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A new familial disease of saccadic oscillations and limb tremor provides clues to mechanisms of common tremor disorders

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Tremor disorders pose fundamental questions about disease mechanisms, and challenges to successful neurotherapeutics: What causes motor circuits to oscillate in disorders in which the central nervous system otherwise seems normal? How does inheritance 'determine' the clinical phenotype in familial tremor disorders? Here, we address these questions. Analogies between the neural circuits controlling rapid eye movements (saccades) and those controlling limb movements allow us to translate the interpretations from the saccadic systems to the limb movement system. Moreover, the relatively well understood neurophysiology of the ocular motor system offers a unique opportunity to test specific hypotheses about normal and abnormal motor control of both eye and limb movements. We describe a new familial disorder—'micro-saccadic oscillations and limb tremor (μSOLT)'—in a mother and daughter who had tiny saccadic oscillations of the eyes and tremor of the hands. This unique oscillatory movement disorder resembles other common tremor disorders (such as essential tremor) that occur in patients who have an otherwise normally functioning central nervous system. We hypothesize that μSOLT is caused by an inherited abnormality that results in abnormal membrane properties causing reduced external inhibition in the premotor neurons that generate the high-frequency discharge (burst) for saccades and for ballistic limb movements. To test this hypothesis, we recorded hand tremor and eye movements in two patients with μSOLT and particularly during natural circumstances when inhibition of the premotor saccadic burst neurons is removed (e.g. eye closure). We then simulated a conductance-based model for the premotor commands which included excitatory and reciprocally inhibitory burst neurons. The structure of this physiologically realistic model was based upon known cell types and anatomical connections in the brainstem (for saccades) and the thalamus (for limb movements). The physiological phenomenon of post-inhibitory rebound in premotor burst neurons makes the circuit inherently unstable and prone to oscillate unless prevented by external inhibition. Indeed, with simulated reduction of external inhibition (in this case glycinergic), saccadic oscillations and limb tremor were reproduced. Our results suggest that a single-inherited deficit can alter membrane properties, which impairs inhibition in an inherently unstable neural circuit causing the eye and limb oscillations in μSOLT. This concept has broad implications for understanding the mechanism and designing rationale pharmacotherapy for abnormal oscillations and may be applicable to other common disorders in which there are no structural abnormalities in the brain such as essential tremor.

Keywords: ion channels; saccade; tremor; computational simulation; oscillations

Abbreviations: EBN = excitatory burst neurons; IBN = inhibitory burst neurons; IO = inferior olive; μSOLT = micro-saccadic oscillations and limb tremor; OPN = omni-directional pause neurons; PIR = post-inhibitory rebound; TC = thalamo-cortical; TR = thalamic reticular; VA = ventral anterior; VL = ventral lateral

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Introduction

Many tremor disorders, e.g. essential tremor and tremor with cervical dystonia, occur in a structurally sound central nervous system without any consistent patho-anatomical correlates. A similar situation often exists for tremor-like,

saccadic oscillations of the eye (unwanted, back to back saccades that occur one upon the other, without an intersaccadic interval and disrupt clear vision) (Leigh and Zee, 2006). Saccadic oscillations may occur transiently in normal subjects, e.g. during eye closure, with convergence and sometimes they can be produced voluntarily (Ramat *et al.*, 2005; Miura and Optican, 2006). Abnormal saccadic oscillations also occur, often dramatically, in patients with neurological disorders when they are called flutter or opsoclonus. Such pathological saccadic oscillations have been attributed to autoimmune (post-infectious and paraneoplastic) or various toxic and metabolic processes (Leigh and Zee, 2006).

We discovered a novel disorder of saccadic oscillations and fine hand tremor in a mother and her biological daughter who have had these symptoms since childhood. We call this novel disorder 'familial micro-saccadic eye oscillations and limb tremor (μSOLT)'. Because the patients had no other 'neurological abnormalities', we assume their eye oscillations and tremor have a genetic basis and offer an analogy to other common familial tremor disorders such as essential tremor. Prior hypotheses to account for saccadic oscillations have had in common the idea that the instability arises from an abnormality in the feedback circuits that control the amplitude of saccades (Zee and Robinson, 1979; Wong *et al.*, 2001). But these models do not explain the wide range of frequencies of oscillations that have been reported both for normal subjects (with a presumably normal nervous system) and patients with abnormal oscillations with an otherwise normally functioning central nervous system. Recently we have suggested a different mechanism to account for the appearance of saccadic oscillations in normal subjects, which allows oscillations of different frequencies to emerge depending upon changes in the properties of burst neurons themselves (Ramat *et al.*, 2005). The key concept is that the instability arises because (i) brainstem inhibitory burst neurons (IBN) for agonist and antagonist muscles are reciprocally interconnected, providing an anatomical substrate for self-sustaining oscillations and (ii) burst neurons show post-inhibitory rebound (PIR), a spontaneous burst of activity after a neuron is released from sustained inhibition (Enderle and Engelken, 1995; Ramat *et al.*, 2005; Miura and Optican, 2006).

Here, we study μSOLT using a 'neuromimetic' model of saccade generation in which (i) the circuit consisted of premotor excitatory, excitatory burst neurons (EBN) and IBN and their known anatomical interconnections, (ii) membrane kinetics were determined by specific subsets of membrane ion channels and (iii) reduction in external inhibition, glycinergic in the case of the saccadic system, lead to the appearance of saccadic oscillations during fixation and excessive rebound excitation after sustained inhibition (increased PIR). We then used a similar approach, based on an analogous central circuit within the thalamus that generates ballistic limb movements, to simulate their limb tremor.

Finally, this study presents a unique approach to study common tremor disorders such as essential tremor. Oscillations in the motor system in disorders like μSOLT or essential tremor pose fundamental questions about the mechanisms of disease in the motor system: What causes motor circuits to oscillate in the disorders in which the central nervous system otherwise seems normal? How does inheritance 'determine' the clinical phenotype in familial tremor disorders? Answers to these questions will influence the practice of clinical neurology and neurotherapeutics. We address some of these questions in the present study. The relatively well-understood neurophysiology of the eye movement system offers a unique opportunity to test specific hypotheses about normal and abnormal motor control. Moreover, analogies between the neural circuits controlling ballistic eye movements (saccades) and those controlling limb movements allow us to translate the interpretations from the eye movement systems to the limb movement system. Thus, the discovery of novel disorders of eye and limb movements such as μSOLT presents an opportunity to understand more common movement disorders, such as essential tremor of the head and limb in which there are no gross structural abnormalities within the brain.

Methods

Eye movements and limb tremor recordings

Both patients and healthy subjects signed appropriate consent material before enrolling in the study. The study was approved by The Johns Hopkins Institutional Review Board. Eye movements were recorded with the magnetic field/search coil technique in the adult patient and in healthy subjects. An infrared monitor (Ober saccadometer) was used to quantify the eye oscillations in the daughter. Limb tremor was recorded using three axis accelerometer (Tremormometer, FlexAble Systems, Fountain Hills, AZ).

For the mother, the movements of both eyes were recorded around all three axes of rotation (horizontal, vertical and torsional). The output signals of the coils were hardware filtered with a single pole RC filter with bandwidth of 0–90 Hz, and then sampled at 1000 Hz with 12-bit resolution. Readers are referred to Bergamin *et al.* (2001) for more details of the eye movement calibration and recording procedures. Tremor recording methodology is reviewed in detail in Caliguiri and Tripp (2004).

Recording paradigms

Eye movements

Three-dimensional eye positions were recorded as healthy subjects and patients with μSOLT fixed their gaze on the target of interest, closed their eyes, or made 20° and 40° saccades in horizontal and vertical directions.

Hand tremor

Hand tremor were recorded as subjects outstretched their arms (postural tremor), rested hands comfortably on the table in front of them (resting tremor) and attempted to reach an object of interest (kinetic tremor).

Results

The neurological phenotype of familial micro-saccadic oscillations and limb tremor (μSOLT)

The only symptoms of our patients were occasional brief episodes of blurring of vision, and hand tremor, both noted since early in childhood. The visual symptoms and hand tremor were accentuated during periods of stress or anxiety. The only neurological findings on simple visual inspection were intermittent brief bursts of eye oscillations and the hand tremor. For example, the video clip (see Supplementary material) of the daughter shows a brief burst of eye oscillations during otherwise normal smooth pursuit of the examiner's finger. During ophthalmoscopy, however, both patients showed nearly continuous, small-amplitude, high-frequency oscillations of the eye. Neither patient showed abnormalities of saccades; they appeared to be generated promptly, and were accurate and fast. Quantitative recordings showed that the saccades made by the patients were of normal velocity and amplitude [for example, compare Fig. 1A (normal subject) and Fig. 1D (mother)]. During attempted steady fixation (Fig. 1E), however, there were nearly continuous, small-amplitude, high-frequency saccadic oscillations (~18 Hz) around all three axes of rotation (horizontal, vertical and torsional) [compare with the normal subject (Fig. 1B)]. The daughter showed similar behaviour during fixation and the frequency of the saccadic oscillations was the same. Pursuit eye movements, convergence and vestibulo-ocular responses were normal. Both the mother (Fig. 1F) and daughter also showed a small-amplitude, high-frequency (~12 Hz) tremor of their outstretched arms (postural limb tremor). A kinetic tremor was also present in both with a frequency of (~11 Hz). The postural and kinetic tremors had similar amplitudes (postural: 18 mm/s^2 and kinetic: 18 mm/s^2). There was no resting or head tremor.

Computational analysis of saccadic oscillations in μSOLT

We next develop a hypothesis to account for the saccadic oscillations of our patients. First, we discuss the key anatomical and physiological features of the neural circuits that underlie normal saccade generation, and then show how oscillations might emerge.

Normal generation of saccades

Sherringtonian reciprocal innervation—agonist excitation and antagonist inhibition—underlies the patterns of innervation to the ocular motoneurons for saccades. Three classes of neurons—excitatory burst neurons (EBN) within the pontine paramedian reticular formation, inhibitory burst neurons (IBN) within the rostral medullary reticular formation and omni-directional pause neurons (OPN) within the pontine raphe—comprise the premotor horizontal saccade circuit (Fig. 2). For example, when we want to make a rightward saccade, premotor neurons on the right side of the brainstem send excitation to motor neurons for rightward saccades and send inhibition to motor neurons for leftward saccades (Fig. 2A, green lines are excitatory, red lines are inhibitory neurons, grey lines are inactive neurons). When we are not making saccades, the EBNs and IBNs on both the left and right are held off by tonic glycinergic inhibition from the OPN. Each OPN pauses for saccades in all directions. Removal of OPN inhibition at the initiation of the saccade allows a high-frequency burst of neuronal activity in the premotor burst neurons to drive saccades (Ramat *et al.*, 2007). Part of the burst following the silencing of the OPN discharge reflects a 'rebound' increase in neural activity after transient inhibition and is thought to be due to PIR (Enderle and Engelken, 1995; Ramat *et al.*, 2005; Miura and Optican, 2006). Projections from the IBNs on the right side inhibit EBNs on the left, and *vice versa*, also through glycinergic transmission (Horn *et al.*, 1996). We proposed that these connections form a 'short-latency, mutually inhibitory' circuit between the left and right sides that is inherently unstable (Ramat *et al.*, 2005).

How saccadic oscillations might appear in normal subjects

How does mutual inhibition between neurons with PIR lead to network oscillations? Figure 2B schematizes a neural circuit formed by two mutually inhibitory neurons (neuron-A and -B). Suppose a small increase in neural activity, either from spontaneous fluctuation (noise) in neural activity or a small spontaneous saccade, causes a brief pulse of activation (dashed line) that starts neuron-A firing. When neuron-A discharges, it inhibits neuron-B. After the input to neuron-A ceases, its discharge drops, removing the inhibition from neuron-B. Neuron-B, in turn shows a PIR increase in its firing rate. After neuron-B inhibits neuron-A, the same PIR occurs in neuron-A. Thus, PIR in reciprocally inhibited neurons is enough to cause oscillations (Fig. 2C). These oscillations are normally prevented by tonic glycinergic inhibition from the OPNs. If the activity of OPN could be diminished physiologically in normal subjects they might show transient saccadic oscillations. In fact, OPN activity is diminished naturally when normal subjects close their eyes (Fig. 3A), combine vergence with a saccade (data not shown) or make pure vertical or pure horizontal saccades (Fig. 3B). In the last case, for example, a large horizontal saccade is accompanied by (vertical) saccadic oscillations in the orthogonal direction since different sets of burst neurons generate horizontal and vertical saccades but all OPN cease discharging for saccades in all directions. These experimental results suggest that instability in the saccadic burst generating system, introduced by the removal of OPN inhibition, is the cause of saccadic oscillations in normal subjects.

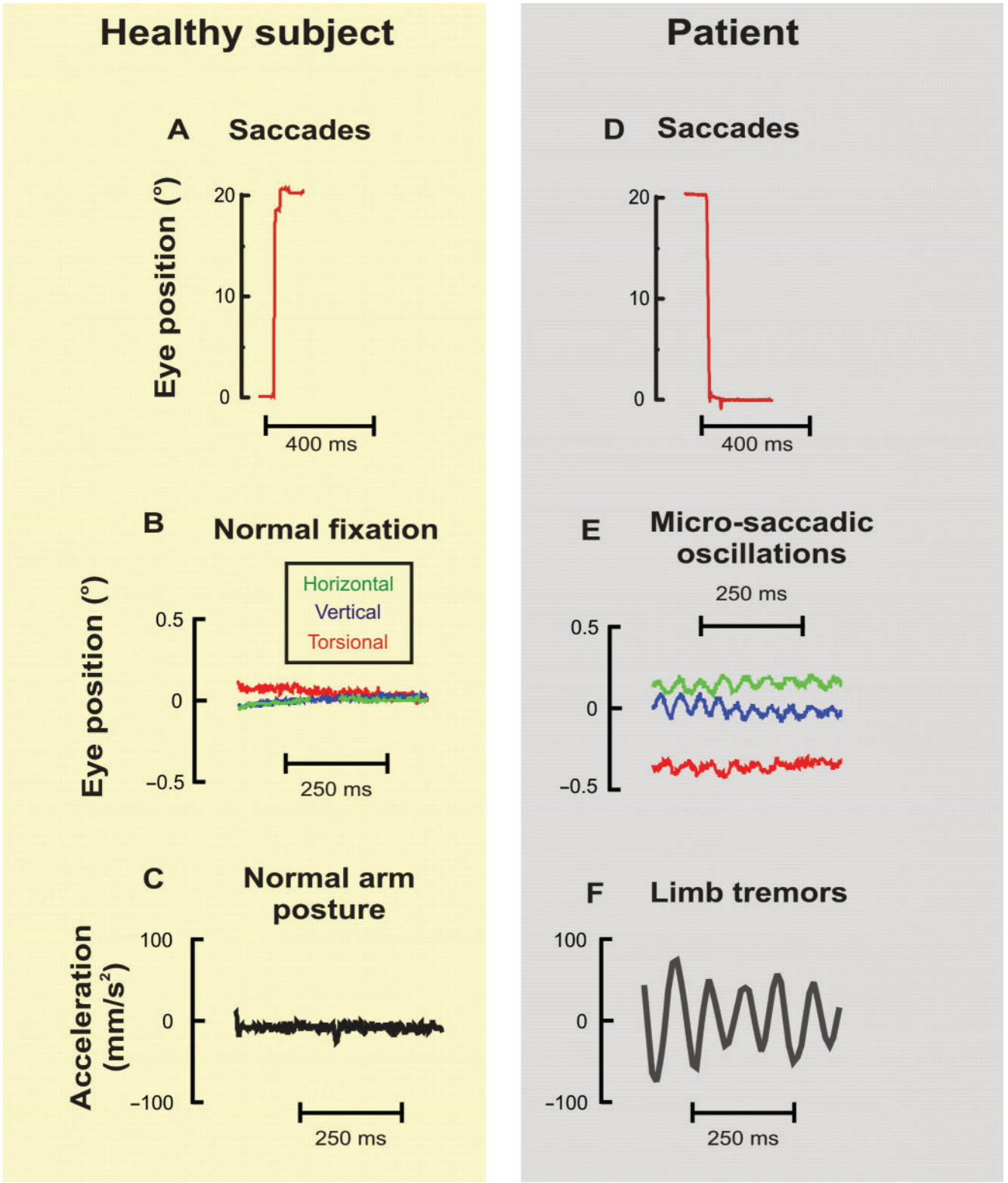

Fig. 1 Saccades in patients with familial μSOLT are normal in speed and accuracy [compare normal subject (**A**) and patient (**D**)]. Gaze holding in patients with μSOLT is also normal (**D**). During attempted steady fixation there are micro-saccadic oscillations in the patient [compare **E** and **B** (normal subject)]. Hand tremor is seen in patients with outstretched hands (postural limb tremor) (**F**), but not in the normal subject (**C**).

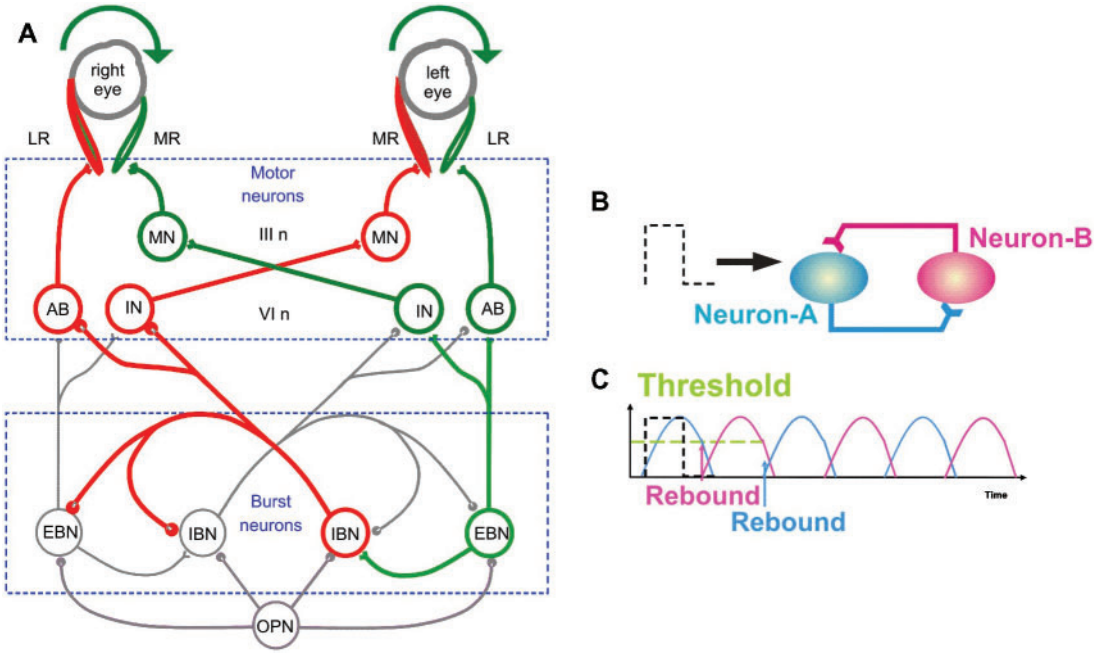

Fig. 2 (**A**) Brain stem circuit for controlling horizontal saccades. The premotor neurons are located in the pons and rostral medulla. The excitatory burst neurons (EBN) project to the ipsilateral abducens nucleus that contains interneurons (IN) and motor neurons (AB) that relay excitation to neurons innervating the agonist muscles (green) and also to the region of the ipsilateral IBN. Axons of the IBN cross the midline and project to the contralateral internuclear (IN) and abducens motoneurons (AB) innervating antagonist muscles (red), and to the region of the contralateral excitatory and inhibitory burst neurons (red). When a rightward saccade is called for, the axons from right EBN carry impulses to excite motoneurons (or relay interneurons (IN)) innervating the agonist muscles rotating the eyes to the right (green). At the same time, right IBN inhibit the antagonist muscles rotating the eyes to the left (red). The mutual inhibition between the burst neurons across the midline predisposes the neural circuit to instability and can lead to saccadic oscillations (uncalled for back-to-back saccades). (**B** and **C**) Demonstration of oscillations in a two-neuron circuit. Neuron-A inhibits neuron-B and vice versa. A small pulse (physiologically evoked, e.g. by a tiny spontaneous saccade) to neuron-A increases its discharge and thus inhibits neuron-B. Once the discharge of the neuron drops (at the end of the pulse) inhibition from neuron-B is removed. This results in a rebound increase in the neuron-B firing rate. Since neuron-B also inhibits neuron-A, the same phenomenon of post-inhibitory rebound repeats for neuron-A.

Following on the idea that a decrease in OPN inhibition leads to saccadic oscillations, we developed a hypothesis to explain the *abnormal* saccadic oscillations in our patients. We propose that a single deficit (such as, reduction of the conductance of the strychnine-sensitive glycine channel) could change the properties of the burst neuron membrane resulting in reduced inhibition of this circuit by the OPNs and making the neural network oscillate, even when steady fixation is desired. In other words, we are suggesting that the defect here is a 'channelopathy' in which there is a deficit in the ability of the glycine channel to conduct chloride ions—i.e. reduced glycinergic conductance.

Simulations of saccadic oscillations in normal subjects and in μSOLT

To address this hypothesis quantitatively, we simulated a conductance-based single-compartment model of burst neurons within a local feedback loop model of the saccadic system (Miura and Optican, 2006). The general schema for the major conductances is shown in Fig. 4. The mathematical details of the simulation are detailed in the Appendix. Using normal membrane properties and a normal profile of expression of ion channels the model simulated behaviour in normal subjects with stable fixation with eyes open, normal amplitude and velocity saccades, and by turning off the OPN, saccadic oscillations (comparable to Fig. 3A, during eye closure). The pathological oscillations of μSOLT were simulated by reducing the chloride conductance through the strychnine-sensitive glycine channel of the model premotor burst neurons. The model simulated small-amplitude saccadic oscillations during fixation with eyes open. As expected the saccades themselves remained normal (Fig. 5A and C). The model also simulates oscillations during eye closure (Fig. 5B) and oscillations in the orthogonal direction during horizontal or vertical saccades (Fig. 5C).

One important difference was noted between the behaviour of the patients and that of the normal subject. The amplitude of the oscillations in the patient was larger than in the normal subject during eye closure and along the orthogonal axis during large purely vertical or horizontal saccades. In addition, during saccades and eye closure, the amplitude of the oscillations in the patient increased above

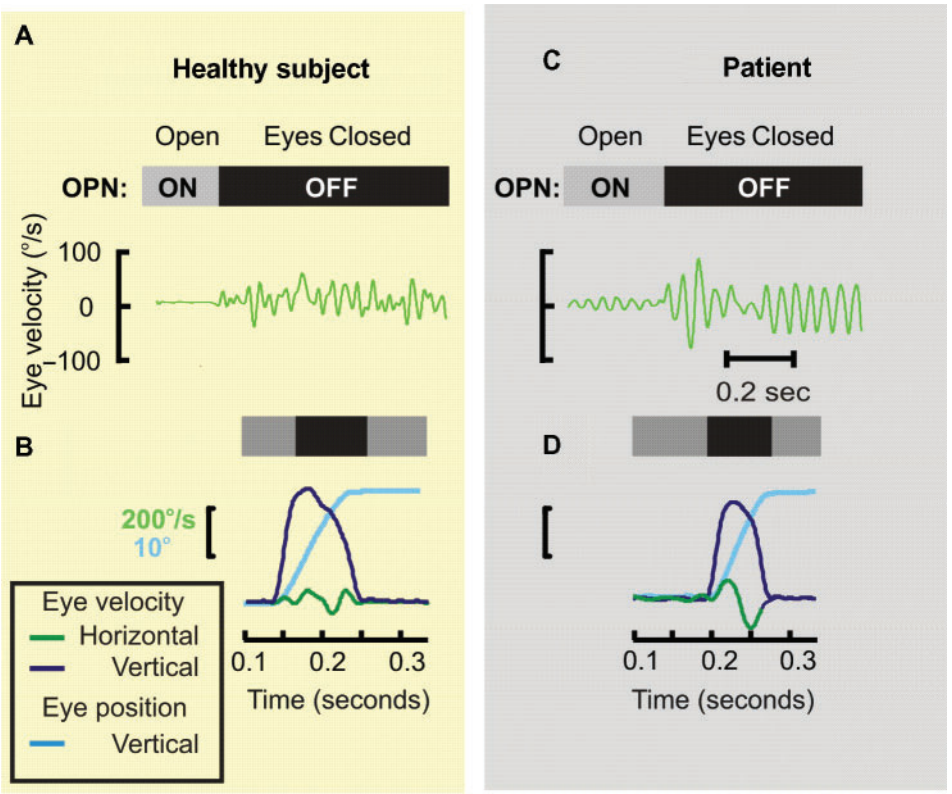

Fig. 3 Saccadic oscillations: (**A**) when a normal subject closes their eyes or (**B**) makes a vertical saccade. OPNs are turned off under these conditions, allowing the system to oscillate. Similar conditions for the patient with μSOLT (**C** and **D**). Oscillations are larger in amplitude in the patient than in the normal subject. Horizontal axis: green, vertical axis: blue.

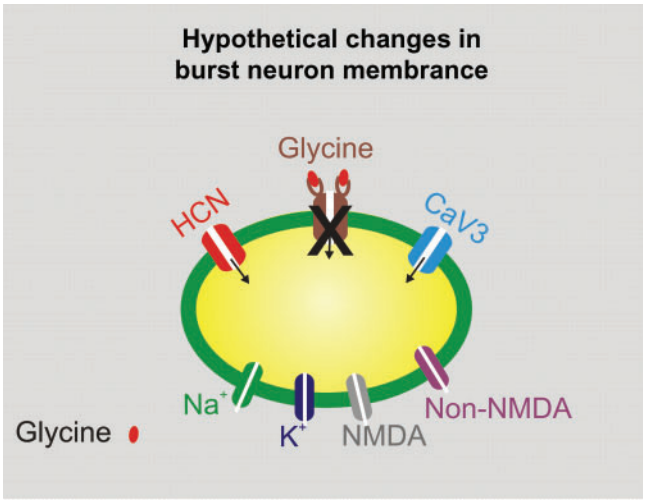

Fig. 4 Membrane channels used in the conductance based model of premotor burst neuron. Ion channels comprising traditional Hodgkin–Huxley model of cell membranes were used to generate the action potential. In order to simulate the physiologically realistic neural behaviour, pacemaker ion channels such as hyperpolarization-activated cation currents (I_H) and low-threshold calcium current (I_T) were also included. Neural modulation as a response to neuro-transmitter release was accomplished by simulated NMDA and non-NMDA excitatory glutamatergic channels as well as glycine or GABA sensitive inhibitory channels. A defect in glycinergic inhibition (black cross symbol) causes saccadic oscillations.

that during fixation. Our simulations also showed this behaviour because the pathologically decreased glycinergic inhibition reduces the level of hyperpolarization of the burst neuron membrane, which reduces the threshold for neural firing. This, in turn, leads to increased membrane excitability and thus an increased strength of PIR. Therefore, during attempted steady fixation, although OPNs are turned on, there is decreased effect of glycinergic inhibition on the burst neurons and micro-oscillations occur. Furthermore, the increased membrane excitability and reduced threshold results in pathologically amplified effects of turning off the OPNs resulting in an increased amplitude of oscillations during orthogonal saccades and eye closure.

Computational analysis of limb tremor in μSOLT

How do we explain limb tremor in μSOLT?

Limb tremors are traditionally thought to arise from oscillation in central neurons or from mechanical abnormalities in the reflex-arc. The similar frequency and amplitude of kinetic and postural tremor in our patients suggest a central origin for the limb tremor in μSOLT. There are at least two possible mechanisms, not necessarily mutually exclusive, underlying oscillations in the central

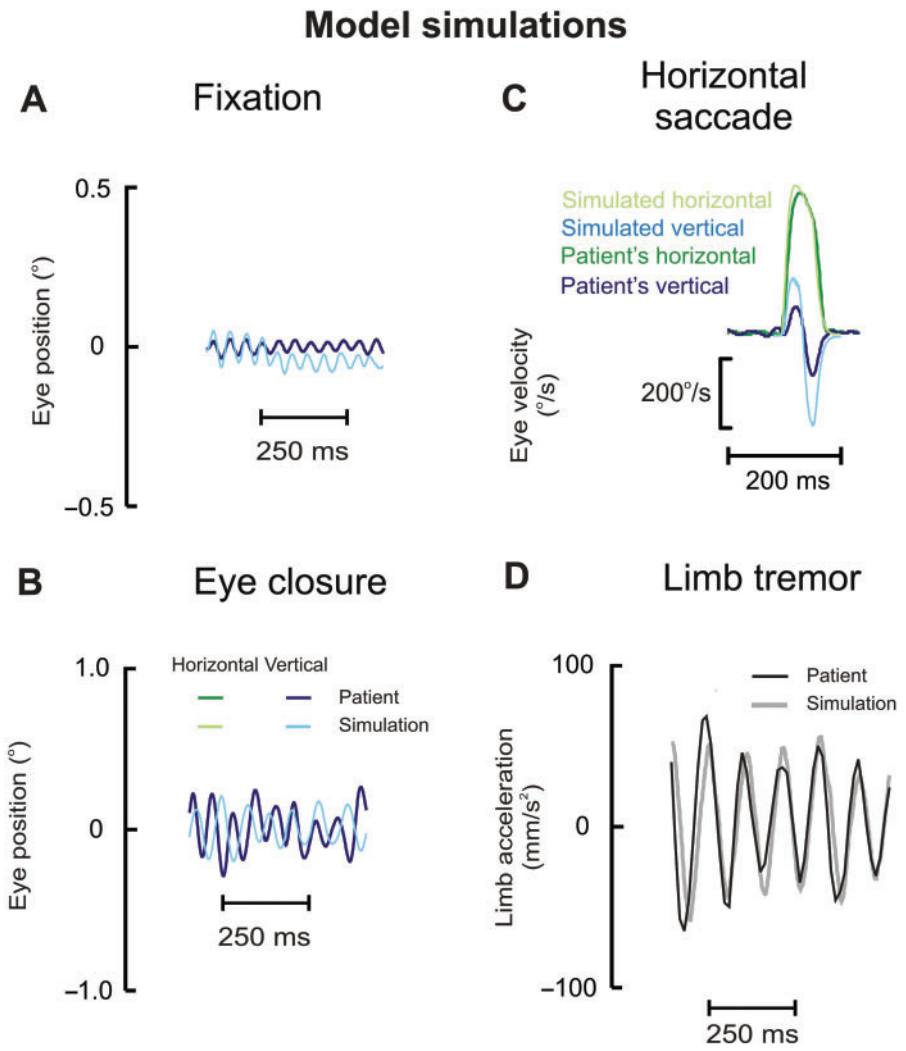

Fig. 5 Simulations of saccadic and limb oscillations in μSOLT: Simulated eye oscillations during (**A**) fixation, (**B**) eye closure and (**C**) a single cycle of a vertical oscillation during a horizontal saccade. (**D**) Simulation of limb tremor by the same model with membrane kinetics consistent with thalamic burst neurons. Light blue, light green and grey traces illustrate simulated oscillations, while blue, green and black traces represent actual recordings from the μSOLT patient.

neurons generating limb movements; one may reside primarily in 'olivo-cerebellar' circuits and the other in 'thalamo-cortical' (TC) circuits.

An olivo-cerebellar mechanism for tremor

One mechanism for tremor is that a group of neurons within a single nucleus develops an abnormal oscillatory mode. In this mode, a neural discharge is followed by a prolonged hyperpolarization which then terminates in rebound spikes. Thus, each neuron oscillates independently. Synchronization of such independently oscillating neurons could result in rhythmic activity that becomes strong enough to cause gross motor oscillations. Electrotonic coupling through connexin gap junctions can facilitate such synchronization in premotor nuclei such as the inferior olive (IO) (Sotelo *et al.*, 1974). Cells of IO also express ion channels that carry I_H (hyperpolarization activated cation current) and I_T (low-threshold calcium current). Moreover, I_h is thought to influence the synchronization of oscillations in IO (Bal and McCormick 1997). Octanol, which reduces synchronized oscillations in IO, also reduces essential tremor (Sinton *et al.*, 1989; Shill *et al.*, 2001). Glycinergic inhibition to IO is sparse, yet present (De Zeeuw and Berrebi 1995). Therefore, impaired glycineric inhibition resulting in increased PIR (as explained earlier) could cause increased synchronized oscillatory behaviour in IO manifesting as limb tremor in µSOLT.

A thalamo-cortical mechanism for tremor

The mechanism presented earlier, which is based on electrotonic conduction within the IO, cannot easily explain the saccadic oscillations of our patients. So, we invoked a second possible mechanism for tremor: increased instability due to reduced 'external' inhibition in a neural circuit

characterized by reciprocal innervation. We then asked if there might be a comparable circuit for limb movements to that for eye movements that would be susceptible to and produce limb oscillations. Reciprocal innervation is also noted in spinal as well as more central neurons controlling limb movements (Sherrington, 1907). The globus pallidus internus (GPi), sends inhibitory GABAergic projections to the motor thalamus [ventral anterior (VA) and ventral lateral (VL) thalamic relay nuclei], which relays the output to the motor cortex (Parent and Hazrati, 1995). The thalamus may participate in the generation of limb movements similar to the way the brain stem reticular formation generates premotor commands for saccades. TC relay neurons send glutaminergic excitatory projections to the thalamic reticular (TR) neurons (as EBNs send excitatory projections to contralateral IBNs in the ocular motor system). The TR neurons also have inhibitory feedback projections to TC neurons (as IBNs send inhibitory projections to EBNs). TR neurons mutually inhibit each other (as IBNs mutually inhibit each other across the midline) (Takada and Hattori, 1987; Sherman and Guillery, 2001; Guillery and Harting, 2003; Pinault, 2004). The globus pallidus (GP) sends inhibitory projections to TC and TR neurons which could be analogous to OPN-induced inhibition of the saccadic burst neurons. Inhibitory neurotransmitters of the GP system are GABA and glycine (Takada and Hattori, 1987). Furthermore, thalamic relay and GPi neurons also exhibit PIR (Llinas and Jahnsen, 1982; Nambu and Llinas, 1994). Hence, analogous to the neural circuit formed by the premotor neurons of the saccadic system, a neural network formed by the TC and TR neurons for controlling limb movements is also reciprocally inhibitory and might also be inherently unstable, and physiologically under control by external inhibition. Therefore, we propose that similar mechanisms—impaired external inhibition upon an inherently unstable circuit that normally generates reciprocally innervated movements—underlie limb tremor in familial μSOLT.

A conductance-based model of thalamic burst neurons simulates limb tremor

A conductance-based computational model of thalamic burst neurons was simulated to generate limb tremor. While the circuitry and structure of this model was analogous to that for generating saccades there are key differences between the two models in the expression profile of different subtypes of ion channels carrying hyperpolarization activated cation current (I_H). For limb tremor, the expression profile of the simulated ion channel subtypes carrying I_H conductance was made consistent with the presumed profile in TC and thalamic relay neurons (Monteggia *et al.*, 2000). This model simulated the hand tremor and had a similar frequency to that observed in the patient (Fig. 3E). Note the considerable difference between the frequency of oscillations of the hands (12 Hz) and eyes (18 Hz) in our patients (Fig. 5E).

Discussion

Impaired inhibition in inherently unstable neural circuits generating rapid ballistic movements

Here, we report a novel familial disorder characterized by tiny saccadic oscillations with hand tremor. The symptoms began early in the life and were not associated with any known acquired cause of eye oscillations such as toxic, metabolic, infectious or immune-mediated disease. This suggests an inherited origin. We attribute the saccadic and limb oscillations in this familial disorder to an unmasking of the inherent instability associated with reciprocally inhibitory premotor neural circuits that underlie reciprocal innervation of agonist–antagonist muscle pairs.

We simulated micro-saccadic oscillations with a membrane-based model of premotor burst neurons innervating agonist-antagonist muscle pairs. The key features of this model are PIR and the inherent instability of the burst neuron circuit which results from reciprocal inhibition between the neurons that innervate agonist and antagonist muscle pairs. The model reproduced micro-saccadic oscillations when glycinergic inhibition of this circuit was reduced.

We speculate that thalamic circuits relaying signals related to ballistic limb movements may be functionally analogous to the premotor saccadic burst generator. We emphasize that we know much less about the direct, premotor contribution of thalamic circuits to the generation of limb movements than we know about the circuits that generate the premotor commands for saccades, but we think it likely that similar principles underlie the generation of ballistic limb movements. Therefore, the same pathological deficit—removal of external inhibition from mutually inhibitory circuit might also explain the limb tremor in our patients. Using a slightly different, but physiologically plausible, set of ion channel kinetics than used to simulate saccadic oscillations, the same simulated membrane deficit—reduced external inhibition of reciprocally innervating neural circuits—reproduced the characteristics of limb tremor.

Why do eye and limb oscillations have different frequencies?

There was a considerable difference in the frequency of the eye oscillations and the limb tremor (18 and 12 Hz, respectively). This difference in frequency is likely related to differences in the central and peripheral mechanisms responsible for eye versus limb movements and to the mechanical properties of the eye and limb (the motor 'plant'). For example, (i) there is no stretch reflex in the extra-ocular muscles (Keller and Robinson, 1971), but there

is in the limbs, (ii) the dynamics of the limb motor 'plant' (i.e. the physical properties of the muscles and connective tissues in the limbs) are different between the eye and limbs. It is possible that the relatively larger mass of the fingers and hand is associated with a lower-frequency physiological and an enhanced physiological mechanical-reflex tremor. The hand tremor in our patients, however, was presumably of central origin, and its frequency therefore would not be affected by limb inertia. Unfortunately, we could not study the limb tremor in our patients with mass loading, as described by Deuschl *et al.* (2001).

For the purposes of our simulations we optimized the mechanical time constants in the simulated limb motor 'plant' according to the tremor of our patient. Another important factor in determining both the amplitude and the frequency of oscillations could be the strength of PIR. The expression pattern of ion channel subtypes carrying hyperpolarization-activated cation currents (I_H) and low-threshold calcium currents (I_T) determines the membrane kinetics and thus the strength of PIR (Llinas, 1988; McCormick and Pape, 1990; Sekrinjak and du Lac, 2002; Nelson *et al.*, 2003; Perez-Reyes, 2003). Indeed a simulated increase in activation of I_T and/or I_H changes the frequency and amplitude of the oscillations (Fig. 6). In particular, a stronger I_T simulates a larger oscillation amplitude, whereas a stronger I_H simulates a higher oscillation frequency. The relative pattern of expression of the four subtypes of ion channel carrying I_H can also influence the oscillation frequency. A larger proportion of I_H channel subtype with the shortest activation kinetics (HCN1) causes a higher frequency of oscillation. On the other hand, a larger proportion of the I_H subtype with the longest activation kinetics (HCN4) causes a lower frequency of oscillation. Compared to thalamic burst neurons, the premotor saccadic burst neurons presumably have a relatively larger proportion of I_H channel subtypes with faster activation kinetics (Monteggia *et al.*, 2000). In addition, inherent synaptic delays associated with the feedback loops from motor neurons to premotor neurons could also contribute to differences in frequency between the eye oscillations and the hand tremor.

In spite of the many possible differences between the neural networks that generate eye and limb movements, and the differences in the effector organs, we suggest a common pathophysiology for oscillations in these networks in our patients: a reduced efficacy of the external inhibition on the premotor burst neuron networks that generate eye and hand movements. The differences in the membrane ion channel kinetics expressed in different neurons (here thalamic burst neurons and pontine burst neurons) is an important factor in determining the different frequencies of eye and limb oscillations.

An approach to study more common tremor disorders such as essential tremor

This combined clinical, biophysical and computational approach to a rare disorder of the ocular motor system may have broader implications for understanding the pathophysiology of and developing rational therapies for abnormal oscillations in other motor systems. Essential tremor, tremor associated with dystonia and the rapid saccade-like head oscillations that are sometimes associated with saccadic eye oscillations might have origins in altered membrane properties related to neurons that burst during head, eye and limb movements. We suggest that, on the one hand, oscillations in these other motor systems might be caused by reducing the effect of inhibitory input, either on an acquired (toxic or immune mediated) or on an inherited basis. On the other hand, the specific complement of ion channel subtypes may determine the characteristics of the oscillations. This, too, would be under genetic control and might explain why there is a wide range of frequencies of saccadic oscillations across normal individuals (Ramat, *et al.*, 2005) but within a family of normal subjects (including our family), the frequency of saccadic oscillations is almost the same (Neppert and Rambold, 2006).

While these hypotheses remain to be proven, they nevertheless suggest new genetic, experimental and clinical approaches to disorders of movement, and especially those in which there are no gross structural abnormalities within the brain. Treatment with pharmacological blockers targeted towards these ion channels may offer therapeutic benefits. For example, although counterintuitive, *interfering* with the function of a normal ion channel to decrease membrane excitability in the face of impaired external inhibition might reduce oscillatory behaviour. Such an approach is similar to that for inherited epilepsy when seizures presumably are caused by an abnormal ion channel, while treatment targets another, presumably intact channel (Cannon, 2006). Indeed, propranolol—a commonly used beta-blocker that reduces membrane excitability (McCormick and Pape, 1990) is an effective drug for treating essential tremor (Gilligan, 1972). Alternatively, *enhancing* the function of an impaired ion channel might also reduce oscillatory behaviour. For example, ethanol, which enhances the conductance through the glycine channels, ameliorates essential tremor (Eggers and Berger, 2004).

Supplementary material

Supplementary material is available at *BRAIN* online.

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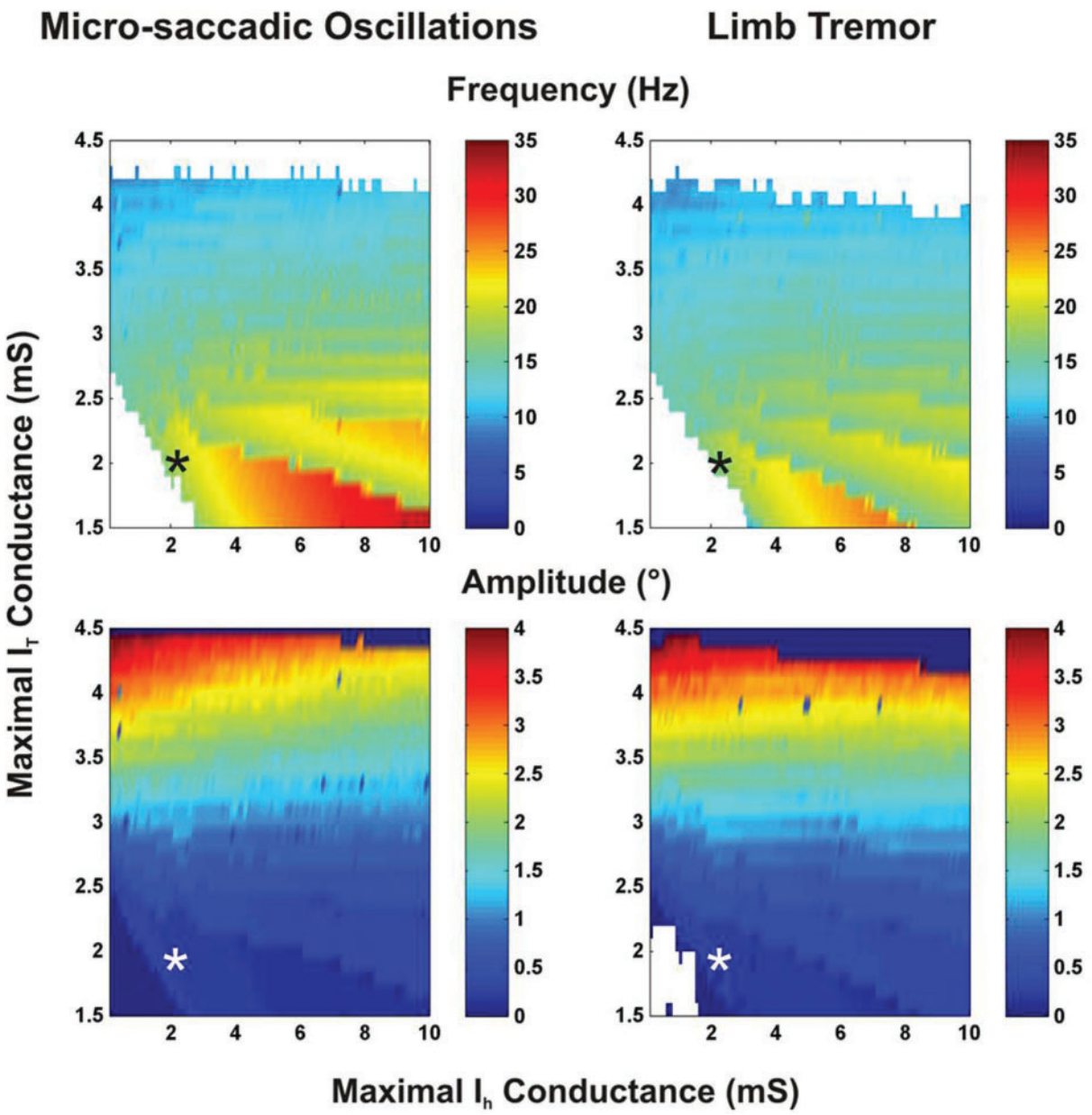

Fig. 6 Oscillations occur in the model after reducing glycinergic inhibition. The frequency of the oscillations is determined primarily by the kinetics of the I_H and I_T currents, which in turn depend upon the distribution of ion channel subtypes (upper panels). Kinetics of the four I_H subtypes have a 15-fold range, thus the frequency depends on the proportion of each subtype expressed in the burst neurons. Saccadic oscillations involve pontine burst neurons, which express more of the subtypes of I_H channels with faster activation kinetics (Monteggia et al., 2000). This contributes to the higher frequency of saccadic oscillations. Limb tremor may involve thalamic burst neurons, which express a larger proportion of the slower I_H channel subtypes (Monteggia *et al.*, 2000). This contributes to the lower frequency of limb tremor. The amplitude of the oscillations depends primarily on the maximal conductance through I_T channels (lower panels). Black, grey and white asterisk indicate the values of I_H and I_T maximal conductances used in the simula

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Appendix

Computational simulations

The Hodgkin–Huxley equations were implemented to simulate action potentials. A glycine channel was implemented for inhibition, and non-NMDA and NMDA channels for excitation. CaV3 channels (carrying I_T) and four subtypes of HCN channel (HCN1–HCN4; carrying I_H) were included to simulate PIR and to modulate the effectiveness of glycinergic inhibition. Not the activation kinetics of subtypes of ion channels carrying I_H are significantly different from each other, HCN-1 being the fastest and HCN-4 the slowest, with the others in between, as reflected in their activation time constants (Monteggia *et al.*, 2000).

The following equation describes the time evolution of the membrane potential of the brain stem neurons:

$$C\frac{dV}{dt} = -I_L - I_T - I_{Na} - I_K - I_{Gly} - I_{GluNMDA} - I_{GlunonNMDA} - \sum_{j=1}^{4} n_j I_{Hj} \quad (1)$$

where, V is the membrane potential of the burst neuron, C is the membrane capacitance (1 μF/cm2) and n_j is an expression rate scaling factor determining the ion channel expression profile in the burst neuron. I_L, I_T, $I_{H1\text{-}4}$, I_{Na} and I_K, denote the leak current, low-threshold calcium current, hyperpolarization activated current (carried by HCN1–4), fast sodium current and delayed rectifier potassium current, respectively. I_{Gly} and I_{Glu} are synaptic currents mediated by glycinergic and glutamatergic (NMDA and non-NMDA type) synapses. Details of these currents are presented in Miura and Optican (2006). This model added one new channel type, for the HCN currents. The equations for I_H are:

$$I_H = g_H x(V - E_H) \quad (2)$$

$$\frac{dx}{dt} = \lambda\frac{(\alpha - x)}{\tau} \quad (3)$$

$$\alpha(V) = \frac{1}{\left(1 + \exp\left[\frac{(\mu - V)}{\theta}\right]\right)} \quad (4)$$

$$\tau(V) = 0.01 + \frac{1}{(\exp[-14.59 - 0.086\,V] + \exp[-1.87 + 0.0701\,V])} \quad (5)$$

where, g_H (= 9 mS/cm^2) and E_H (= −40 mV) denote the maximal conductance and the reversal potential of this channel, respectively (citation). The four different HCN channels are determined by the parameters in the Table 1 (Moosmang *et al.*, 2001):

Table A1 Determination of HCN channels

HCN	λ	$\mu_{(mv)}$	$\theta_{(mv)}$
1	15.36	94	8.1
2	2.51	99	6.1
3	1.74	96	8.6
4	1.00	100	9.6