Exploring input-output characteristics of the cerebellar granule neuron: role of synaptic inhibition

Priyanka James, Nimshitha Abdulmanaph, Bipin Nair, Shyam Diwakar

EXPLORING INPUT-OUTPUT CHARACTERISTICS OF THE CEREBELLAR GRANULE NEURON: ROLE OF SYNAPTIC INHIBITION, SPIKE TIMING AND PLASTICITY

Priyanka James, Nimshitha Abdulmanaph, Bipin Nair, Shyam Diwakar*
School of Biotechnology, Amrita Vishwa Vidyapeetham (Amrita University)
Amritapuri, Clappana P.O., 690525Kollam, Kerala
India
*e-mail: shyam@amrita.edu

ABSTRACT
Understanding functional role of bursts firing is vital in understanding coding of sensory information [1]. Regulating the burst is related to stimulus properties and neural heterogeneities [2].The granule cells form the largest neuronal population in the mammalian brain and regulate information transfer along the major afferent systems to the cerebellum. Understanding how the granule cell processes information appears critical to understand the cerebellar function. We used a mathematical model of cerebellar granule cell to explore information transmission in mossy fibre - granule cell synapse of the cerebellum. The impact of plasticity changes in excitatory synaptic release probability and variation in intrinsic excitability of granule cell was studied combining the modulatory effects of inhibition. We explored the changes in pre and post synaptic factors to study spiking properties and report their influence on first spike latency and spike amplitude, revealing the indicators of information encoding in individual neurons [3].

KEY WORDS
Computational neuroscience, cerebellum, granule cell, information processing.

1. Introduction

Neurons carry information in the form of spikes [4]. The input spike information is processed by neurons in complex ways and it involves various factors [5]. Through synapses spikes are transmitted between neurons and the process of synaptic transmission involves neurotransmitter release and diffusion, postsynaptic receptor activation and electroresponsiveness. To quantify the information transfer of a whole neuron, we focused on a simple neuron cell, the cerebellar granule cell (GC), with which the excitatory input space could be explored extensively .MFs (mossy fibres) convey afferent signals to GCs following sensory stimulation [6], [7].

Persistent changes in synaptic strength can cause long term synaptic plasticity, which is supposed to provide the cellular basis for learning and memory and typically takes the form of potentiation (LTP) or depression (LTD) ([8], [9], [10]).Synaptic plasticity is bidirectional [11] involving the changes in postsynaptic responsiveness or changes in presynaptic neurotransmitter release. Induction of LTP at MF-GC synapses enhances the spike train response of GCs [9]. However, the input-output relationship and quantified output is unknown i.e. how much the information transmitted is changed and how much is transferred through the spike train average frequency, spike correlation, or number of spikes [10]. This knowledge is useful to understanding granular layer computation, which has been proposed to provide temporal dynamics ([12], [13]) and regulate the input-output relationship through synaptic gain modulation ([14], [15]) and long-term adaptation ([16], [17], [18]).

During mossy-fibre granule cell LTP, there is an enhancement in neurotransmitter release and intrinsic excitability of granule cell ([19], [9]). LTD expression was associated with a decrease in release probability of the mossy fibre, therefore showing changes opposite to those characterizing LTP [11]. Strong and weak postsynaptic activity determines LTP and LTD respectively. Golgi cells exert an effective time-dependent control over the information conveyed by mossy fibre activity [20].

This paper reports the impact of release probability and intrinsic excitability changes on mossy-fibre granule cell relay and related post-synaptic granule cell response. We also predict the regulatory effect of Golgi cell inhibition on granule cell firing.

2. Methods

The granule cell model was adapted from [21] and the simulations were done with the NEURON simulator [22]. Modeling reliability for spiking models was based on the extensive characterization of membrane currents and the compact electrotonic structure of cerebellar granule cells ([23], [21]).

The release probability of excitatory synapses was varied from 0.1 to 0.8 while keeping the release probability of inhibitory synapses unchanged to study the impacts of plasticity and as a second case the release

probability of excitatory synapses were kept unchanged and inhibitory synapse release probability was varied. This was done for various synaptic activation patterns seen in granule cells *in vitro* and *in vivo* in order to understand the effects of release probability changes in granule cell firing. The intrinsic excitability of granule cell was modified and the release probabilities of inhibitory and excitatory synapses were varied to understand the regulatory and modulatory effects of these synapses in granule cell firing.

2.1 Modeling *in vitro* and *in vivo* behavior

In vitro like behaviors were studied by giving single spike as input. *In vivo* like behaviors were characterized by burst. Short burst means 5 spikes per burst and long burst means 9 spikes per burst. First spike latency was measured from the time of stimulus to peak of the spike. In all models, the stimulus was applied at t=20ms. This spiking nature in reference to *in vitro* and *in vivo* conditions was observed in experimental data (Diwakar et al., submitted).

2.2 Simulating LTP/LTD

By modifying intrinsic excitability and release probability [7] 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 [24] for higher and lower intrinsic excitability.

3. Results

3.1 Release probability changes of excitatory synapses affecting MF-GC relay

During Mossy-fibre (MF) granule cell LTP, an increase in neurotransmitter release was observed ([8], [19]). To understand whether increase in release probability could determine changes in EPSP, the release probability (U) of excitatory synapse was modified from control (U=0.416) while the release probability of inhibitory fibre was set at its control value (U_{inh}=0.34). Various synaptic activation patterns were applied as inputs via MF and for each of the activation patterns, number of spikes; first spike latency (from the peak of the spike) and amplitude of the initial spike were measured. Here, EPSPs were measured from the initial membrane potential of –70mV. With inputs from 4 MF excitatory synapses and 2 inhibitory synapses (see Figure 1A) single spike was observed and the spike measured 15.52 mV in 23.225 ms under control condition (U=0.416).

U	Amplitude(mV)	Number of spikes	Spike Timing(mS)
0.1	-65.54		
0.3	6.01	1	25.8
0.416	15.52	1	23.225
0.6	16.3	3	22.425
0.8	16.52	4	22.1

Table 1. Varying release probability (U) of Mf for 4 Mf; 2 inhibitory synapses active with *in vitro* like single spikes as inputs.

A decrease in number of spikes was observed when the release probability of excitatory synapse was decreased below 0.416.When the release probability of the excitatory synapse was increased above 0.416, number of spikes increased, spike amplitude increases and the first spike latency decreased (see Table 1, Figure 1A). The same procedure was repeated with other activation patterns (data not shown) comprising combination of excitatory and inhibitory synapses. *In vitro* model behaviour with increased release probability of excitatory synapse was associated with decreased first spike latency, increase in amplitude and number of spikes.

GCs tend to discharge bursts *in vivo* [6]. *In vivo* model with the reduction in release probability of MF synapses below 0.416 (control) was associated with decrease in number of spikes, decrease in spike amplitude and increase in first spike latency. When the release probability was increased above the control (U=0.416), number of spikes remained unchanged and small changes in spike amplitude and spike latency were observed (see Figure1 B).

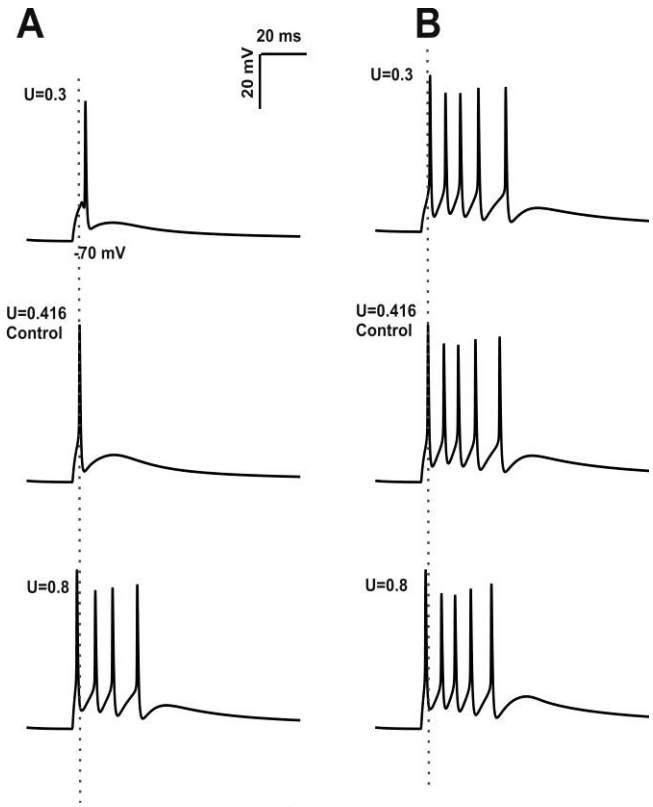

Figure 1. Changes in Mossy fibre release probability**.** A. EPSP obtained by *in vitro* like input through 4 MF synapses, 2 inhibitory synapses of granule cell with varying release probability. First spike latency was reduced (see the grey dotted line) and number of spikes was increased by the increase in release probability. B. 4 MF synapses, 2 inhibitory synapses activation of granule cell *in vivo* with changes in release probability. At U=0.1, change in initial spike delay and decrease in number of spikes was clearly observed. During control condition (U= 0.416), increase in number of spikes and shortening of first spike latency was seen (the grey dotted line passing through the centre of first spike).When the release probability of excitatory synapses was increased to 0.8, number of spikes remains constant, small change in initial spike latency was seen.

3.2 Spike variations and first spike latency during LTP

During LTP, granule cells show an increase in intrinsic excitability and enhanced neurotransmitter release

U	Amplitude of first spike (mV)	Number of spikes	First spike latency (ms)
Control(0.416)	15.52	2	23.225
0.6	21.788	3	22.4
0.8	21.637	4	22.075

([25], [8], [19]).

Table 2. Spiking properties for single spike input via MF, inhibitory synapses during LTP. 4 excitatory synapses and 1 inhibitory synapses provide inputs to the granule cell. Note the change in latency and increase in spike count and amplitude.

Combining variations in intrinsic excitability, the effect of increase in excitatory synapse release probability was studied for various combinations of excitatory and inhibitory synapses.To test the effects of increase in mossy fibre release probability during LTP, the release probability of excitatory synapse was increased (U>0.416) from control. As release probability was changed with the same synaptic pattern (4 excitatory synapses and 1 inhibitory synapse activating the cell), number of spikes increased (Table 2, Figure 2A).

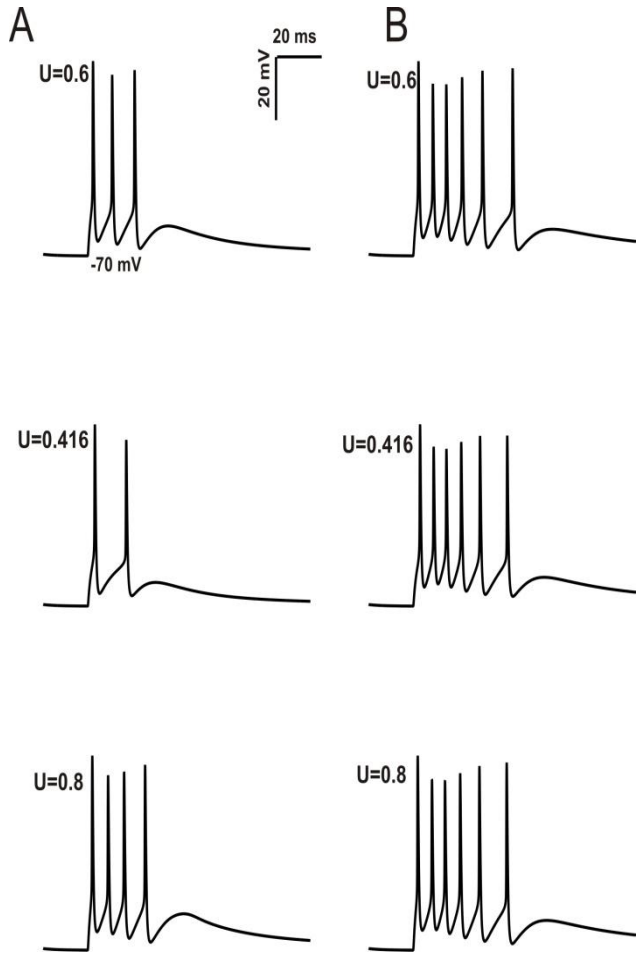

Figure2. Simulating LTP A. 1 inhibitory synapse 4 excitatory synapses activating granule cell with high intrinsic excitability. Varying release probability of mossy fibre for single spike input shows difference in number of spikes A. Single spike input via MF B. Burst like input via MF. Note the number of spikes remains constant.

U	Amplitude of initial spike	Number of spikes	First spike latency (ms)
Control(0.416)	15.55	6	23.125
0.6	21.725	6	22.4
0.8	21.635	6	22.075

Table 3. Spiking properties for burst input via MF, inhibitory synapses during LTP. 4 excitatory synapses and 1 inhibitory synapses provide inputs to the granule cell. No change was observed in spike count but significant changes are seen in first spike latency.

In vivo behaviour of granule cell model showed, when the release probability of MF was increased above the control (U=0.416), number of spikes remained constant and small variations in spike amplitude and first spike latency was observed but the increase in intrinsic excitability caused an increase in spike amplitude (see Table 3, Figure 2 B).

3.3 Predicting the effects of LTD *in vitro* and *in vivo*

Plasticity in MF-GC has been known to be bidirectional [26]. The expression of LTD was associated with decrease in release probability of the excitatory synapses, thereby showing directionally opposite characteristics of LTP [11]. LTD arises due to weak, asynchronous, sporadic activity in pre and postsynaptic neurons [8]. To test effects of LTD induction, intrinsic excitability of the model was reduced and the release probability of MF synapses was reduced below 0.416(control) and its effect in granule cell firing was observed for various synaptic activation patterns. The release probability of inhibitory synapse was kept constant (U_{inh}= 0.34).With low release probability of MF synapses (U=0.1) *in vitro* granule cell model with low intrinsic excitability did not produce spikes (Figure 3A).

The effect of low release probability of MF synapses and low intrinsic excitability of *in vivo* granule cell was simulated and studied. *In vivo* like inputs to granule cell model under control condition produced 5 spikes and the initial spike measured 15.55 mV in 23.125 ms. Low intrinsic excitability of granule cell affected spike amplitude and first spike latency, but it did not show significant effect on number of spikes. Decrease in release probability of MF synapses affected spike latency, number of spikes and spike amplitude. MF-GC LTD was associated with decrease in spike amplitude and increase in first spike latency (see Figure 3B, Table 4).

U	Amplitude of first spike (mV)	Number of spikes	First spike latency (ms)
0.1	2.24	2	31.95
0.3	8.59	5	24.075
0.416	15.55	5	23.15

Table 4. Release probability changes (U) of MF synapses for cells with *in vivo* like inputs via 4 MF synapses and 2 inhibitory synapses.

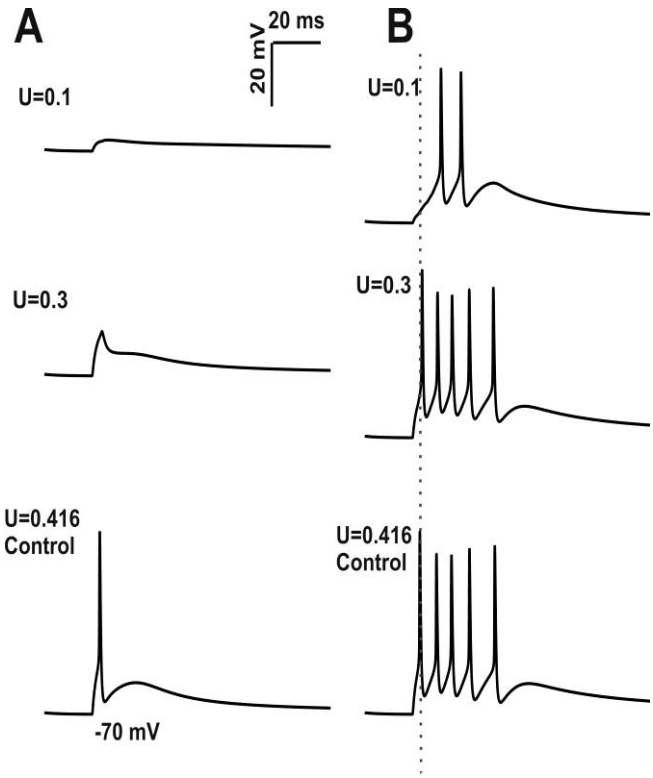

Figure 3. Simulations of changes in mossy fibre release probability and intrinsic excitability. A. *In vitro* behaviour of granule cell model with low release probabilities of MF synapses B. *in vivo* behaviour of granule cell model during low release probability of mossy fibre. First spike latency was increased when there was a reduction in MF synapse release probability coupled with low intrinsic excitability of granule cell (see grey dotted line passing through control). It can be noted that the decrease in release probability of MF synapses was associated with decrease in number of spikes.

3.4 Variation in number of spikes

Spikes compute and carry information since time-dependent signals are usually rate-modulated [27]. To understand information processing in granular networks, number of spikes as rate code is very useful as spontaneous activity in granule cells reflects in Purkinje cells [28].A model-based estimate on the number of spikes with varying inputs and control parameters is reported in Table 5.

			# spikes for 2 excitatory synapses (inputs).	# spikes for 4 excitatory synapses (inputs).
In vitro	Without inhibition	Control	0	2
		LTP	1	4
		LTD	0	0
	With inhibition	Control	0	1
		LTP	0	1
		LTD	0	0
In vivo	Without inhibition	Control	3	7
		LTP	3	7
		LTD	2	6
	With inhibition	Control	0	3
		LTP	0	3
		LTD	1	2

Table 5. Granule neuron spike count. Note the variations in number of spikes at different conditions *in vitro* and *in vivo*. Control is model under normal conditions. More spikes are seen in absence of inhibition.

During LTP *in vitro*, simulations showed sharper increase in number of spikes in the absence of inhibition from Golgi cells. On the other hand, *in vivo* simulations, in the presence of inhibition, showed a steady increase in number of spikes during LTP while LTD simulations predicted a steady decrease (see Table 5).

3.5 Impacts of inhibition during LTD

Variation of inhibitory release probability with low intrinsic excitability was studied for various synaptic activation patterns. The release probability of the Mossy fibre synapses were kept at control value (U = 0.416) for all the activation patterns and the release probability of inhibitory synapses were varied from 0.1 to 0.8.

Bi-directional nature of plasticity [26] was noticed as inhibitory strengths increased. *In vitro* like behavior of granule cell model with increased inhibition and low intrinsic excitability showed delayed spike timing and change in spike amplitude. When inhibition was predominant (both in number of synapses and release probability change), the granule cell did not generate spikes (See Table 6, Figure 4A).When the release probability of inhibitory synapse was increased number of spikes remained unchanged for bursts of spikes as input (see Table 7, Figure 4B) while spike latency and amplitude tend to remain unchanged.

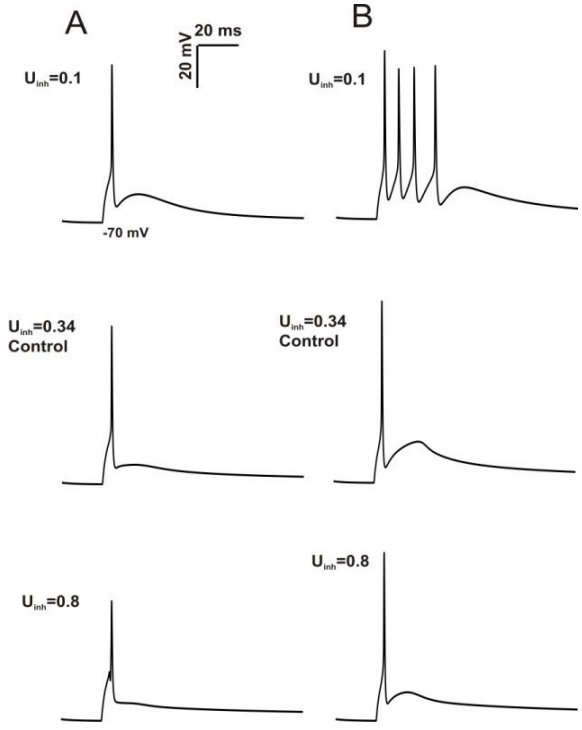

Figure 4. Spiking characteristics of granule cell with single and burst as input. For *in vitro* like behaviour first spike delay was reduced with lesser inhibition. The first spike delay remains constant irrespective of the release probability change in inhibitory synapses for in vivo like behaviour.

U_{inh}	Amplitude of first spike (mV)	Number of spikes	First spike latency (ms)

0.1	3.61	1	24.775
Control(0.34)	3.76	1	24.675
0.8	-14.24	0	25.15

Table 6. Spiking properties for single spike input via MF, inhibitory synapses during LTD. 4 inhibitory synapses and 3 excitatory synapses provide inputs to the granule cell. Note decrease in spike count and spike amplitude.

Inhibition with LTD affected spike count as shown in Table 6 and 7.

3.6 Modulation of spiking during plasticity

To understand the information encoding in terms of number of spikes and spike latency [3], we explored the parameter space of mossy fibre excitations and spatial inhibition for granule cell under control, LTP and LTD conditions. Absence of inhibition sets the upper limit of spikes in case of control, LTP and LTD simulations. As inhibition increased slight decrease in number of spikes was observed. Intrinsic excitability mainly modulated the first-spike latency in most patterns. Higher intrinsic excitability reduced first spike delay (see Figure 5). The study (Figure 5 for *in vitro* single spike input) revealed how LTP/LTD with inhibition could influence granule neuron information flow.

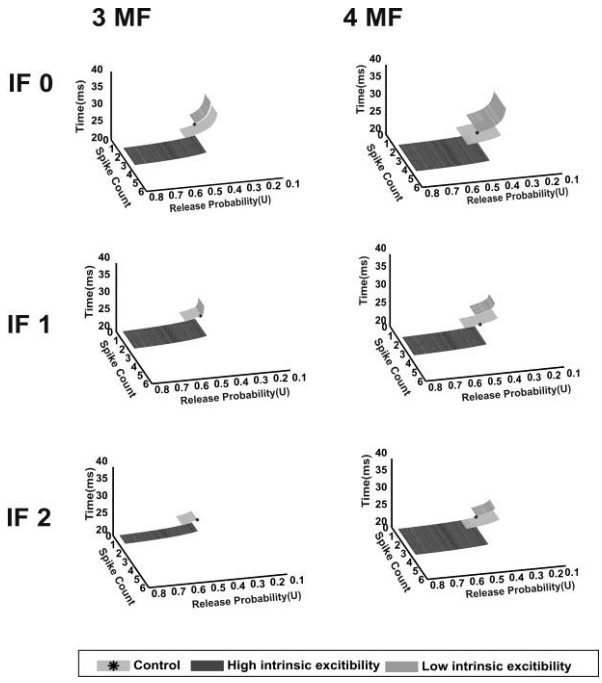

Figure 5. Quantifying information transfer in spiking neurons for single spike (*in vitro*) input. Light grey region shows number of spikes, first spike delay for release probability changes (U=0.35-0.45). Black dot (over light grey) shows the control (U=0.416) value. Black area shows model variation with high intrinsic excitability. The grey area shows model with low intrinsic excitability. X axis indicated various release probabilities; Y-axis shows number of spikes, Z-axis shows first spike latency (ms). MF: Mossy Fiber Synapses, IF: Inhibitory Fibre.

A similar observation with *in vivo* like inputs was observed (data not shown). However, in case of in vivo data there was a sharper modulation by intrinsic excitability and hence the variation in number of spikes.

U_{inh}	Amplitude of first spike (mV)	Number of spikes	First spike latency (ms)
0.1	8.624	4	24.05
Control(0.34)	14.83	1	24
0.8	8.531	1	24.05

Table 7. Spiking properties for burst input via MF, inhibitory synapses during LTD. 3 excitatory synapse and 4 inhibitory synapses provide inputs to the granule cell. Distinct change was observed.

4. Discussion

This paper provides extensive analysis of information transmission in granule neuron. The input-output parameter space analysis beyond current experimental techniques has been explored. Part of information is transmitted as rate, part as time precision and that high correlation among the MF inputs was characteristic of most informative stimuli. Importantly, the transmitted information was regulated by inhibition especially during induction of LTP and LTD.

In vivo simulations of granule cell showed that increase in the release probability of MF synapses alone does not make significant changes in firing and yet a decrease in MF synapse release probability can reduce granule cell firing. Increase in release probability and intrinsic excitability of granule cell showed increases in spike amplitude and decrease in spike latency whereas firing frequency remains same [9]. During low release probabilities of MF synapses, number of spikes is reduced compared to control.

Decrease in release probability of MF synapses along with low intrinsic excitability affected the spike latency, number of spikes and spike amplitude. MF-GC LTD is associated with decrease in spike amplitude and increase in first spike latency. The change in number of spikes was not significant. Low intrinsic excitability in granule cell combined with high release probability of inhibitory synapses showed a decreased number of spikes and decreased spike amplitude, but the spike latency was preserved. Low release probability of inhibitory synapses showed increased number of spikes, with low spike amplitude due to low intrinsic excitability. Theoretical network models predict that information in the MF-GC relay is a relevant parameter that could optimize cerebellar performance for certain tasks and under appropriate learning rules [16] [18]. Hence, plasticity at this relay may be an important element of tremendous storage capacity in the learning of coordination of actions, sensorimotor or cognitive, in which the cerebellum participates.

Given the pervasiveness of temporal information in external stimuli and the generality of the time-dependent mechanisms studied in the current paper, all temporal responses needed for forming coarse temporal code can be stipulated. This, in turn, will help estimate overall behaviour in firing in the underlying granular layer network. Also selective inhibition in GC may help to quantify and to reveal mechanisms of coincidence detection and spatial pattern separation as described in Motor learning theory [14].

Acknowledgement

This project derives direction and ideas from the Chancellor of Amrita University, Sri Mata Amritanandamayi Devi. This work is supported partially by the Sakshat project of National Mission on Education through ICT, Department of Higher Education, Ministry of Human Resource Department, Government of India.

References

[1] Leda Roggeri, Bruno Rivieccio, Paola Rossi, & Egidio D'Angelo, Tactile Stimulation Evokes Long-Term Synaptic Plasticity in the Granular Layer of Cerebellum, *The Journal of Neuroscience*, June 18, 2008.

[2] O. Avila-Akerberg ,R. Krahe & M.J Chacron , Neural heterogeneities and stimulus properties affect burst coding in vivo, *Neuroscience, 168*(1), 2010, 300-13.

[3] Rufin VanRullen, Rudy Guyonneau & Simon J. Thorpe , Spike times make sense , *Trends in Neurosciences, 28*,2005.

[4] G. Foffani , M.L Morales-Botello & J .Aguilar , Spike timing, spike count, and temporal information for the discrimination of tactile stimuli in the rat ventrobasal complex, *J Neurosci*. ,*29*(18),2009, 5964-73.

[5] P. Bezzi , V. Gundersen , J.L Galbete , G. Seifert , C. Steinhäuser , E. Pilati & A. Volterra , Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate, *Nat Neurosci*., *7*(6) , 2004, 613-20.

[6] P. Chadderton, T. W. Margrie & M. Hausser, Integration of quanta in cerebellar granule cells during sensory processing. *Nature, 428*(6985), 2004, 856–860.

[7] Szwed M. et al, Encoding of vibrissal active touch, *Neuron, 40*, 2003, 621–630.

[8] Egidio D'Angelo, Paola Rossi, David Gall, Francesca Prestori,Thierry Neius, Arianna Maffei & Elisabetta Sola, Long-term potentation of synaptic transmission at the mossy fibre-granule cell relay of cerebellum ,*Progress in Brain Researc , 148*,2005.

[9] Thierry Nieus, Elisabetta Sola, Jonathan Mapelli, Elena Saftenku, Paola Rossi, & Egidio D'Angelo, LTP Regulates Burst Initiation and Frequency at Mossy Fibre–Granule Cell Synapses of Rat Cerebellum: Experimental Observations and Theoretical Predictions, *J Neurophysiol, 95*, 2006, 686–699.

[10] R. Krahe & F. Gabbiani, Burst firing in sensory systems, *Nat Rev Neurosci, 5*, 2004, 13–23.

[11] Anna D'Errico, Francesca Prestori & Egidio D'Angelo, Differential induction of bidirectional long-term changes in neurotransmitter release by frequency-coded patterns at the cerebellar input, *J Physiol*, 2009.

[12] V. Braitenberg, Is the cerebellar cortex a biological clock in the millisecond range?, *Prog Brain Res, 25*, 1967, 334–346

[13] JF. Medina & MD. Mauk, Computer simulation of cerebellar information processing, *Nat Neurosci 3*, 2000, 1205–1211.

[14] J. S. Albus, A theory of cerebellar function, *Math Biosc, 10*, 1971, 25–61.

[15] S.J. Mitchell & R. A. Silver, Shunting inhibition modulates neuronal gain during synaptic excitation, *Neuron, 38*(3), 2003, 433–445.

[16] N. Schweighofer, K. Doya & F. Lay, Unsupervised learning of granule cell sparse codes enhances cerebellar adaptive control, *Neuroscience, 103*, 2001, 35–50.

[17] C. Hansel, D. J. Linden & E. D'Angelo, Beyond parallel fibre LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum, *Nat Neurosci., 4*, 2001, 467–475.

[18] Philipona & Olivier J.M.D. Coenen, Model of granular layer encoding in the cerebellum, *Neurocomputing, 58–60*, 2004, 575–580.

[19] Elisabetta Sola, Francesca Prestori, Paola Rossi, Vanni Taglietti & Egidio D'Angelo, Increased neurotransmitter release during long-term potentiation at mossy fibre–granule cell synapses in rat cerebellum, *J Physiol 557.3*, 2004.

[20] Sergio Solinas, Lia Forti, Elisabetta Cesana, Jonathan Mapelli, Erik De Schutter & Egidio D'Angelo, Fast-reset of pacemaking and theta-frequency resonance patterns in cerebellar Golgi cells: Simulations of their impact in vivo, *Frontiers in Cellular Neuroscience,1*, December 2007.

[21] Shyam Diwakar , Jacopo Magistretti, Mitchell Goldfarb, Giovanni Naldi & Egidio D'Angelo, Axonal Na Channels Ensure Fast Spike Activation and Back-Propagation in Cerebellar Granule Cells , *J Neurophysiol, 101*, 2009., 519-532.

[22] ML. Hines & NT. Carnevale (1997), The neuron simulation environment, *Neural Comput, 9*, 1179–1209.

[23] E. D'Angelo, T. Nieus, A. Maffei, S. Armano, P. Rossi, V. Taglietti, A. Fontana & G. Naldi, Theta-frequency bursting and resonance in cerebellar granule cells: experimental evidence and modeling of a slow K_-dependent mechanism, *J Neurosci, 21*, 2001, 759–770.

[24] S. Diwakar et.al. Unpublished.

[25] S. Armano, P. Rossi, V. Taglietti & E. D'Angelo, Long-Term, Potentiation of Intrinsic Excitability at the Mossy Fibre– Granule Cell Synapse of Rat Cerebellum, *The Journal of Neuroscience, 20*(14), 2000, 5208–5216.

[26] E. D'Angelo & CI. De Zeeuw, Timing and plasticity in the cerebellum: focus on the granular layer, *Trends in Neurosciences, 32*, 2009.

[27] M.Merkel & B.Lindner, Synaptic filtering of rate-coded information, *Phys Rev E Stat Nonlin Soft Matter Phys*,2010.

[28] H.Lu, M.J. Hartmann & J.M.Bower,Correlation between purkinje cell single-unit activity and simultaneously recorded field potentials in the immediately underlying granule cell layer, *J Neurophysiol, 94*,2005,1849-1860.