CHAPTER 3.12

Inferior olive hypertrophy and cerebellar learning are both needed to explain ocular oscillations in oculopalatal tremor

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Abstract: A new model of cerebellar learning explains how the cerebellum can generate arbitrary output waveforms to adjust output timing in the classical delay conditioning. This model can also reproduce the low frequency ocular oscillations seen in oculopalatal tremor (OPT). A novel circuit in the cerebellum uses both interneurons (INs) and Purkinje cells (PC) to control timing. Brain lesions that cause OPT give rise to hypertrophy of the inferior olive (IO) and an increase in conductance through gap junctions among IO neurons. When our model is changed in this way, the heavily coupled IO becomes an oscillator and generates synchronous spike trains at 1–2 Hz. These synchronized spikes do not produce the large amplitude, aperiodic waveforms of OPT. However, the synchronized IO signal goes to the cerebellar cortex (flocculus) directly, on climbing fibres, and indirectly, on mossy fibres from the vestibular nuclei. This creates a pathological association between the IO pulse trains on mossy and climbing fibres in PC. Variable pendular ocular oscillations emerged from the cerebellum model after learning this association. Since electrotonic coupling of IO cells depends on connexin proteins, drugs that block gap junctions, such as anti-malarial agents, might provide a novel therapy for OPT.

Keywords: Purkinje cell; interneuron; interaction; waveform; mGluR; classical conditioning; gap junction

Introduction

The role of the cerebellum in generating timed movements has been demonstrated in animal studies of *classical delay eyeblink conditioning* (e.g., Thompson, 1986). In this paradigm (see Fig. 1A), the animal receives a conditioned stimulus (CS), such as a tone. After a certain delay (inter-stimulus interval, ISI), an unconditioned stimulus (US), such as an air puff directed at the cornea, causes a reflexive blink (unconditioned response, UR). After training, the CS causes the eyelid to blink (conditioned response, CR) at the time when the subsequent US is expected. In this study, we propose a new model of cerebellar timing. The model emphasizes the interaction between the Purkinje cell (PC) and its connected interneurons (INs) in generating modulated waveforms of PC activation (Fig. 1B; Hong and Optican, 2005). It is hypothesized that the delayed

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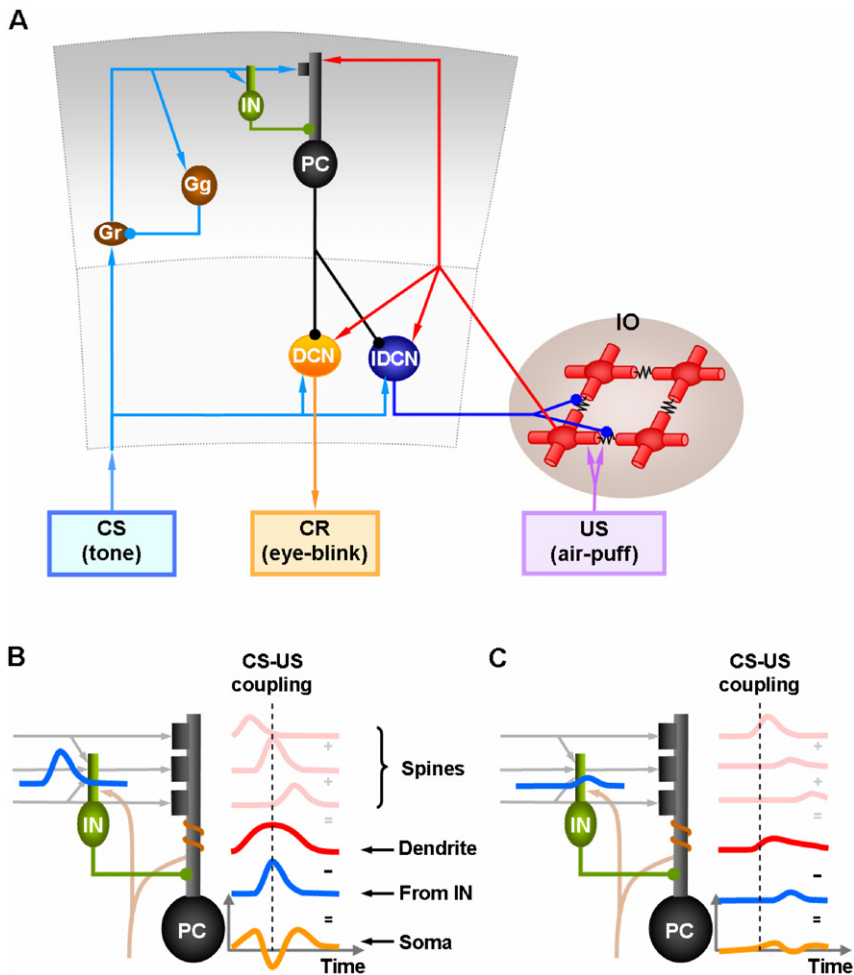Fax: 301-402-0511; E-mail: LanceOptican@nih.gov

Fig. 1. Schematic of model. (A) Cerebellar modules and IO. See the text for detailed explanations. The zigzags (black) between IO neurons (red) represent gap junctions. Excitatory and inhibitory pathways are represented by triangular heads and round heads, respectively. (B) and (C) PC–IN pairs learn the timing of CS–US coactivation. When the CFs activate after the PF signal with a certain delay, the PC–IN pairs with a matching internal delay timing, such as the one in (B), increase their mGluR-mediated excitability. The "CS–US coupling" with a vertical broken line in (B) and (C) shows the timing. After learning, the increased excitability leads to a big modulation of activity in the PCs of those pairs (the orange wave). This synchronized modulation of a population of PCs leads to the generation of timed CR. For the PC–IN pairs with different internal delay timings, such as the long delay timing in (C), the CS–US coactivation does not change their excitability. Theses pairs remain relatively inactive therefore do not contribute to CR. (See Color Plate 3.12.1 in color plate section.)

coupling of the CS and US delivered via PF and CF, respectively, induces a simultaneous increment of dendritic excitability in INs as well as in the PC, thus leading to an increased modulation of PC activity. This waveform modulation leads to a timed pause of the PC population and makes the deep cerebellar nucleus (DCN) neurons generate the CR signals. We hypothesize that the delayed PC–IN interaction is due to the intracellular activation of the mGluR. This is based on the observations that (1) the decrement of mGluR-mediated excitability in PCs may affect the

long-time scale component of motor execution (Coesmans et al., 2003), (2) the group I mGluRs in the INs have similar signalling properties to those in PC (Karakossian and Otis, 2004). More details are provided in Discussion.

Clinical observations also implicate cerebellar circuitry in a variety of motor activities, including eye movements. One clear example is found in patients with oculopalatal tremor (OPT) where the disruption of the deep cerebellar inhibitory projection to the inferior olive (IO) causes a gradual development of involuntary irregular eye movements. The main pathologic finding with OPT is hypertrophy of the inferior olivary nucleus, which may be seen on MRI (Goyal et al., 2000). The olivary nucleus is enlarged, due to hypertrophy of neurons that contain increased acetyl-cholinesterase reaction product. Such changes begin within a month after the lesion and maximize in about six months, being accompanied by astrocytosis and synaptic and axonal remodelling.

Animal studies have clarified several aspects of how the clinical syndrome develops. An important projection runs from the deep cerebellar nuclei through the superior cerebellar peduncle, decussates, turns caudally at the red nucleus to form the central tegmental tract, and makes inhibitory synapses on inferior olivary neurons (Bengtsson et al., 2004). Disruption of this inhibitory pathway leads to increased activity of inferior olivary neurons. Furthermore, chemical lesions of the deep cerebellar nuclei lead to hypertrophy of inferior olivary neurons with changes in their connectivity (Ruigrok et al., 1990).

In this study, we use progressive development of soma-somatic gap junctions between adjacent inferior olivary neurons and learning in the cerebellar cortex to simulate the involuntary ocular eye movements of OPT.

Methods

This computational study constructs a model of the cerebellar circuit utilizing leaky integrator-type equations for all of the cell types except the IO neurons. IO neurons use more elaborate spiking equations to simulate realistic CF activity including the low frequency ($\sim 2\,Hz$) baseline noisy spikes. Figure 1A illustrates our simplified circuit of the cerebellum. There are four major divisions in the circuit: Two inputs (MF and CF), cerebellar cortex, and DCN. MFs provide inputs that represent certain events (CS in Fig. 1), such as a tone signal in the classical eyeblink conditioning paradigm. This input is transmitted to the cerebellar cortex via granule cells and also to the DCN by MF collaterals. CFs, which constitute another major input system, originate in the IO and transmit US signals. The activity of DCN represents the output of the cerebellum and also gates the IO-mediated learning in the cerebellar cortex with its negative feedback via inhibitory DCN→IO projections.

Normal model

In our model, the timing mechanism is localized to the PC–IN pair. Figure 1B summarizes the key concepts of signal processing in a PC–IN pair. First, it is assumed that repeated CF–PF coupling trains the PC spines and the IN dendrite to increase their excitability. After this training, the increased excitability makes the spines of the PC and the dendrite of the IN increase their potentials upon PF input due to the mGluR-induced long latency calcium activation (the blips in Fig. 1B). The terms *increment* or *decrement of excitability* will be used here for long-time scale plastic changes to differentiate these from the well known LTP and LTD phenomena, which historically refers to the changes in the fast AMPAR pathways. It is assumed that whereas the IN shows a narrow activation profile (blue trace in Fig. 1B), the PC dendrite generates a relatively broad activation profile (red trace) because of the variability in latencies of the slow-acting intracellular calcium components among the dendrite's many spines (three pink traces). At the PC dendrite level, the excitatory potential coming from the PC spines (red trace) and the inhibitory potential coming from the IN (blue trace) interact. This interaction generates a waveform, or temporally modulated pattern, of potential in the PC soma (orange trace in Fig. 1B). We assume that PC–IN

pairs have a wide range of mGluR-induced activation latencies that span the possible range of delay timing (~ 4 s), similar to the range of PC latencies in the population-based spectral timing model (Fiala et al., 1996). The modulation of the waveforms of PC activations via the PF–CF coactivation happens only in those PC–IN pairs whose latencies match the timing of the coactivation. For example, a PC–IN pair whose internal timing longer than the CS–US coupling timing, as illustrated in Fig. 1C, will not increase the excitabilities, and the PC's activation will not be modulated. This way only the PC–IN pairs having the right internal timing will be recruited by the CS–US coupling leading to a timed decrease of discharge in the PC population. This decrement of inhibition lets the DCN generate the CR.

Model with hypertrophic inferior olive

To further validate this cerebellar model, the circuitry has been adapted to explain OPT which results from an injury that disrupts the DCN→IO inhibitory pathway. Our first hypothesis of the mechanism of these ocular oscillations is as follows: (1) The brain stem or cerebellar injury damages the inhibitory pathway from the DCN to the IO; (2) this causes IO neurons to gradually form soma-somatic gap junctions with their neighbours as part of a progressive hypertrophy (e.g., Ruigrok et al., 1990); (3) the soma-somatic gap junctions increase the communication among the connected IO neurons and as a consequence they start to fire synchronously; (4) the synchronized signals reach the eye movement-related DCN or the vestibular nuclei (VN).

After the implementation of this first hypothesis, which by itself failed to explain the OPT phenomena (see Results), a further modification was made to the model to examine our second hypothesis: The cerebellar cortex acts upon the sequences of pulses coming from the IO and modulates the eye movements. Inputs to PC consist of climbing fibre signals (carrying the synchronized olivary discharge) and parallel fibre signals (via IO→VN→ granule cells). Thus, the inferior olivary signal, which projects to both the VN and the cerebellar

cortex, could in turn also affect the discharge of PC via a mossy fibre projection from the VN (Zhang et al., 1993). (cf. Fig. 1 in the following article by Liao et al. for the circuit carrying these two signals.) This possibility led us to apply the hypothesis for motor learning in eyeblink conditioning explained above. In the case of OPT, the repeating IO pulses create periodic CF and PF inputs to PC–IN pairs at approximately the same time. The periodic conjunction of the PF–CF signals can train the PC–IN pairs, which after learning can pause after the PF input at the time of the expected CF input. This makes the PCs oscillate with the ongoing IO pulses, which in turn modulate the activity of the VN. The eye movement circuit, which receives its input from the VN, was simulated using a first-order ocular motor plant.

Results

Simulation of classical delay conditioning

Figure 2 shows the simulations of the classical delay eyeblink model with ISIs of 250 ms, 500 ms, and 750 ms. The troughs of the PC population and the peaks of the DCN neuron occur near the arrival time of the US (dashed lines). Individual model PCs often showed a variety of responses (data not shown), often with multiple peaks similar to the patterns in vivo. However, their population response shows a smooth trough around the arrival time of the US (Fig. 2, top).

Simulation of hypertrophic inferior olive in OPT

The lesion of the DCN→IO pathway induces the hyperactivation and ensuing over expression of gap junctions among IO neurons. The model simulates this process by initially selecting a few IO neurons at random as seed points to spread the soma-somatic gap junctions to their nearest neighbours. Figure 3 shows the effects of progression of the hypertrophy in the IO (downward arrows). The large dots indicate the hypertrophic neurons that have soma-somatic gap junctions with their neighbours. Note that as the simulated
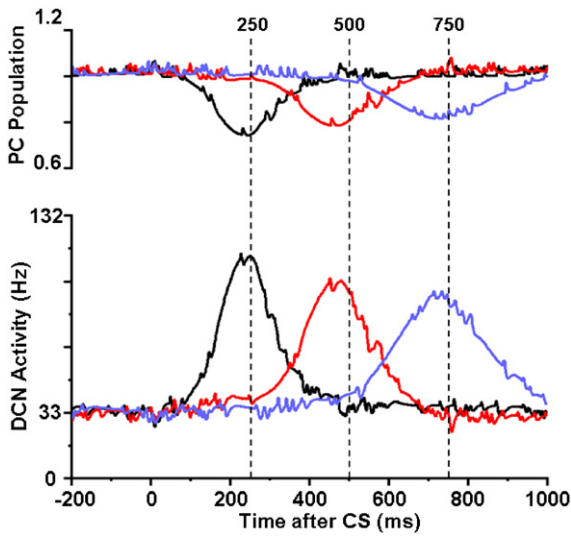
Fig. 2. Simulation of delay eyeblink conditioning. Upper panel: Input given to the simulated DCN neuron from the PC population with different ISIs (black curve: 250 ms, red: 500 ms, blue: 750 ms). Lower panel: DCN activities at different ISIs. PC population reduces the inhibitory output near the arrival of the air-puff US signals (dashed lines). The model DCN peaks near the arrival of the US with appropriate delays because of the reduction of PC inhibition. (See Color Plate 3.12.2 in color plate section.)

hypertrophy progresses, the spiking activity among neighbouring IO neurons becomes more synchronized. This finding can also be appreciated in the upper trace of each panel, showing percent synchrony (Synch), which becomes almost 100% in the bottom panel. As a consequence, the spike histogram in the bottom panel (Hist) now shows narrow periodic peaks.

Simulation of OPT without cerebellar learning

Figure 4A shows the result of the simulation when the model does not include learning in the cerebellar cortex. The simulation produced periodic eye movements, but they were small, regular, and jerky, in contrast to ocular oscillations in OPT patients (Fig. 4C). Thus, although our model of the hypertrophied IO accounted for the slow development of OPT and produced oscillations at $1.5 \sim 2$ Hz, it could not account for the actual waveform of the ocular oscillations of OPT.
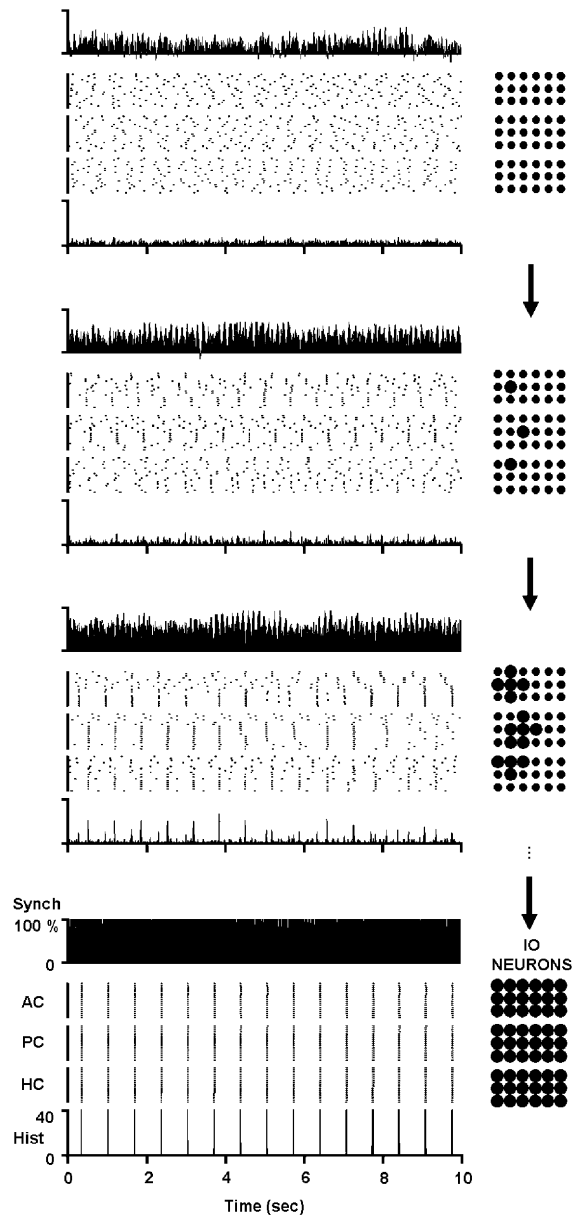

Fig. 3. Results of model IO activity before (top), during (middle two), and after (below) the development of soma-somatic gap junctions. The model IO has three groups of neurons (each group is a 6×3 array of dots on the right side of the figure) corresponding to anterior (AC), posterior (PC), and horizontal canals (HC). To simulate the hypertrophy in IO neurons, soma-somatic gap junctions were slowly introduced into the system (from top to bottom). The big dots represent hypertrophic IO neurons. Notice the increasing synchrony (Synch; top trace), the temporal alignment in the spike rasters (middle traces), and the peaks in the spike histogram (Hist; bottom trace).
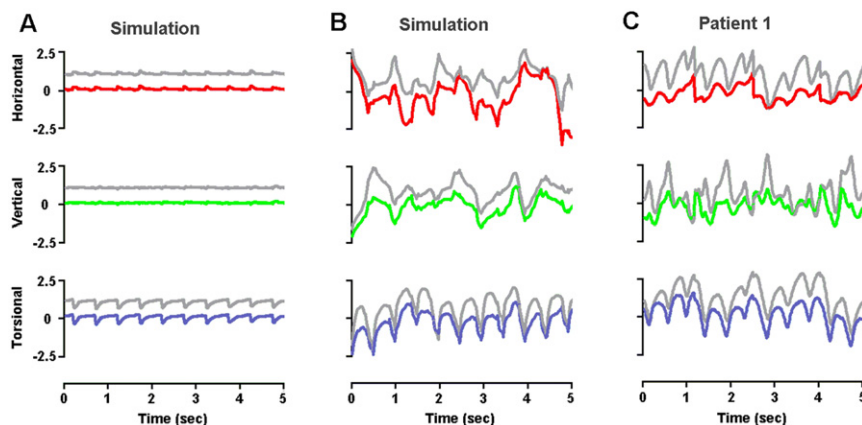
Fig. 4. Three-dimensional eye movement simulations of the OPT without (A) and with (B) the cerebellar cortical learning. (A) The model's simulation with IO hypertrophy but without cerebellar cortical learning shows small, jerky periodic eye movements. (B) When the simulation includes both the IO hypertrophy and the cerebellar cortical learning, the model makes larger, smoother, and more irregular movements, which are qualitatively similar to eye movements recorded in a patient with OPT (C). Gray traces represent right eye, coloured traces represent left eye. (See Color Plate 3.12.4 in color plate section.)

Simulation of OPT with cerebellar learning

When the model includes the cortical circuit that learns the temporal CS–US coactivation as seen in the classical eyeblink condition above, the resulting ocular oscillations (Fig. 4B) are now qualitatively similar to those observed in affected human subjects. Specifically, they are larger, smoother, and more variable than the movements in Fig. 4A. Each of these effects can be attributed to a specific mechanism in the cerebellum. First, the larger amplitude can be attributed to the pause of the PC after the arrival of a mossy fibre pulse, but at the expected arrival time of the next climbing fibre pulse; this, in turn, disinhibits the VN, which fire more vigorously. Second, the waveform is smoother because the distributed PC pauses at around the time of the expected climbing fibre signal. Since the timing of the climbing fibre signal after the parallel fibre signal is variable, the PC population learns the probability distribution of the delay. The variability of activity over the population of PC due to this learning has the effect of smoothing the output of the deep cerebellar nuclei. Third, the irregularity of the waveform can be attributed to mixing of two signals that arrive on PC via mossy fibres: the self-fed mossy

fibre signal (via PC to VN to parallel fibres), and the IO signal, also projecting to the VN and then onto the cerebellar cortex. It should be emphasized that the coupling of large CF–PF signals from the IO is a pathological situation created by the abnormal soma-soma electrotonic gap junctions that synchronize many IO neurons. In the normal state the IO is not synchronized and does not generate large, periodic pulses (Fig. 3, top panel).

Discussion

The model described in this article simulates cerebellar timings in classical delay eyeblink conditioning and in the abnormal oscillatory involuntary eye movements in OPT. Unlike most models that assume PCs are the sole player in cerebellar timing, the current model hypothesizes that (1) the interplay between IN and PC generates the PC pause and (2) that the slow-activating mGluR-mediated $[Ca^{2+}]_i$ change in PC (Finch and Augustine, 1998) and in IN (Karakossian and Otis, 2004) determine the timing of the pause. The main reason for us to propose this PC–IN interaction model is the findings by Schreurs et al.

(1997, 1998) pointing out an increased excitability of PC dendrites (in Larsell's lobule HVI) after natural acquisition of classical delay eyeblink conditioning, in contrast to the decreased excitability seen in some in vitro studies. This creates a paradox: if learning increases PC excitability, how can they pause at the right time? Our model suggests a solution to this paradox by proposing a learning mechanism that generates a simultaneous increment of activity in both PC and IN. This concept is a temporal version of the shaping of spatial waveforms in the visual system. For example, in V1 excitatory and inhibitory neurons both increase their activation in response to a relevant input, which shapes the spatial waveforms that define the (centre-surround) receptive fields of visual neurons. We speculate that the modulation of both PCs and INs endow the cerebellar cortex with the versatility to learn almost any temporal output pattern. This point has been demonstrated in the simulation of OPT.

The consistent correlation between the hypertrophic IO and OPT (e.g., Goyal et al., 2000) led us to hypothesize that OPT was caused by the physiological changes in hypertrophic IO neurons. Hypertrophy causes the affected neurons to make extensive electrotonic connections with their neighbours via abnormal soma-somatic gap junctions. This causes a widespread, or even global, synchronization among the IO neurons that induces a behavioural tremor.

We hypothesize that the vestibular circuit is involved in the generation of the OPT. One piece of evidence comes from a recent experiment by Yoshida et al. (2004), who showed that malfunction of the flocculus and connected circuit can generate oscillatory eye movements (albeit of a higher frequency than OPT). Also IO to VN connections are known to exist (e.g., Balaban, 1988). One interesting fact of the vestibular circuit is that the vestibular inputs from head rotation may inhibit the vestibular part of the IO, the dorsal cap of Kooy and the ventrolateral outgrowth, which is known to control eye movements (e.g., Balaban and Beryozkin, 1994). This leads us to suggest that large head rotation could reset the phase of the eye oscillations in the OPT, via the vestibular inhibitory pathway (VN→IO). The simulation result of this prediction is shown in Fig. 2 of the following article (Liao et al., 2008). The clinical analysis of our study in the following article (Liao et al., 2008) shows that an impulsive head rotation indeed resets the phase of ocular oscillations in OPT patients.

A major insight gained from this study was the pivotal role played by the development of soma-somatic gap junctions on neurons in the inferior olivary nucleus (e.g., Ruigrok et al., 1990). Gap junctions are common in the adult nervous system, and there is debate about their role in dendritic coupling of the normal IO. If development of gap junctions between cell bodies is indeed the key event in turning the IO into a synchronized pulse-generator that drives OPT, then drugs that block gap junctions (e.g., anti-malarial drugs) might have some therapeutic effect. For example, Martin and Handforth (2006) found that the gap junction blocker mefloquine (Cruikshank et al., 2004) suppressed harmaline-induced tremor in rats. However, they did not see any reduction in tremor in their human subjects who took a very low dose of mefloquine. In higher doses, mefloquine has serious side effects, but safer drugs, such as quinine, are also known to block connexin-36 and may be able to reduce tremor. Rash et al. (2004) reported that the connexin-36 labelled gap junctions were localized in proximity with NMDA receptor clusters in IO neurons. This raises a possibility that NMDA receptors may amplify the communication between IO neurons (Du et al., 1997). Thus, a targeted suppression of NMDA receptors may also reduce the synchronization of IO neurons, thereby alleviating the symptoms. This suggests studying the effect of a combination of drugs on tremor, one to block the gap junctions (e.g., anti-malarial drug) and another to block the NMDA receptors (e.g., memantine). This dual action may lessen the synchrony among IO neurons by (1) reducing the initial gap junction currents and (2) disabling the amplification of the currents afterwards. It is possible that once the pulse-generator (hypertrophic IO) is silenced, the learned gratuitous timed pause of the PCs in the cerebellar cortex would undergo extinction, further alleviating the symptoms.

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