule:

$$\Delta w_i \propto [Ca^{++}]_o - \theta \quad (2)$$

When the extracellular concentration $[Ca^{++}]_o$ is low (below some set-point θ), the forward weight w_i is more likely to depress as a result of less calcium influx into the postsynaptic dendrite. This is consistent with a variety of learning rules that have been developed over the past decades, mostly involving covariance (60), which evolved into the BCM rule (61), the ABS model (57), evidence from frequencies that induce LTD vs LTP (62), and biochemical pathways (54). The above theories are all consistent with the single hypothesis that the magnitude of postsynaptic calcium influx will determine the direction of the weight change.

With this in mind, it seems reasonable to think of the spike in the GC dendrites as "assaying" the calcium concentration in the glomerulus. This is consistent with the experimental evidence that LTD is induceable even at inactive synapses if postsynaptic $[Ca^{++}]_i$ is raised to the appropriate level by antidromic or heterosynaptic activation (57). Thus, equation 2 is only applicable as a learning rule when the postsynaptic cell is spiking, i.e., when $s_j>0$.

The limitation of equation 2 is that it does not individuate the many different connection strengths in a glomerulus, i.e., all the weights in a given glomerulus will change in the same manner. To make individual weight changes for the *j* different connections, we now make the first main assumption in our model, which will await experimental verification. We assume that the set-point for each weight can change as a function of the weight itself, such that equation 2 becomes:

$$\Delta w_i \propto [Ca^{++}]_o - \theta_i = [Ca^{++}]_o - (\theta - \lambda w_i) \quad (3)$$

where λ is a constant of proportionality. This is an interesting learning rule, as it asserts that a potentiated synapse will need less total extracellular calcium to potentiate further. This may be thought of as follows: a potentiated dendrite has more calcium-fluxing

channels, which means that it can reach a higher total level of influx given the same extracellular concentration.

The relationship of forward weights to backward weights

The participation of each GC dendrite in determining the $[Ca^{++}]_o$ in the glomerulus (i.e., its backward weight) will be weighted by several factors. The main factors will be the concentration and distribution of calcium-permeable channels on the dendrite (both ligand- and voltage-gated), the amplitude spike in the dendrites, and the total surface area of dendrite within the glomerulus.

We now make the assumption that forward and backward weights will be proportional. The simplest justification of this assumption is to concentrate on the postsynaptic NMDA receptors: an increase in their number will cause both a stronger response to glutamate.

There is evidence for glutamate spillover in the glomerulus: the activation of a mossy fiber terminal influences mGlu receptors on neighboring Golgi cell axons in a frequency dependent manner (63). This establishes that mGluRs on inhibitory interneuron axons sense the glutamate of neighboring excitatory synapses, but it also leads to the possibility that a resting level of glutamate, shared in the cleft, will bind to NMDA receptors, making them, effectively, like voltage-gated calcium channels; i.e., when the dendrite is depolarized, the NMDA-R already has ligand bound. It has also been shown that certain AMPA receptors on granule cells are calcium permeable (64, 65). Therefore, an upregulation of GluRs may be commensurate with higher calcium influx. This assumption, critical to the next step of our analysis, awaits experimental verification.

Having made the assumption that $p_j \alpha w_j$, we may substitute a weighted sum of the postsynaptic activity into equation 2:

$$\Delta w_i = \lambda w_i - k\sum_j p_j s_j = \lambda w_i - \alpha \sum_j w_j s_j \qquad (4)$$

Equation 4 is very interesting because it includes a measure of all the postsynaptic activity. This is a novel learning rule in that it allows the weight change to be a function of a summation of postsynaptic activity.

This theory embeds two main assumptions that have yet to be experimentally verified or ruled out. The first is that the LTD/LTP set-point (θ) is a function of the current weight (equation 3). The second assumption is that forward weights are proportional to backward weights ($w_j \alpha p_j$), i.e., a dendrite with strong synaptic efficacy will also consume more calcium.

RESULTS

Example of our main simulation result are shown in Figure 3. In this Monte Carlo simulation of extracellular dynamics, different GC firing rates set the extracellular calcium levels in the glomerulus to different set points. Specifically, to understand the effect of normal background activity on the baseline $[Ca^{++}]_o$, we simulated a glomerulus that communicates with 40 randomly active GCs (Poisson firing rates; 5 Hz and 35 Hz). The ECS concentration drops to its new set point in ~500 msec in Fig 3A. However, depending on the pattern of firing, that set point can be approached more rapidly (Fig 3B).

Why do granule cells maintain low firing rates?

Because of the sensitivity of NT release on extracellular calcium, a granule cell is likely to bounded away from high firing rates. To understand this, note that a high spike rate traveling up the dendrites will presumably veto all afferent transmission. When there is no more NT release to drive the granule cells, the firing rate will necessarily return to lower values. So we see that baseline firing will be bounded away from high firing rates.

This lower calcium level in Fig 3 would reduce the probability of further neurotransmitter release, which would in turn reduce further firing of the GCs. Thus, the limited supply of Ca++ immediately biases the system toward a sparse encoding.

Why are glomeruli ensheathed?

Despite the fact that the ECS constitutes 20% of the volume of the brain, it is not continuous everywhere. In a closed volume, such as a glomerulus ensheathed by glial cells, it is possible that a relatively fixed amount of external calcium is shared by the terminals and dendrites of that volume. In this way, recovery time would be much slower than those in the examples presented here – recovery would depend in large part on extrusion rates. A mathematical analysis of astrocytic partial-ensheathing of a synapse is consistent with this notion (3).

Why are GC dendrites digitiform?

A question that might be asked about the GC dendrites is why they have four digits spread throughout the glomerulus, instead of only one. A speculation, in light of the current framework, is that the spreading of the dendrite allows much faster equilibration of the calcium signal. In other words, when a spike travels up a GC dendrite and consumes calcium, a digitiform dendrite allows the local calcium changes to become quickly global, with all parts of the glomerulus sensing the same $[Ca^{++}]_o$. Analytic analyses in progress indicate that the speed of equilibration with four digits is ~16 times faster than equilibration with one digit.

DISCUSSION

Although synaptic transmission is often thought of as the only means of communication between nerve cells, it is almost certainly an incomplete description. It appears that some structures may be specialized to take advantage of limited resources. Such mechanisms have the property of bi-directional information transfer, which is not thought to occur with synaptic transmission. In this way, a pre-synaptic cell could have a measure of the spiking activity of the pre-synaptic cells.

The importance of the glomerular is suggested by the demonstration in cultures of dissociated mouse cerbellar cells that natural histogenetic mechanisms persist after dissociation and reaggregation of cerebellar cells, which retain specificity of their

synaptogenic capabilities both with regard to appropriate cell types and the morphological form that the synapses take. Specifically, mossy fibers still formed synaptic glomeruli [Orkand, 1984 #24].

Beginning with a model of the glomerulus, we have shown that calcium consumption and diffusion is predicted to lead to rapid, local changes in limited pool of external calcium in the GECS.

The exact size of the calcium signal depends on several parameters. For example, the cleft width might be used by the system as a control parameter: changing the gap between elements can amplify or squelch the calcium signal.

Spillover

In a continuous ECS, an extracellular calcium signal will not travel far through the tissue – the signal will remain approximately as local as neurotransmitter signals such as glutamate (1, 2, 5). This is because diffusion from neighboring regions of the ECS will quickly equalize the decrement. However, in the case of a limited ECS, as in an ensheathed glomerulus, the signal will remain local (21). The calcium depletion parallels the evidence for glutamate spillover in glomeruli (63). Specifically, spillover is likely to boost the efficacy of active excitatory fibres by locally reducing the level of inhibition (63). By the same reasoning that calcium changes will be of larger magnitude and longer duration than they would be in a more open ECS.

Other calcium-consuming channels

Both voltage-gated conductances, and ligand-sensitive calcium-permeable channels, causing extensive calcium consumption (66). Although we only concentrate on voltage-gated channels here, it should be noted that NMDA, ATP, and nicotinic Ach receptor channels show a fractional Ca++-current of 12%, 6.7%, and 4%, respectively (67); additionally, certain AMPA receptors are permeable to calcium (46, 68). Such channels have widely different flux rates; for example, when NMDA receptors are activated by the coincidence of glutamate and depolarization, the local Ca++-consumption lasts 80 - 100

msec. Such a slow calcium sink could generate a substantially different calcium levels for the surrounding cells.

Other methods of reading extracellular calcium

However, aside from "reading" $[Ca^{++}]_o$ through levels of influx, a cell might also detect the external levels directly. An intriguing example is the recently cloned Ca^{++}-sensing receptor (CaR) (19, 69-71). The activation of the CaR has a steep sigmoidal dependence on $[Ca^{++}]_o$. In parathyroid cells, a 2-3% change in $[Ca^{++}]_o$ can activate the CaR, since the middle of the sigmoid is positioned at the physiological range of concentrations (71). In brain the CaR is widely distributed, being particularly abundant in neurons in cerebellum, hippocampus, subfornical organ, and cingulate cortex (47), as well as in glia (72). This strongly suggests that the alteration of calcium levels in a cleft can be directly sensed by a G-coupled protein whose function, and this has been directly demonstrated (19). This metabotropic way of reacting to changes in external calcium may live on a slower time scale than the influx and binding of calcium to enzymes.

On the other hand, there may exist other ways of reading $[Ca^{++}]_o$ levels that are faster than activation of enzymes through influx. For example, rapid functional effects are sometimes expressed in channel dynamics: in squid giant neurons, external calcium levels quickly modulate both the gating and selectivity of K^+-channels (19, 73).

An evolution of coding strategy?

An interesting side note is that the glomeruli form over weeks after birth in rabbit (74), chick (75), and in rats (42). We propose that this may represent an evolution of coding strategy.

Moreover, there is an interesting temporal relationship between the dendrites and the glial ensheathment: electron microscopic analysis in the developing rat shows that between postnatal days 15 – 45 (P15-45), the size of the mossy fiber rosettes do not change, but the glomerulus increases enormously due to the continuing multiplication of postsynaptic

dendrites (42). The formation of neuroglial sheaths, which occurs after the third postnatal week, corresponds to the waning phase of dendrite extension (22).

Neurotransmitter release

The sensitivity of neurotransmitter release to external calcium (76-79) suggests that such a decrement will influence the probability of synaptic transmission. If the presynaptic terminal were invaded by an action potential just after a volley of calcium-consuming back-propagating spikes in the GC dendrites, the presynaptic release probabilities would be diminished.

In conclusion, the ensheathment of the glomerulus may force neighboring granule cell dendrites to share a resource that is in limited supply on short temporal and spatial scales. The resting levels of external calcium are not sufficiently high to protect against large decrements in this important resource. Instead, it seems as though the tissue is engineered so that external calcium levels are meant to fluctuate dramatically; given the functional importance of external calcium, we are led to hypothesize that external calcium fluctuations are an important class of information-bearing signal.

APPENDIX

The granular layer: model

A linear relationship between the activity of the mossy fiber inputs **x** and the granule cells activity **s** is assumed and the overall effect of Golgi cell inhibition on granule cells is represented by a bias weight factor $\mathbf{w_o}$. The granule cells activity is given by $\mathbf{s = W\,x - w_o}$, where $\mathbf{s, W, x, w_o \geq 0}$, and is assumed to be sparse and as independent of each other as possible. Their activity is therefore assumed to have a prior probability density function that has high kurtosis and multiplicative: $\mathbf{f_s(s)} = \prod_i f_s(s_i)$, where $f_s(s_i)$ is chosen to be the same exponential density for all granule cells, $f_s(s_i) = \alpha \exp(-\alpha s_i) = \gamma_1(s_i)$ where the function γ_1 is the gamma probability density of order 1. Due to the positive constraint on the granule cell's firing rates $\mathbf{s \geq 0}$, the prior is

more precisely $f_s(s_i) = \alpha e^{-\alpha s_i} U(s_i)$ where $U(\cdot)$ is the step function. To simplify the derivation of the learning rules this prior was approximated by two exponential priors: $f_s(s_i) = \alpha \exp(-\alpha s_i)$ for $s_i > 0$, and $f_s(s_i) = \alpha \exp(-\beta s_i)$ for $s_i \le 0$, where $\beta >> \alpha$.Taking the limit as $\beta \to \infty$, the original prior with the step function is recovered.

Role of Golgi cells

If the mossy fibers inputs have the form $\mathbf{x} = \hat{\mathbf{x}} + \mathbf{x_o}$, where $\mathbf{x_o}$ is the mean of $\mathbf{x}$ and $\hat{\mathbf{x}}$ has zero mean, $\mathbf{x_o}$ will be large and positive since the firing rates of the mossy fibers are positive and may be large. As a result, in order for the granule cells activity $\mathbf{s = W\,x - w_o}$ to be sparse, its average will be close to $\mathbf{0}$ if the bias weight vector is near $\mathbf{w_o} \sim \mathbf{W\,x_o}$ and therefore positive. The bias weight $\mathbf{w_o}$ represents the Golgi cell role of setting the threshold of granule cells so that their activities $\mathbf{s}$ remain sparsely distributed with a peak in their probability distribution at $\mathbf{0}$, so that the granule cells are most of the time inactive during their lifetime.

Bayesian derivation with maximum likelihood

The objective is to maximize the probability density of the input mossy fiber data **(X)** given the model. The likelihood function in terms of M observations x_k of $\mathbf{x}$ is $\mathbf{f_x(X \mid W, w_o)} = \prod_{k=1}^{M} \mathbf{f_x(x_k \mid W, w_o)}$. Assuming a complete representation where, say, $n = 4$ granule cells receive the same $n = 4$ mossy fibers, the n-dimensional mossy fibers input can be written as $\mathbf{x = W^{-1}(s + w_o)}$ in the linear regime of $\mathbf{s = W\,x - w_o}$ by inverting the network. Dropping the index $\mathbf{k}$, the density of a single data point is obtained by marginalizing over the states of the network,

$\mathbf{f_x(x \mid W, w_o)} = \int \mathbf{f_x(x \mid s, W, w_o) f_s(s) ds}$ where $\mathbf{f_x(x \mid s, W, w_o) = \delta(x - W^{-1}s + W^{-1}w_o)}$ and where $\boldsymbol{\delta}(\cdot)$ is the n-dimensional delta function.

Learning rules

The learning rules for the weights $\{w_{ji}\}$ and $\{w_{Oj}\}$ are derived by taking the gradient of the log likelihood above and multiplying the results by $\mathbf{W}^T\mathbf{W}$. For an active mossy fiber $x_i > 0$, and

for an active granule cell $s_j > 0$,

$$\Delta w_{ji} \propto w_{ji} - \alpha \sum_j s_j w_{ji}$$

$$\Delta w_{Oj} \propto +\alpha$$

for an inactive granule cell $s_j = 0$,

$$\Delta w_{ji} \propto w_{ji} + \beta \sum_j s_j w_{ji}$$

$$\Delta w_{Oj} \propto -\beta$$

The synaptic weight update rules change sharply depending on whether the granule cell is active or not. Notice that a backward summation $X_i = \sum_j s_j w_{ji}$ from granule cell activity s_j must be computed at the i th glomerulus and that the particular connectivity at the glomerulus makes its computation possible (see below). The summation X_i is unique to the i th glomerulus, and is the same for all weight changes Δw_{ji} at that glomerulus, but the difference $w_{ji} - \alpha \sum_j s_j w_{ji}$ is unique to each synapse at that glomerulus.

Although these learning rules were derived for the complete case, Girolami *et al.* showed that the same learning rules hold whether **s** forms a complete or an undercomplete representation of **x**. In our case, complete and undercomplete refer to whether the number of granule cells with the same mossy fiber inputs is equal or smaller than the number of mossy fiber inputs **x** to the granule cell **s**.

The backward summation $X_i = \sum_j s_j w_{ji}$ is biologically plausible in the granular layer of the cerebellum due to the unique convergence of information at the glomeruli. Because the granule cells are electrotonically compact (refs here 9, 17, 33), the spiking activity at the soma is assumed to be reflected at the dendrites.

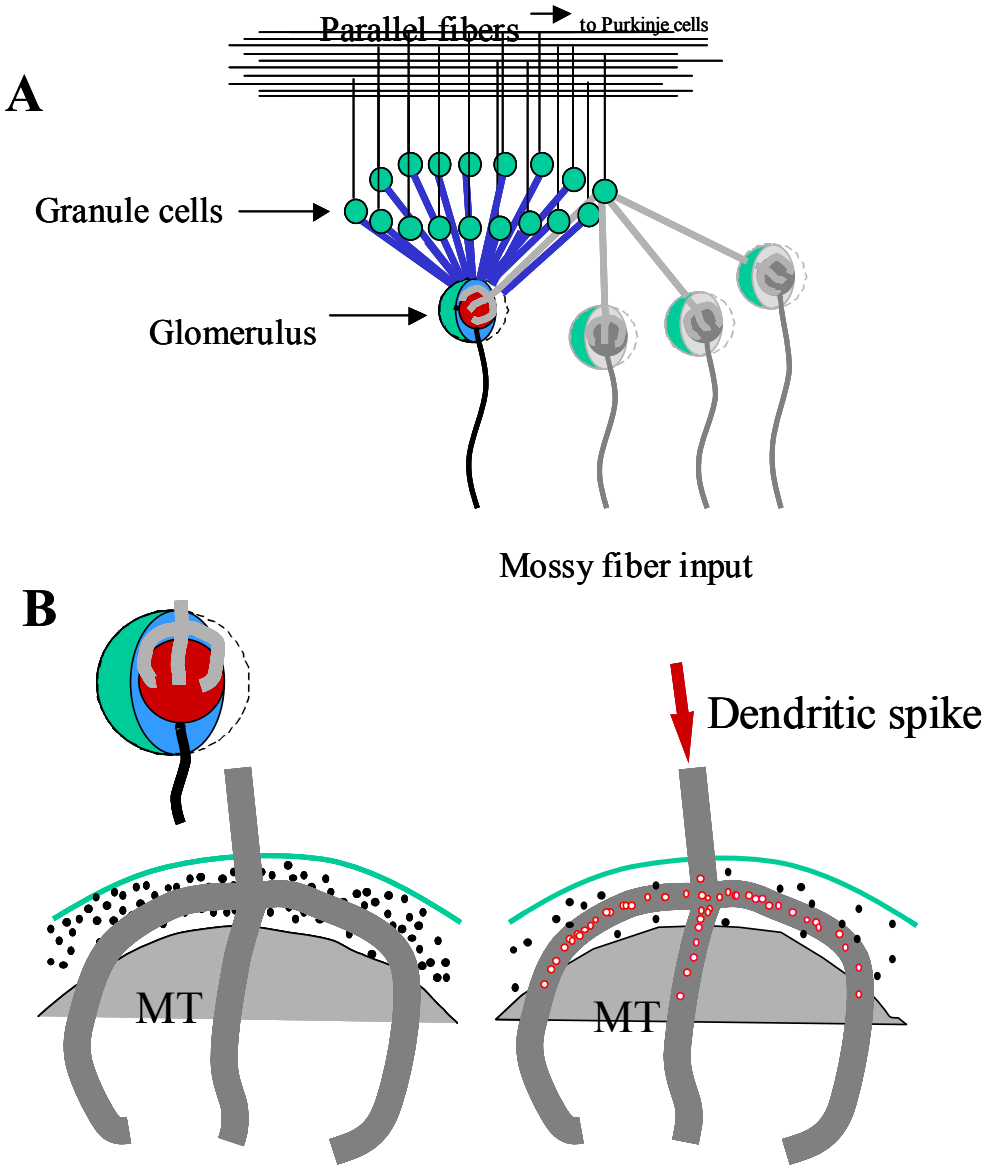

Figure 1. Back-propagating GC spikes may be encoded as external calcium changes in the glomerulus.

A. Illustration of the interface of the mossy fiber and granule cell layers.

B. Calcium in the glomerular cleft is shared. Experimental data suggests that a back-propagating spike travels relatively unattenuated along dendritic branches, especially in the electrotonically compact granule cells. The occurrence of the back-propagating dendritic spike is associated with large influxes of calcium through voltage-gated channels. This influx is mirrored by a peri-dendritic efflux of calcium from the glomerular extracellular space. The total calcium concentration in the glomerulus would decrease when a BP-spike arrives along a GC dendrite.

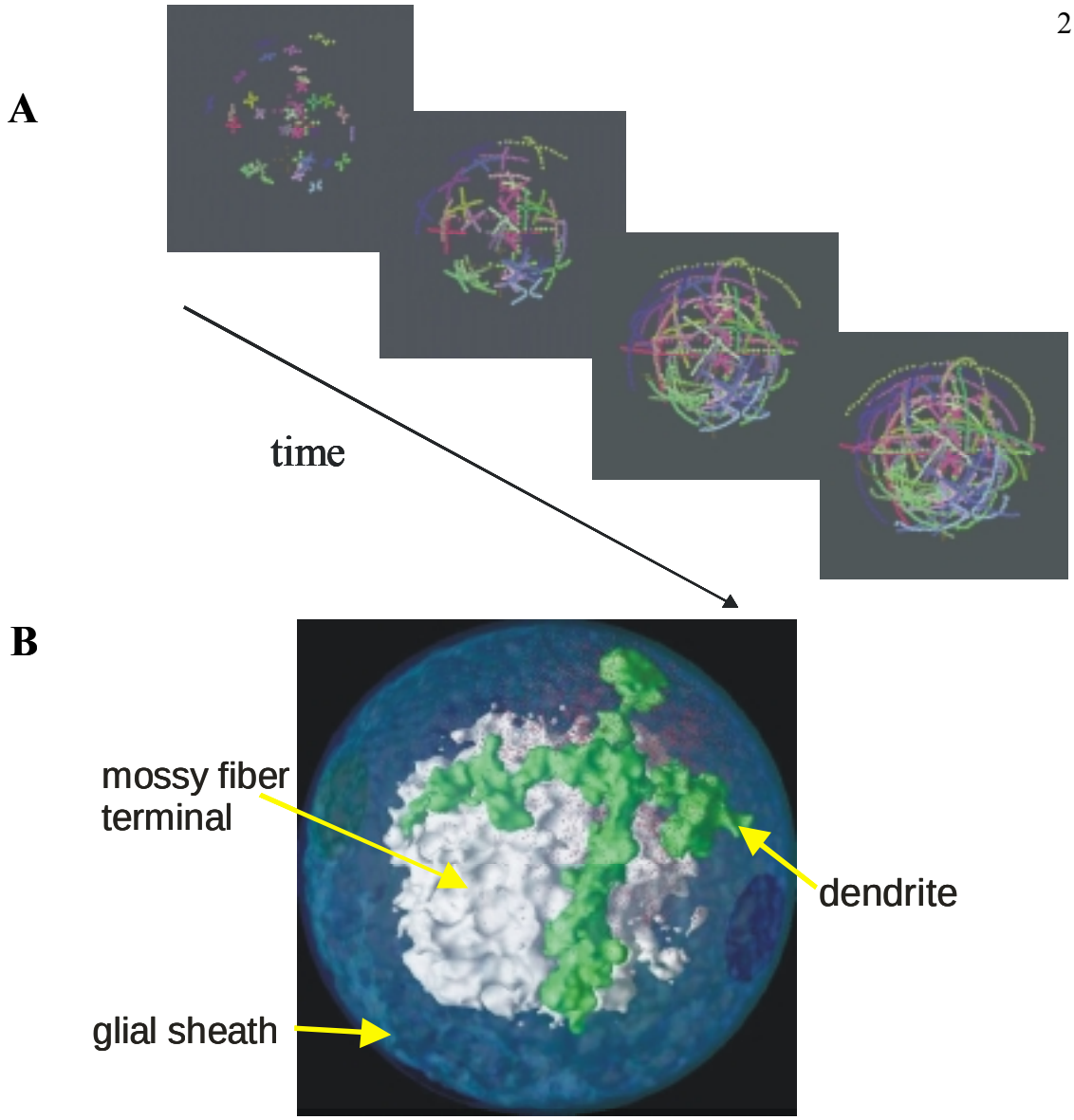

Figure 2. Simulating a 3D glomerulus.

A. In our Neural Growth Simulator, dendritic tips determine their paths by local avoidance rules. Each tip attempts to take a step under the constraint that it cannot come within a fixed distance of any other dendrite. After 80 steps of the simulation, growth is stopped, and the dendrites 'inflate' to fill any available voxels.

B. When the voxels have all been committed, a Marching Cubes algorithm constructs each dendrite into a 3D polygon mesh. The coordinates of the polygon mesh are read into MCell, where calcium ions, channels, and pumps are distributed appropriately (see Methods).

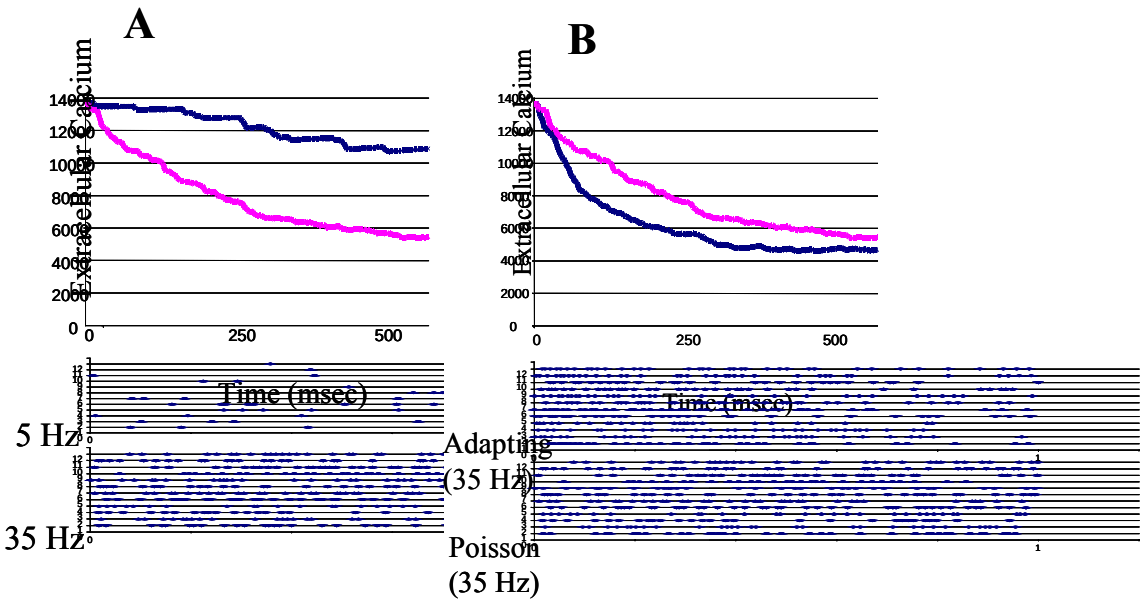

Figure 3. Changes in granule cell firing rates modulate the extracellular calcium signal.

A. Simulations of calcium dynamics performed with the Monte Carlo simulator MCell, using explicity modeled calcium channels, pumps, and 13,688 calcium atoms. In these simulations, a single mossy fiber terminal articulates with 40 GCs. The GCs have an Poisson average firing rate of 5 Hz (top trace) or 35 Hz (bottom trace). Shown below are the spike trains of 12 of the GCs.

B. If the GCs have an adapting firing rate, a burst of firing will slow down. In this simulation, there are the same number of total spikes as in Fig 3A, but here the rate begins quickly and slows. In this situation, the new calcium set point is approached more quickly.

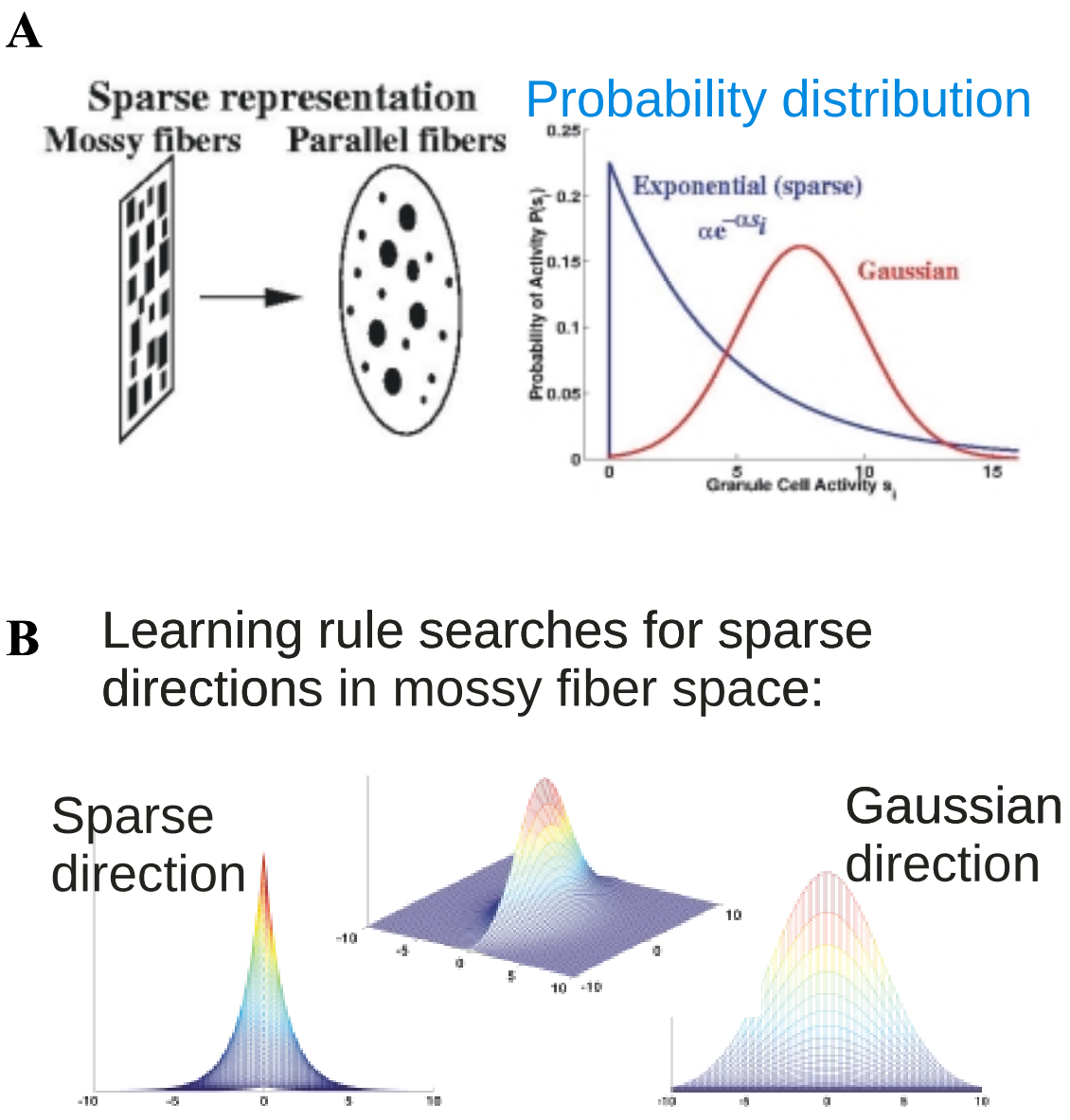

Figure 4. Principles for coding and plasticity at the granule cells.

A. Illustration of the goal of the learning rule: to maximize the mutual information between the mossy fiber inputs and the granule cell outputs under a sparseness constraint on the firing of the GCs.

B. The learning rule searches for sparse directions in mossy fiber space.

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