

# Cerebellar Cortex: Computation by Extrasynaptic Inhibition?

Dispatch

Erik De Schutter

**In the cerebellar cortex, inhibitory inputs to granule cells exhibit prominent tonic and spillover components resulting from the activation of extrasynaptic receptors. A recent study shows how extrasynaptic inhibition affects information flow through cerebellar cortex.**

The cerebellar cortex contains more granule cells than there are neurons in the rest of the brain, but comparatively little is known about their function compared to the more striking Purkinje cells. Cerebellar granule cells are, however, a popular preparation for pharmacological studies of receptors and channels. A long series of studies of granule cell receptors for the inhibitory neurotransmitter  $\gamma$ -amino butyric acid (GABA) has now led to some fundamental insights into how a characteristic anatomical specialization of the cerebellar cortex, the glomerulus, may work [1]. Glomeruli are formed around the large axonal terminals of glutamatergic mossy fiber afferents (Figure 1). Each terminal is contacted by dendrites from 50–60 distinct granule cells. The glomeruli also contain GABAergic synapses that inhibitory Golgi cells make with the granule cells, and glutamatergic contacts between the mossy fibers and Golgi cells. The structure has a radius of about 2.5  $\mu\text{m}$  and is enwrapped by glial sheathing. Granule cells have one to eight dendrites, each participating in a different glomerulus.

The first indication that GABAergic inhibition of granule cells has unusual properties came from the discovery that it has a strong tonic component [2,3]. This tonic GABA current is much larger than that evoked by spontaneous events and can be completely blocked by the GABA<sub>A</sub> receptor antagonist bicuculline; its fraction of the total GABA current increases from 5% in young to 99% in mature rats [3]. An attractive hypothesis is that this phenomenon is caused by spillover of GABA molecules between neighboring Golgi-to-granule cell synapses of the same glomerulus. The initiating event would still be action potential-evoked GABA release, but because the release happens at relatively distant synapses, diffusion and the summation of multiple events will result in delays which will act to filter out the synaptic transients. If the tonic current is indeed caused by GABA spillover, then one would expect it to be blocked by tetrodotoxin or low external calcium. These manipulations partially block the tonic current in young animals and not at all in adult ones [1–3], so it is assumed that the ambient

GABA concentration in glomeruli can activate some GABA<sub>A</sub> receptors.

Several lines of evidence support the view that spillover transmission also makes a contribution. First, two different kinds of synaptic current can be recorded in a granule cell following Golgi cell stimulation: fast responses with time courses similar to spontaneous events, and much slower responses with a slow decay [4]. Second, granule cells in the cerebellum and cochlear nucleus are the only cells that express the  $\alpha_6$  subunit of the GABA<sub>A</sub> receptor [5]. Receptors containing the  $\alpha_6\delta$  subunit combination have a 50-fold higher affinity for GABA than other GABA<sub>A</sub> receptors, and they do not desensitize upon prolonged presence of agonist [6]. The  $\delta$  subunit is found exclusively in extrasynaptic locations on the dendrites and somata of granule cells [7]. The tonic and evoked slow currents recorded from granule cells both have the pharmacological profile of a receptor containing  $\alpha_6$  and  $\delta$  subunits, being furosemide-sensitive but diazepam- and neurosteroid-insensitive [1, 4]. Taken together, these observations strongly suggest that the slow evoked currents reflect the activation of extrasynaptic  $\alpha_6\beta_{2/3}\delta$  receptors by GABA molecules that have ‘spilled over’ from activated synapses on other granule cells in the same glomerulus. The diffusion boundaries caused by the glomerular sheath may further promote extrasynaptic interaction between granule cells.

It was always assumed that the extrasynaptic activation of GABA<sub>A</sub> receptors might have an important role in cerebellar processing, but until recently there has been little experimental evidence for such a notion. In mature animals, where most of the spontaneous GABA<sub>A</sub> current is tonic, blocking all GABA<sub>A</sub> receptors with bicuculline leads to increased responses of granule cells to current injection [3]. Much progress has now been made by using furosemide to specifically block GABA<sub>A</sub> receptors that have an  $\alpha_6$  subunit in cerebellar slices from adult animals [1]. Using this approach, it is estimated that 97% of the charge evoked during Golgi cell stimulation flows through extrasynaptic GABA<sub>A</sub> receptors! This fraction includes the tonic current, which accounts for 75% of the charge transfer. These measurements were, however, made at 29° and tonic inhibition may make a much smaller contribution at body temperature [8]. Specifically blocking extrasynaptic GABA<sub>A</sub> receptors with furosemide causes a leftward shift of the firing curve of granule cells, as expected for the removal of a shunting inhibition [9], but it has no effect on the excitability of other neurons in the cerebellar cortex.

But how will the altered excitability of granule cells that results from extrasynaptic inhibition affect information transfer in the cerebellar cortex? This is not easy to predict as additional properties of the circuitry

need to be taken into account. An increase in granule cell activity resulting from a blockade of extrasynaptic inhibition would also enhance Golgi cell activity through excitatory contacts made by parallel fibers [10], which may lead to increased synaptic GABA release. Besides its inhibitory effect on granule cells, this may also activate GABA<sub>B</sub> receptors on mossy fibers. It has been shown that such receptors are activated by GABA spillover and reduce evoked mossy fiber responses in granule cells at low stimulation frequencies [8]. Multiple effects on Purkinje cells are possible, as they are both directly activated by increased parallel fiber activity and inhibited by stellate/basket cells which also receive parallel fiber input.

In their recent study, Hamann *et al.* [1] measured the effect of blocking extrasynaptic GABA<sub>A</sub> receptors with furosemide on the input and output elements of the pathway. They found that the resulting enhanced excitability of the granule cells increases the number of spikes they fire in response to mossy fiber stimulation by about 100%. Purkinje cells also increase their firing frequency in response to mossy fiber stimulation by about 100%. These increases reflect an increase in the size of evoked excitatory postsynaptic potentials (EPSPs), corresponding to a larger number of co-activated parallel fiber synapses. In conclusion, blocking extrasynaptic inhibition increases the flow of neural activity through cerebellar cortex — and conversely, the tonic inhibition normally present reduces this flow.

What may be the functional impact of the reduction in activity transmission caused by extrasynaptic inhibition? Hamann *et al.* [1] refer to David Marr's [11] seminal work on cerebellar motor learning to suggest that decreasing the number of granule cells activated by mossy fiber input increases the storage capacity of the cerebellum. This is actually a simplification of what Marr really wrote — that Golgi cell inhibition should keep “the numbers of active parallel fibres ... reasonably small over quite large variation in the number of active mossy fibres” [11]. In other words, there should be a dynamic component to the inhibition of granule cell activity, stronger when many mossy fibers are active and weaker when few are firing. This was called ‘automatic gain control’ by Albus [12]. The anatomy seems to favor such a role for Golgi cells, as the combined direct mossy fiber and indirect parallel excitation makes them sensitive both to input to the granular layer and the resulting activity of granule cells.

But at the physiological level, it is more difficult to reconcile the properties of Golgi cells with a gain control function [13]. Recordings *in vivo* show that Golgi cells typically fire a few accurately timed spikes in response to natural stimulation [10], followed by a long pause due to afterhyperpolarization [14]. Moreover, spike-evoked inhibition by Golgi cells *in vivo* is strong enough to cause the theoretically predicted [15] synchronization of Golgi cell activity along the parallel fiber beam [16,17]. The Golgi cell firing pattern may not be suitable for gain control, as it would promote rebound firing by granule cells during the

Golgi cell afterhyperpolarization [13], unless one can assume mechanisms which prolong the effect of each Golgi cell spike. While the synaptic GABA<sub>A</sub> channels already have relatively slow kinetics, the much slower spillover mechanism seems well suited to having a function in gain control. As a result of spillover, increased Golgi cell activity — through either direct mossy fiber activation or indirect parallel fiber activation — will reduce excitability of all granule cells participating in a glomerulus. This would provide for rather a slow gain control mechanism, which may not prevent fast swings in granule cell activity [13].

But how does the stronger tetrodotoxin-resistant tonic inhibition fit into this picture? It could provide a constant baseline, raising the threshold for spike initiation in granule cells. But if this is desired, it seems more straightforward to reduce the intrinsic excitability of granule cells, as observed in transgenic mice that lack the GABA<sub>A</sub> receptor  $\alpha_6$  and  $\delta$  subunits and so have no tonic current [18]. A more attractive idea is that tonic inhibition itself is regulated somehow. This could occur at the receptor side, for example by phosphorylation depending on  $\beta$  subunit expression [19], or by changing the baseline GABA concentration in the glomerulus. Reduced tonic inhibition may explain the evidence for spike-mediated inhibition *in vivo* [13,17], which is difficult to reconcile with the *in vitro* observation that only 3% of the GABA<sub>A</sub> current is spike mediated [1]. If tonic inhibition is regulated, it could have additional effects beyond controlling information flow through the cerebellum. Long-term potentiation (LTP) of mossy fiber-to-granule cell synapses can only be reliably evoked *in vitro* when inhibition is blocked [20]. Any mechanism that reduces tonic inhibition will enhance LTP of this synapse. If it turns out that extrasynaptic inhibition can be regulated separately in each individual glomerulus, there could be very interesting implications for the possible computational functions of the glomerulus.

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Figure 1. Synaptic elements in a glomerulus of the cerebellar cortex.

Excitatory synapses are represented by triangles and inhibitory ones by circles.