Modeling spatio-temporal processing in cerebellar granular layer and effects of controlled inhibition

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MODELING SPATIO-TEMPORAL PROCESSING IN CEREBELLAR GRANULAR LAYER AND EFFECTS OF CONTROLLED INHIBITION ON PLASTICITY

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ABSTRACT
The cerebellum input stage has been known to perform spatio-temporal transformations and combinatorial operations [1] [2] on input signals. In this paper, we developed a model to study information transmission and signal recoding in the cerebellar granular layer and to test observations like center-surround organization and time-window hypothesis [1] [3]. Detailed biophysical models were used to study synaptic plasticity and its effect in generation and modulation of spikes in the granular layer network. Our results indicated that spatio-temporal information transfer through the granular network is controlled by synaptic inhibition [1]. Spike amplitude and number of spikes were modulated by LTP and LTD. Both *in vitro* and *in vivo* simulations indicated that inhibitory input via Golgi cells acts as a modulator and regulates the post synaptic excitability.

KEY WORDS
Cerebellar granular layer, network, modelling, plasticity, inhibition.

1. Introduction

Cerebellar granular layer forms the input stage of the cerebellum in which information coming from the peripheral and central systems converge through the mossy fibers. The granular layer has by far the smallest (~ 5μm) and the most numerous neurons (~ 10^{11}) in humans. Understanding how the granular layer process information appears critical to understand the cerebellar function, since signals coming into upper cortical layers are provided by the granular layer. The granule cells form the largest neuronal population in the mammalian brain and regulate information transfer along the major afferent systems to the cerebellum. The granule layer receives excitatory input primarily from mossy fibers and inhibitory inputs from interneurons like Golgi cell. The Mossy fiber input excites both the granule cell and inhibitory interneurons like Golgi cell. The granule cell is a small neuron with three-five dendrites.

In this paper, we focus on the impact of excitation on granule cells. The study also includes effect of inhibition on the granular layer circuitry and population activity in terms of combinatorial operations on granular layer network [2]. In this work, we used detailed models of neurons for estimating overall spiking in granular layer network. We also expanded the network to use biophysical models such as [5], [6], [7] to build a biophysical realistic model.

The characteristic study on our network model was the effect of inhibition. Our granular layer network model contained 1680 granule cells (GrC), 1 Golgi cell (GoC) excited by 218 Mossy-fiber (MF) rosettes, where 140 homogenous Mossy-fiber rosette exciting granule cells through 140 glomerular connections and the 78 homogenous MF rosettes for Golgi cell excitation [2][8]. Varying strengths of excitatory inputs from the center to the periphery were applied to the model to understand the concept of center-surround activation patterns. Another goal was to understand the effect of combinatorial operations on the granular layer network. Combinatorial operations included combined excitation and inhibition which forms the spatiotemporal pattern in granular layer network *in vitro* and *in vivo*. All modeling and simulation were made using the NEURON environment [9].

The paper reports the effect of inhibition on information transfer along the granular layer network. An estimate of number of spiking cells with and in the absence of inhibition was obtained. This estimate is the upper cut-off of the overall excitation in any particular region of interest. Spike properties of underlying cells and modulation of spikes were also studied. Variations due to synaptic plasticity in the granular network and modulation of LTP-LTD are also reported.

2. Methods

The study carried out in this paper involved the use of computational models of neurons, modified ion channel properties for intrinsic excitability and analysis of simulation data.

2.1 Neuronal models

Mathematical neuron models of granule cell [4], [5] and Golgi cell model [6] [7] were used in this network study. A single compartmental model was used to represent granule cell and was adapted from [4] with 13-state sodium channel model from [5]. Modeling reliability for spiking models was based on the extensive characterization of membrane currents and the compact electrotonic structure of cerebellar granule cells [4] [5]. The model used AMPA and NMDA components as excitatory MF-GrC synapses and GABAergic synapses for the Golgi cell- GrC relay [6].

2.2 Granular layer network

Granular layer spiking network model consisted of 140 homogenous Mossy fibers rosettes (MF), 1680 Granule cells (GrC) and 1 Golgi cell (GoC). In this network, about 48 GrC receive 1 excitatory input from the same mossy fiber and each granule cell received four excitatory connections from four different mossy fibers. Along with these excitatory inputs given to GrC, mossy fibers also give excitatory input to GoC whose ratio is about 78:1 giving overall glomeruli connectivity pattern. The network topology is shown below in Figure 1. The study have been made by varying the level of inhibition uniformly over the network of 1680 Granule cells and by varying the release probability of the GABAergic synapses from 0.1 to 0.8. *In vitro* like behavior was studied by giving single spike as input. *In vivo* like behavior was characterized by burst (5 spikes/burst).

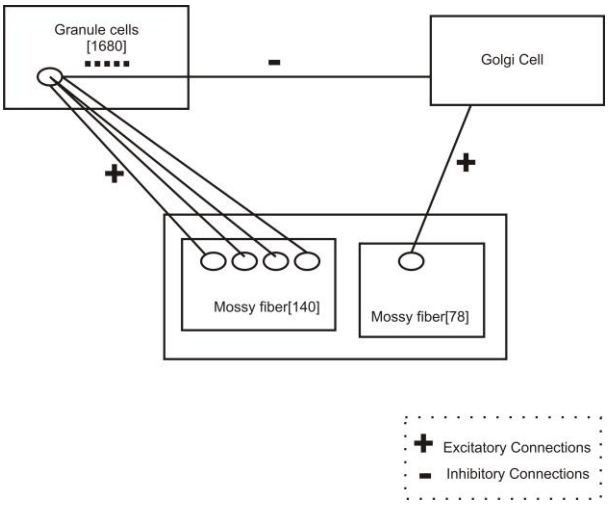

Figure 1. Granular layer Network topology. Granule cells (GrC) and Golgi cell (GoC) receive excitatory inputs from mossy fibers. GrC receive inhibitory input from a single GoC.

2.3 Center-surround "spot" pattern

Stimulating mossy fibers with an electrode at a particular point activates granule cells in the network in a center-surround activation pattern [2]. Within a 'spot', cells which are in close proximity to the electrode will receive high excitation and the periphery layer cells receive less excitation. Our model of the granular network shows the pattern activated as a 'spot'. The center-surround pattern showing the decreasing strengths of excitation can be noticed from the center to the periphery.

2.4 Simulating LTP/LTD

By modifying intrinsic excitability and release probability [10], we simulated plasticity in the granule cells. We modified intrinsic excitability by changing ionic current density or gating. We modified the on-off gating characteristics of sodium channel to modify sodium activation and inactivation parameters [11] for higher and lower intrinsic excitability.

3. Excitation, Inhibition and Plasticity in granular layer network

LTP and LTD in granule cells [10] comprises of variation in release probability and intrinsic excitability. The network model was tested with high and low intrinsic excitability observed by changes to sodium channel properties and release probabilities of MF synapses, thereby granule cell LTP and LTD were simulated respectively.

In the case of *in vitro* like behavior of network, with release probability of MF synapses 0.416 (control) the cells receiving 4 and 3 excitatory inputs produced 2 and 1 spikes respectively.

With the lesser release probabilities of MF synapses ($U<0.416$), the cells did not produce spikes irrespective of excitatory inputs (see Figure 2A). When $U=0.3$, 48 cells in a population of 648 cells (in a spot) receiving 4 excitatory inputs produced a single spike (see Figure 2B) with decreased spike amplitude and increased spike latency. When $U>0.3$, an increased number of spikes were observed (see Figure 2C) where the spike amplitude, first spike latency remained unchanged. In network, number of spiking cells observed was 192 and the number increased to 432 when the release probability of MF synapses $U>0.5$. Even the number of spikes became saturated with the higher release probabilities of MF synapses ($U=0.7$, 0.8) (see Figure 2D).

The modulation in intrinsic excitatory from normal to higher excitability showed a significant increase in spike amplitude (~6%) where the first spike latency and number of spikes remained unchanged. Number of spiking cells increased from 192 to 432 cells when $U>0.4$. Cells with lower intrinsic excitability and varying release probabilities of MF synapses ($U<0.416$) did not produce spikes (see Figure 2A). With 0.3 release probability, 48 cells in a population of 648 cells (in a spot) receiving 4 excitatory inputs produced a single spike (see Figure 2B) with decreased spike amplitude and consistent first spike latency.

On an average, each granule cell received excitatory connections from 4-5 mossy fibers [12]. Golgi cells converging through lateral connections onto some granule cell subsets could generate combined inhibition [2] [3]. The strength of the inhibition depends on the number of inhibitory connections and synaptic release probability.

The granule and Golgi cell will receive excitatory inputs from mossy fiber (MF) at the same time. The inhibitory input from Golgi cell reaches the granule cell with a delay of ~4ms compared to the mossy fiber input through GABAergic synapses [3]. The inhibition-based time-windowing in granule cells allow one or more spikes and is seemingly regulated by varying inhibitory inputs. The effects of inhibition on the circuitry were tested on the *in vitro* model where one spike/burst in

MF was used as stimulus and on the *in vivo* model where 5 spikes/burst at 500Hz was used as stimuli.

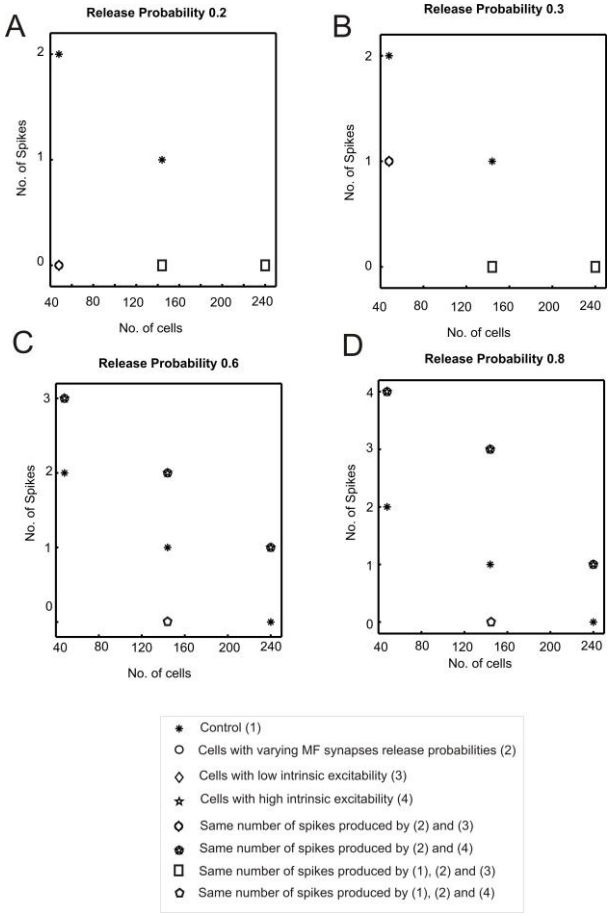

Figure 2. Number of spiking cells vs number of spikes. In the figure star represents control condition (U=0.416), circle represents cells with varying release probabilities, diamond represents low intrinsic excitability condition. Circle and diamond together indicates that same number of spikes were produced by both cells with varying release probabilities and low intrinsic excitability. Rectangle indicates that same number of spikes were given by star, diamond and circle. Pentagram represents high intrinsic excitability condition. Circle and pentagram together indicates that same number of spikes were given by both cells with varying release probabilities and high intrinsic excitability. Polygon indicates that same number of spikes were given by pentagram, star and circle.

The increase in inhibitory connections to granule cells in the underlying network model decreased number of spikes (data not shown), spike amplitude (if the spike rises after the inhibitory signal reaches the Granule cell) and spike latency. Amplitude decreased gradually (for *in vitro* case) (data not shown) and no significant change with the first spike latency for varying GABAergic release probabilities, $U_{inh.}$

3.1 Effects of inhibition on the *in vitro* GrC network

When we changed the value of inhibitory (GABAergic) synapse release probability U_{inh}, the change in the number of spikes were seen varying between 2 and 1 for lower and higher release probabilities of U_{inh} (data not shown).

For neurons with 3 excitatory synapses, change in GABAergic release probabilities showed a significant change in spike amplitude with U<control, where the single spike was preserved. When the inhibitory release probability U_{inh} > 0.5, then the cells with 4 excitatory inputs with 1-4 inhibitory synapses allowed single spike while other spikes were suppressed. First spike latency did not show significant changes (data not shown).

The impact of synaptic excitatory inputs to generate a spike was 3 and any lesser number of inputs favoured generation of EPSP [5]. The number of spiking and non- spiking cells remained unchanged at 852 and 828 respectively. The number of spiking cells (with excitatory release probability constant at control value) can vary between cells with single and double spikes on modulating the GABAergic release probability values.

The increase in inhibition decreased the spike amplitude and increasd first spike latency.

3.2 *In vivo* network and spatial inhibition

In the *in vivo* case, an increase in inhibition level decreased number of spikes under control condition (inhibitory release probability=0.34) (Figure 3C). Variation in GABAergic release probability modulated the number of spikes, where the spike amplitude and first spike latency remained the same (see Table I). As inhibitory release probabilities increased, the number of spikes decreased for the same amount of excitation (see Table I and Figure 3).

When the GABAergic release probability and number of GABAergic synapses were increased, a decrease in number of spikes was observed. The change in decrease of spikes is gradual with the change in the number of inhibitory synapses for lower inhibitory release probabilities (Figure 4A). It was observed that there were minimum 2 spikes for those cells with 2 excitatory synapses and varying number of inhibitory synapses (inhibitory synapses 0-4). As inhibitory release probability was increased, there was decrease in number of spikes with increasing number of inhibitory synapses became distinct (Figure 4 A-G). Those cells with 2 excitatory synapses and with 4 inhibitory synapses did not produce spikes (Figure 4B).

Cells with 1 or 2 excitatory synapses and high inhibitory release probabilities did not produce spikes in the network. The number of spiking cells remained unchanged when GABAergic release probability U_{inh} > 0.2, with a variation in number of spikes.

Table I. Variation of U_{inh}

Release probability	Spike amplitude	First spike latency	Number of spikes
0.1	14.83	24	5
Control (0.34)	14.83	24	4
0.8	14.83	24	3

a. Increase in release probability of GABAergic synapses did not show a significant change in the spike amplitude and first spike latency. The data in the table are explained with respect to cells with I1E3 (receiving 3 excitatory and 1 inhibitory inputs) activation pattern, for *in vivo* like behaviour.

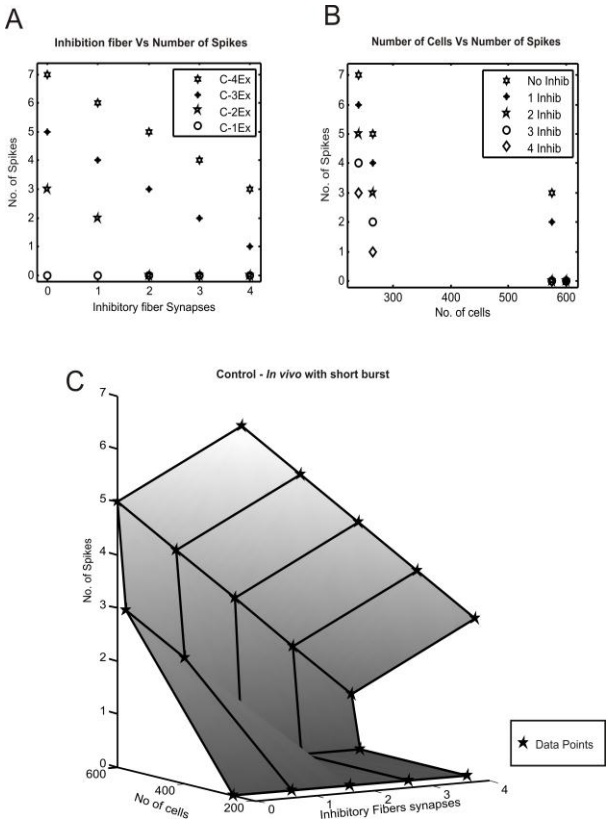

Figure 3. Variation in number of spikes and spiking cells with respect to increasing active inhibitory synapses (0-4) with release probability of GABAergic synapses as 0.34. A shows the variation in number of spikes with respect to the number of active GABAergic synapses. B shows the variation in number of spikes with respect to the number of cells with different activation pattern. C is a 3D-plot showing the variation in number of spikes in granule cells in network with respect to the increase in the number of active GABAergic synapses *in vivo* (input given was 5 spikes/burst).

Main observations *in vivo* for short (5 spikes/burst) and long burst (9 spikes/burst) inputs were: 1) Change in the firing frequency of spiking cells and 2) Level of inhibition (number of inhibitory synapses per cell) under which the cells with 2 excitatory inputs did not generate spikes (data not shown).

The increase in inhibition decreased number of spikes in the network. The inhibitory inputs modulated the spike count, spike amplitude and first spike latency although changes were distinct only with increased inhibition.

4. Center-surround excitation in populations of granule cells

The network model was then used to simulate combinatorial properties of the granular layer network and network activity during double MF- bundle stimulation [2] was explored.

The '*spots*' are maps of excitatory activity as seen in the cerebellar granular layer [2] when MF rosettes were stimulated. In the model configuration, the center of the spot received stronger excitatory inputs and the consecutive peripheral neurons received weaker excitatory input thereby expressing a center-surround configuration (see Figure 5 A).

In this modeled network, about 144 cells received 4 excitatory inputs, 432 cells received 3 excitatory inputs, 144 cells received 2 excitatory inputs and 432 cells received 1 excitatory input. The impacts on a spot by varying release probabilities and intrinsic excitability, thereby modeling LTP and LTD in both *in vitro* and *in vivo* cases were studied. The cells in the granular layer network received GABAergic synaptic inputs with respect to the number of excitatory inputs given to the cells in the granular layer network (see Table II).

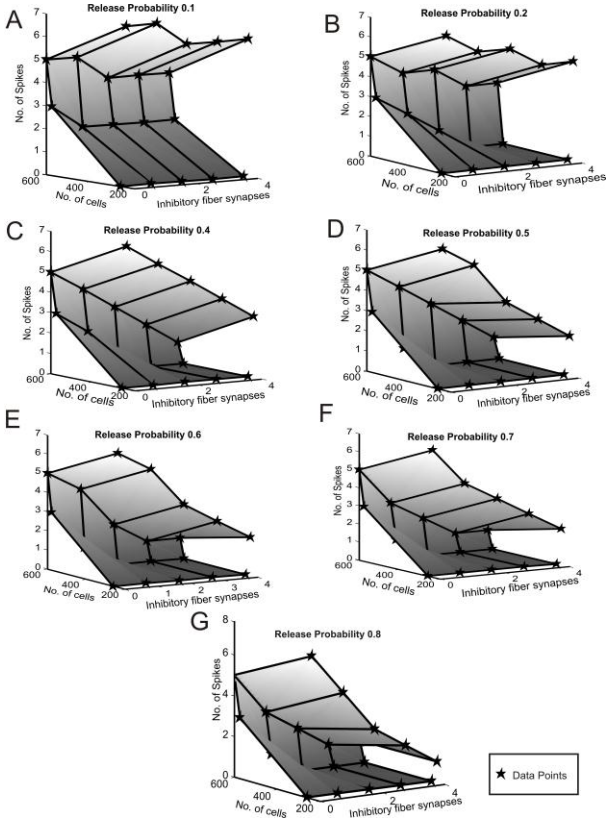

Figure 4. *In vivo* like behaviour (input given was 5 spikes/burst), firing pattern seen in granule cell network by varying the GABAergic release probabilities. A-G shows the variation in number of spikes in the network granule cells (with different excitatory inputs) with respect to the increase in active inhibitory synapses at different GABAergic release probabilities like 0.1, 0.2, 0.4 – 0.8 respectively.

Table II. Activation pattern given to cells in granular layer network.

# cells	# excitatory inputs	# inhibitory inputs
144	4	1
432	3	2
144	2	3
432	1	4

a. Number of cells contributing to the spot corresponding to activation patterns shown as combination of excitatory and inhibitory synapses

When all the inhibitory synapses of granule cells in the network (with *in vivo* like behaviour) were switched off, the cells receiving 4 excitatory inputs produced burst of 7 spikes, cells receiving 3 excitatory inputs produced burst of 5 spikes, cells with 2 excitatory inputs produced a short burst of 3 spikes, cells with 1 excitatory input produced EPSP (see Figure 5B, Table III). The effects of inhibition on the circuitry were

tested on *in vivo* model where 5 spikes/burst at 500Hz in MF was used as stimuli. Inhibitory synapses were activated in the network, where the number of active inhibitory synapses to a cell varied according to its excitatory inputs. Through the GABAergic inhibitory synapses, 1 spike was provided along with the excitatory stimuli. Different combinations of activation pattern to the cells in the network are listed in Table II.

Table III. Effect of inhibition over the number of spikes in spiking cells of network

No. of cells	# active MF synapses	# spikes	
		Network without inhibition	Network with inhibition
144	4	7 spikes/burst	6 spikes/burst
432	3	5 spikes/burst	3 spikes/burst
144	2	2 spikes/burst	EPSP
432	1	EPSP	EPSP

a. Cells with 4 excitatory inputs produced 7 spikes/burst when inhibitory synapse was switched off and produced 5 spikes/burst when it was switched on.

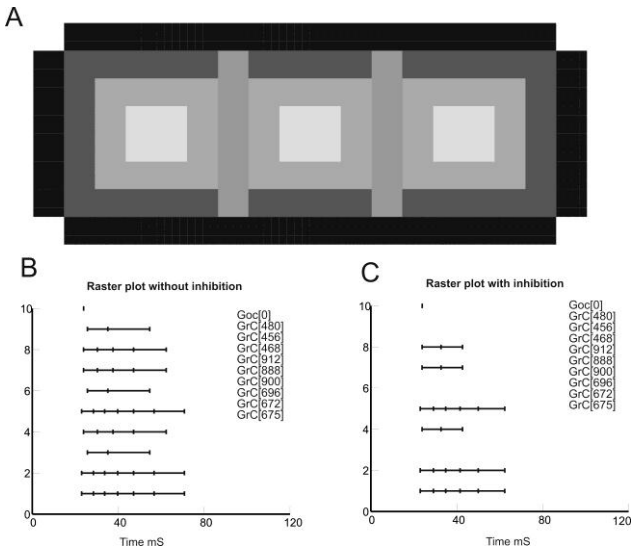

Figure 5. Center-surround "*spot*" activation. A. Varying intensities of grey color in the spot corresponds to varying levels of excitation as mossy fiber inputs to granule cells in the network. The white color in the middle of the spot form I layer, with the cells receiving 4 excitatory inputs. The light grey layer represents II layer cells which receive 3 excitatory inputs. The dark grey colored layer form the III layer cells receiving 1 excitatory input and the cells of III layer present in the overlapping region of the two spots received 2 excitatory inputs (represented in grey color). The black layer represents the outermost layer of the spot form the IV layer (no excitatory inputs given). Each spot contains 648 granule cells in which 96 granule cells (III, IV layer) are present in the overlapping region of the two spots. The spots are activated by double mossy fiber bundle simulation [2]. B. Raster plot of the network with no active GABAergic synapses. C. Raster plot of the network with active GABAergic synapses.

The number of spiking cells decreased from 720 to 576 after activating the GABAergic synapses. The cells with 2 excitatory inputs lost its spikes with 3 active inhibitory synapses (see Figure 3C, 5C). Cells with 4 and 3 excitatory synapses, decreased number of spikes was observed (see Table III).

5. Computational cost

Although simulations were performed on a medium scale, computational costs are very high for using the multi-compartmental neuron. Table IV shows the computational costs for a simulation of 200ms using the single and multi-compartment neuron models and for the network model with those neurons.

Table IV. Computational cost for neuron models

Without Inhibition				With Inhibition			
Single Cell		Network		Single Cell		Network	
SC	MC	SC	MC	SC	MC	SC	MC
0.75	12.19	498.86	9861	0.77	12.17	739.83	9696.1

a. All values are in seconds. The simulation time in all cases was for 200ms.

Results show slightly higher computational cost with inhibition and excitation in model neurons. Estimating the computational cost is essential for studying scaling while building larger networks.

6. Discussion

The study showed modulatory impacts of varying inhibitory and excitatory release probabilities on the activities of granule cells in the granular layer network of the cerebellum. The paper also explores the effects of combined excitation and combined inhibition [2]. Both *in vitro* and *in vivo* simulations indicate inhibitory input cannot completely alter the excitation rather it acts as a modulator that regulates the post synaptic excitability.

The variations of excitatory inputs (without combination of inhibition) showed differences in number of spikes and spike amplitude and did not show variations in first-spike latency. The most promising outcome in variation of spikes and spike behaviour was with the induction of LTP/LTD where both intrinsic excitability and excitatory release probabilities change the nature of information flow.

This study on granule neuron excitation and inhibition is one of the first detailed simulation works where a model has been used to explore the parameter space and test plasticity. The presynaptic mechanism coexisted with postsynaptic regulation of ionic channels, which played a major role in determining the granule cell output firing frequency. Intrinsic bursting and modulatory effects of inhibition can be seen by mechanistic control of number of spikes in a granule cell.

Impact of excitation on single neurons affected network activity. With increased excitation, along with an increase in spikes, first-spike latency also decreased. During LTP, there was no significant change in the first spike latency (*in vitro*). With higher intrinsic excitability and with same release probability of excitatory synapses, spike amplitude was increased. This will also impact the local field potential and could probably explain the observations *in vitro* [13]. *C*ombining higher intrinsic excitability and with higher release probability, there was gradual increase in

number of spikes. In both *in vitro* and *in vivo* simulations, the number of spikes was dependent on the release probability of the synapses while higher or lower intrinsic excitability caused slight change in spike amplitude.

The key role of local circuit inhibition for determining granular layer combinatorial operations was supported by several observations. Increasing active inhibitory connections saw lesser number of spikes in the network. *In vivo* bursts along mossy fibers combined with inhibitory input showed a consistent reduction of 1 spike as inhibition increased. An indicative study on computational costs for network model was also incorporated.

Understanding how inputs are processed by the network reveals the function of the granular network. The studies on intensity of mossy fiber synapses and inhibitory synapses helped to understand spatio-temporal operations [2]. Combining granule neurons and Golgi cell will help to reveal coincidence detection properties and spatial pattern separation [14]. This work is a preliminary start in modeling to understanding long-sought spatiotemporal filtering predicted by the Motor learning theory [15].

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