

## ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Issue: *Basic and Clinical Ocular Motor and Vestibular Research***The effects of ion channel blockers validate the conductance-based model of saccadic oscillations**Aasef G. Shaikh,<sup>1</sup> David S. Zee,<sup>2</sup> Lance M. Optican,<sup>3</sup> Kenichiro Miura,<sup>4</sup> Stefano Ramat,<sup>5</sup> and R. John Leigh<sup>1</sup><sup>1</sup>Department of Neurology, Case Western Reserve University, Cleveland, Ohio. <sup>2</sup>Department of Neurology, The Johns Hopkins University, Baltimore, Maryland. <sup>3</sup>Laboratory of Sensorimotor Research, National Eye Institute, National Institutes of Health, Bethesda, Maryland. <sup>4</sup>Kyoto University, Kyoto, Japan. <sup>5</sup>University of Pavia, Pavia, Italy

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Conductance-based models of reciprocally inhibiting burst neurons suggest that intrinsic membrane properties and postinhibitory rebound (PIR) determine the amplitude and frequency of saccadic oscillations. Reduction of the low-threshold calcium currents ( $I_T$ ) in the model decreased the amplitude but increased the frequency of the simulated oscillations. Combined reduction of hyperpolarization-activated cation current ( $I_h$ ) and  $I_T$  in the model abolished the simulated oscillations. We measured the effects of a selective blocker of  $I_T$  (ethosuximide) in healthy subjects on the amplitude and frequency of saccadic oscillations evoked by eye closure and of a nonselective blocker of  $I_h$  and  $I_T$  (propranolol) in a patient with microsaccadic oscillation and limb tremor syndrome (mSOLT). Ethosuximide significantly reduced the amplitude but increased the frequency of the saccadic oscillations during eye closure in healthy subjects. Propranolol abolished saccadic oscillations in the mSOLT patient. These results support the hypothetical role of postinhibitory rebound,  $I_h$ , and  $I_T$ , in generation of saccadic oscillations and determining their kinematic properties.

**Keywords:** burst neurons; hyperpolarization-activated cation current; low-threshold calcium current; reciprocal innervations

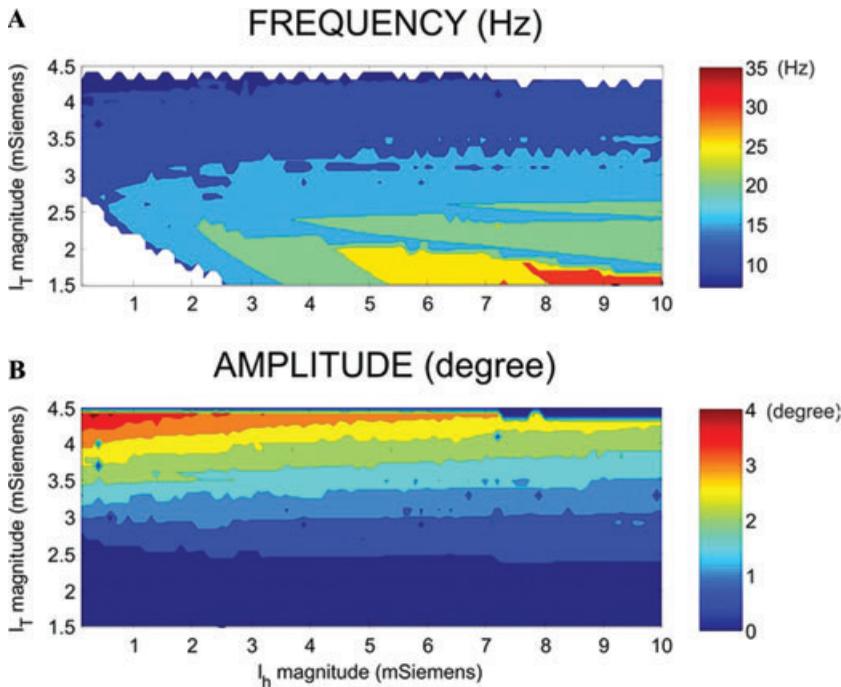
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**Introduction**

Reciprocal innervation of the agonist–antagonist muscle pair and transient removal of the external inhibition generating high-frequency pulse of the neural discharge (postinhibitory rebound, PIR) are the fundamental drives for high velocities of ballistic movements.<sup>1</sup> For example, during saccades, the omnipause neurons (which inhibit the burst neurons during steady fixation) transiently turn off, allowing the ipsilateral excitatory burst neurons (EBNs) to send an excitatory signal to the motoneurons, which innervates the agonist muscle that rotates the eyes to the ipsilateral side.<sup>2</sup> Simultaneously, the ipsilateral inhibitory burst neurons (IBNs) inhibit the motoneurons to the antagonist muscle.<sup>2</sup> It was pro-

posed that PIR generated by the transient inhibition of the OPNs is critical for the bursts of neural discharge in EBNs and IBNs, which provide sufficient force to rapidly turn the globe.<sup>3</sup> During steady gaze, OPNs inhibit EBNs and IBNs on both sides and prevent unwanted saccades.

A conductance-based neuromimetic model predicted that increased excitability of the burst neurons could result in saccadic oscillations.<sup>3</sup> The excitability could be increased either by the removal of inhibition from the OPNs (during eye closure in healthy subjects or decreased glycinergic inhibition during pathological states) or due to pathological depolarizing shifts of the resting membrane potential of the burst neurons (e.g., intoxication, autoimmune disorders, and degenerative cerebellar



**Figure 1.** The effect of  $I_h$  and  $I_T$  on the frequency and amplitude of the simulated saccadic oscillations. Increasing  $I_T$  increases the amplitude but decreases the frequency of simulated saccadic oscillations. For the given change in  $I_T$ , the amplitudes of saccadic oscillations are more affected than their frequency. Hence, the amplitude is more sensitive to changes in  $I_T$  as compared to  $I_h$ . Increasing  $I_h$ , however, increases the frequency of saccadic oscillations.

disorders).<sup>3–6</sup> Hyperpolarization-activated, inward cation current ( $I_h$ ) and low-threshold calcium current ( $I_T$ ) are key determinants of the resting membrane potential and the strength of PIR.<sup>7–11</sup> In the conductance-based model of the burst neurons, the activation kinetics and amplitudes of  $I_h$  and  $I_T$  determined the amplitudes and the frequencies of saccadic oscillations. An increase in  $I_T$  increased the amplitude and decreased the frequency, while increasing  $I_h$  increased the frequency of saccadic oscillations (Fig. 1).<sup>3</sup>

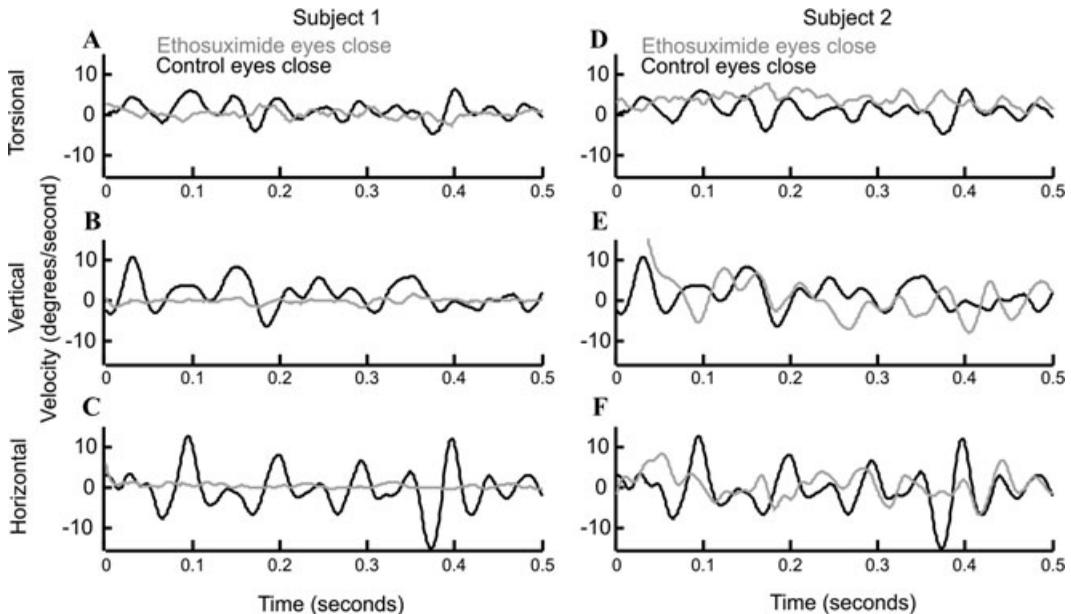
These model predictions motivated two hypotheses: selective blockade of  $I_T$  should reduce the amplitude but increase the frequency of the saccadic oscillations, and nonselective blockers of  $I_h$  and  $I_T$ <sup>7,8</sup> should reduce the amplitude and frequency of saccadic oscillations. To test these hypotheses, we measured the effects of ethosuximide, a selective  $I_T$  blocker,<sup>12</sup> on the properties of physiological saccadic oscillations during eyelid closure in two healthy subjects and the effects of propranolol, a nonselective blocker of  $I_h$  and  $I_T$ , in a patient with syndrome of microsaccadic oscillations and limb tremor (mSOLT).<sup>3</sup>

## Methods

The mSOLT patient and healthy subjects were evaluated at the Johns Hopkins vestibular laboratory. All subjects gave informed consent. The eye movements of the healthy subjects were recorded with the magnetic-field system using dual search coils (Skalar Medical, Delft, the Netherlands). A search-coil system and an infrared camera (Ober Consulting, Poland) were used to measure eye movements of the mSOLT patient.

The subjects fixed their gaze upon a target that was projected straight ahead. Eye movements were recorded both when looking at a stationary target and in a completely dark room with no visual target. Eye movements in healthy subjects were then recorded with both eyes closed but while fixating on the imaginary stationary target. Eye position signals were sampled at 1000 Hz resolution. The data were further processed with custom software written in the Matlab<sup>®</sup> environment.

Data from each axis were processed separately. Saccadic oscillations were best characterized from



**Figure 2.** Horizontal, vertical, and torsional saccadic oscillations evoked by eye closure in two healthy subjects (black traces). The eye velocity is plotted along the  $y$ -axis, while the time is plotted on the  $x$ -axis. Ethosuximide reduces the amplitude and increases the frequency of these saccadic oscillations in both subjects (gray trace).

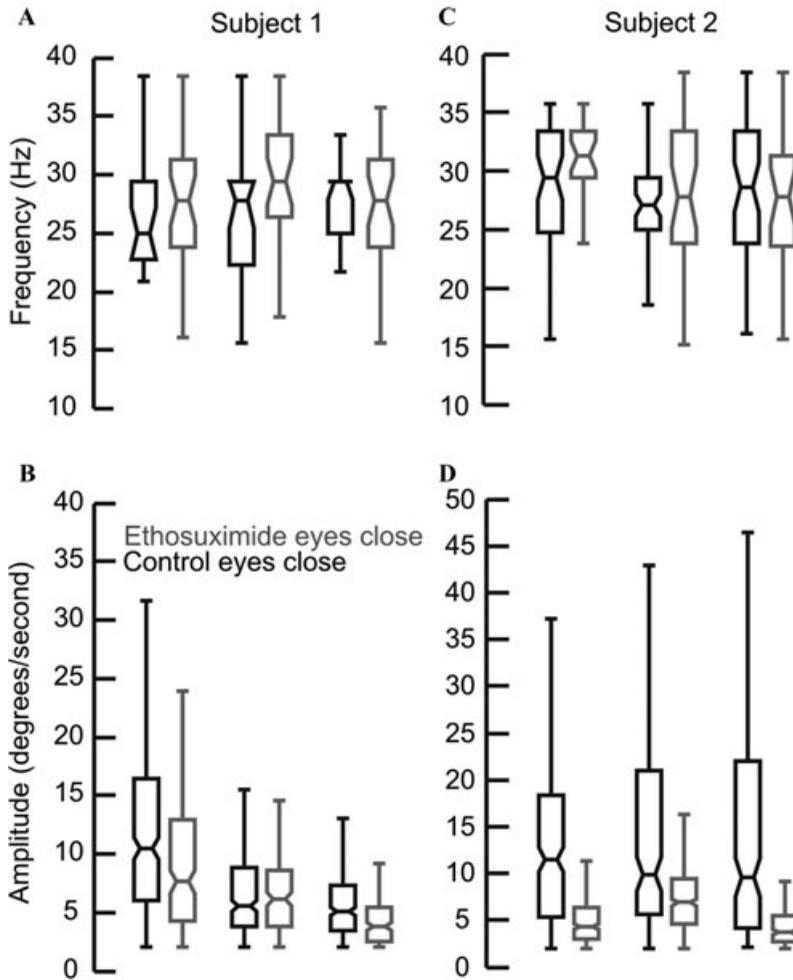
eye velocity records. Oscillations were separated interactively. Cycle-by-cycle analysis was performed in the following steps. The detrended data were normalized with respect to the mean velocity (i.e., normalized velocity = actual velocity – mean velocity). Normalization allowed the velocity to align on the abscissa with the peaks of the cycles remaining positive and the troughs negative. The points of intersection of the velocity data trace, crossing from a negative value to a positive value with the abscissa were recorded. The  $x$  coordinates of these values were called positive zero crossings. The cycle duration was calculated from the time between the positive zero crossings. The inverse of the cycle duration yields the cycle frequency. The average of the peak and trough values of the cycle was considered as the peak velocity of the given cycle. The mean, median, standard deviation, and 95% confidence intervals of the frequencies and peak velocities of all cycles were computed. Saccades were interactively selected in the eye position trace. Saccadic amplitude, velocity, latency, and gain (actual/desired eye movement) were computed.

## Results

### *Effect of ethosuximide on saccadic oscillations during eyelid closure in healthy subjects*

The black traces in Fig. 2 illustrate saccadic oscillations in two subjects with the eyes closed. These oscillations were present around all three axes. Eye movements during attempted fixation on the imaginary target with the eyes closed were recorded in the same subject 45 min after taking 250 mg ethosuximide by mouth (gray traces in Fig. 2). Saccadic oscillations around all three axes were again seen under closed eyelids; however, their amplitude was reduced in horizontal and torsional planes in this example. Frequency was relatively increased.

Figure 3 summarizes the frequency and amplitudes of horizontal, vertical, and torsional saccadic oscillations during eye closure in two healthy subjects. Each gray box–whisker plot represents the effects of ethosuximide on the frequency and amplitude of the saccadic oscillations. The horizontal line in each box and whisker plot represents the median value, while notches represent 95% confidence intervals. The frequency of oscillations increased around all three axes after ethosuximide;



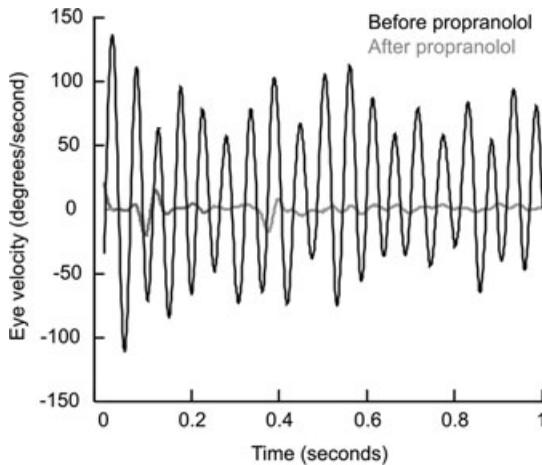
**Figure 3.** Box-whisker plots summarizing the effects of ethosuximide on cycle-by-cycle frequency and amplitude of the saccadic oscillations. The horizontal line in the center of notch represents the median value, the notch represents the 95% confidence interval, the length of the box depicts the inter-quartile interval, and the whiskers represent the range. Nonoverlapping notches represent statistically significant differences between the medians of the compared populations.

however, the difference was not statistically significant (one-way ANOVA,  $P > 0.05$ ). In contrast, there was a statistically significant reduction in the amplitude of horizontal and torsional saccadic oscillations in subject 1 and saccadic oscillations around all three axes in subject 2 (one-way ANOVA,  $P < 0.05$ ).

**Effect of propranolol on saccadic oscillations in a patient with mSOLT**

A young patient with mSOLT was referred to our clinic for occasional brief episodes of blurred vision and fine tremor of the hand. The symptoms were present since early childhood, and were worsened during periods of stress or anxiety. On bed-

side examination, intermittent bursts of saccadic oscillations and limb tremor were the only neurological findings. Ophthalmoscopy revealed nearly continuous, small-amplitude, high-frequency oscillations of the optic disc. Subsequently, the patient’s mother was evaluated and reported similar episodes of blurred vision. On examination, the mother had the same saccadic oscillations and tremor. During a subsequent visit to see the mother, she reported that her primary care provider prescribed propranolol (60 mg by mouth twice a day) for hypertension. The mother now had relief from her intermittent episodes of blurred vision and hand tremor. At this time, the saccadic oscillations were no longer present as examined during ophthalmoscopy.



**Figure 4.** Horizontal saccadic oscillations as the patient (the mother) with microsaccadic oscillation limb tremor (mSOLT) attempts to fixate on a target (black traces). Eye velocities are plotted on the *y*-axis, while the time is plotted along the *x*-axis. Propranolol almost abolished the saccadic oscillations in the patient (the mother) with mSOLT (gray trace).

The black trace in Fig 4 illustrates saccadic oscillations during attempted steady fixation recorded from the mother prior to beginning propranolol. These oscillations were  $23.2 \pm 18.3$  Hz in frequency and  $76.2 \pm 29.4$  degrees/second in amplitude. The treatment with propranolol abolished these oscillations (gray trace in Fig. 4).

## Discussion

We tested the prediction of a neuromimetic model of the saccade generators in which the rapid velocities of saccades are due an abrupt increase in the firing rate, the burst, in reciprocally innervating EBNs and IBNs.<sup>3,13</sup> The model also predicted that a pause in the external inhibition is critical for the high firing rate during the burst, a mechanism known as PIR. Simulation of the pathological enhancement of PIR, by increasing  $I_h$  and/or  $I_T$ , revealed high-frequency back-to-back saccades (i.e., saccadic oscillations). The model also predicted that enhanced  $I_h$  would increase the frequency of saccadic oscillations, without affecting the amplitude (Fig. 1). In contrast, enhanced  $I_T$  would increase the amplitude of saccadic oscillations but decrease their frequency (Fig. 1).<sup>3</sup> Our strategy for testing these model predictions was to determine if a selective blocker of  $I_T$ , ethosuximide,<sup>1</sup> reduced the amplitude of phys-

iological saccadic oscillations and increased their frequency. We also determined whether nonselective blockade of  $I_h$  and  $I_T$ , by propranolol,<sup>7,8</sup> would reduce the amplitude of oscillations. Our findings were consistent with both model predictions; the ethosuximide reduced the amplitude but increased the frequency of saccadic oscillations in both the normal subjects, and propranolol reduced the amplitude of oscillations in the patient with mSOLT.

The model also predicted that the frequency of oscillations is less sensitive to changes in  $I_T$  than is the amplitude. Figure 1 depicts that, in the model, for the given change in  $I_T$ , the amplitude of simulated saccadic oscillations is affected more than the frequency. Consistent with this prediction, the amount of change in the amplitude of oscillations was larger and reached statistical significance as compared to the amount of change in the frequency in both subjects.

There was an inter-axis variability in the effects of ethosuximide in both subjects. This variability could be explained, in part, by differences in the expression profile of CaV3 ion-channels (conducting  $I_T$ ) at anatomically separate brainstem sites of the burst neurons controlling vertical, torsional, and horizontal saccades. The burst neurons for horizontal saccades are found in caudal pons, while those for vertical and torsional movements are located in rostral mesencephalon.<sup>14</sup>

The conductance-based model predicted that mSOLT is caused by an inherited abnormality causing increased membrane excitability or reduced external inhibition of the pontine burst neurons.<sup>3</sup> Our results provided support for this model, since non-selective blockade of  $I_h$  and  $I_T$  with propranolol could theoretically reduce the excitability of the burst neurons, and, in turn, account for the reduction of the amplitude of saccadic oscillations in the mSOLT patient.

According to the model predictions, blockade of  $I_h$  and  $I_T$  should reduce PIR and therefore reduce saccade velocity.<sup>3,15</sup> However, in both our normal subjects, there was no change in saccade velocity. In our model, the strength of PIR required to simulate oscillations had to be supra-physiological; however, to simulate slow saccades, PIR must be significantly less than physiological. Therefore, it is possible that at the given doses, ethosuximide and propranolol might reduce PIR, but not enough to affect saccade velocity.

The recordings in the healthy subjects were done with scleral search coils, under closed eyelids. Does mechanical friction due to lid closure affect the eye velocity or induce coil slippage? We had addressed this issue in an independent study where we had shown that the acceleration and velocity of the eye movement during vestibulo-ocular reflex evoked by head impulses (the mechanism that is not physiologically affected by eye closure) were the same during open and closed eyelids.<sup>16</sup> In the same study, the peak velocities or accelerations of the eye movements evoked by vestibulo-ocular reflex during head impulses are of comparable speed to those of saccades.<sup>16</sup> Therefore, it is unlikely that eye closure induced mechanical hindrance to the movement of the eye or of the search coil mounted on it.

To summarize, the conductance-based model emphasized that increased excitability or disinhibition of the pontine burst neurons can cause saccadic oscillations. The results of the current experiments are in accord with the specific model predictions and in particular with the hypothesis that  $I_h$  and  $I_T$  conductance determines the frequency and amplitude of the saccadic oscillations. There are, however, caveats. The drugs could have effects on other structures related to saccade generation that might influence the frequencies and amplitudes of saccadic oscillations. Electrophysiological experiments investigating the effects of selective ion channel blockers on saccades, injected locally at the location of the burst neurons or omnipause neurons, are needed to further test these ideas.

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### Conflicts of interest

The authors declare no conflicts of interest.

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