Low-Frequency Oscillations in the Cerebellar Cortex of the Tottering Mouse

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INTRODUCTION

The tottering (tg) mouse is of interest because many neurological disorders that are caused by mutations in genes encoding ion channels are also characterized by episodic symptoms such as migraine, seizures, and ataxia (Jen et al. 2004; Kullmann 2002; Pietrobon 2002; Ptacek and Fu 2001). In humans, various mutations within the CACNA1A gene that encodes the α1A subunit of the Ca2,1 channel cause episodic ataxia type 2 (EA2) in which stress, caffeine, and exertion trigger long periods of episodic cerebellar symptoms (Balogh et al. 1997; Jen et al. 2004; Ophoff et al. 1996). The tg mouse has been widely used as an animal model for the Ca2,1 channelopathies (Campbell and Hess 1998; Fureman et al. 2002; Hoebek et al. 2005; Jen et al. 2007; Pietrobon 2002; Walter et al. 2006).

Expressed heavily in cerebellum, P/Q-type Ca2+ channels are located pre- and postsynaptically at parallel fiber (PF–Purkinje cell (PC) synapses and play an important role in transmitter release (Mintz et al. 1995; Westenbroek et al. 1995). P/Q-type Ca2+ channels are also the dominant type of voltage-gated Ca2+ channels on mature PCs (Llinás et al. 1992; Mintz et al. 1992). In tg mice, the mutation results in an approximately 30–40% reduction in P/Q-type current density in PCs with little alteration in channel kinetics (Erkisson et al. 2007; Wakamori et al. 1998). In EA2 patients, the mutations also result in reduced P/Q-type Ca2+ channel function (Guida et al. 2001; Jen et al. 2001; Jeng et al. 2006; Wappl et al. 2002).

Previous studies reported that in the cerebellum of the tg mouse PF–PC synaptic transmission is decreased (Matsushita et al. 2002), GABAergic synaptic efficacy is increased (Zhou et al. 2003), and PC simple spike firing is highly variable (Hoebek et al. 2005; Walter et al. 2006). These abnormalities are consistent with the baseline cerebellar phenotype in the tg mouse (Campbell and Hess 1998; Fureman et al. 2002).

The mechanisms underlying the acute episodes of motor dysfunction in the tg mouse are unknown. The cerebellum is required for expression of the episodic motor attacks in the tg mouse and the cerebellum is highly activated during the attacks (Campbell and Hess 1998; Campbell et al. 1999). In addition to the reduction in P/Q-type Ca2+ channel function, Ca1,2/1.3 (L-type) Ca2+ channels are up-regulated in the tg mouse and are involved in the episodic dystonia (Campbell and Hess 1999; Erickson et al. 2007; Fureman et al. 2002). Activating these channels with an L-type agonist evokes the episodic dystonia and blocking these channels suppresses the induction of the motor attacks (Campbell and Hess 1999; Fureman et al. 2002). The present study used flavoprotein optical imaging

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likely involved in the episodic dystonia. In both the anesthetized and awake tg mouse, the imaging revealed transient, low-frequency oscillations that are likely involved in the episodic dystonia.

Methods

Animal breeding and preparation

Tottering (tg) mice (Cacna1a<sup>tg</sup>) on a C57BL/6J background were bred at the Johns Hopkins University School of Medicine. The presence of stereotyped dystonia was confirmed in each mutant by challenge with 15 mg/kg caffeine (administered intraperitoneally [ip]) before experimentation. Because it was previously shown that dystonic episodes of tg mice are not affected by age or sex (Weisz et al. 2005), male and female tg mice, ages 6–12 mo, were used in these experiments. C57BL/6J+/+ mice of similar age (6–12 mo) and sex were used as wild-type controls. All animal procedures were approved by and conducted in conformity with the Institutional Animal Care and Use Committee of the University of Minnesota and in accordance with the guiding principles for care and use of experimental animals approved by the Councils of the American Physiological Society and the National Research Council.

Both anesthetized and awake mice were studied. Experiments in anesthetized mice enabled rapid and homogeneous application of drugs and manipulation of the extracellular Ca<sup>2+</sup> concentration directly over the folium of interest. Experiments in awake mice allowed for determination of the relation between the oscillations in the cerebellar cortex and the motor attacks. Experimental details on the anesthetized animal preparation and optical imaging techniques have been provided in previous publications and are only briefly described here (Gao et al. 2006; Reinert et al. 2004). The mice were anesthetized by intramuscular injection of a cocktail of ketamine (60 mg/kg), xylazine (3 mg/kg), and acepromazine (1.2 mg/kg), placed in a stereotaxic frame, mechanically ventilated, and body temperature feedback regulated. The electrocardiogram was monitored to assess the depth of anesthesia, allowing anesthetics to be supplemented as needed. After the craniotomy a watertight chamber of dental acrylic was created around the exposed cerebellar cortex that typically included Crus I, II, paramedian lobule (PML), and lobulus simplex. In five tg mice, the craniotomy was enlarged to include lobules VII and VIII. The chamber was filled and periodically rinsed with a gassed Ringer solution (Gao et al. 2006). In the anesthetized mice drugs in normal Ringer solution were applied to the cerebellar surface, including diltiazem hydrochloride (Tocris, Ellisville, MO), 2,5-dimethyl-4-[2-(phenylmethyl)benzoyl]-1H-pyrole-3-carboxylic acid methyl ester (FPL; Tocris), and 6,7-dinitroquinoxaline-2,3-dione disodium salt (DNQX; Tocris).

Optical imaging in the awake mice required two survival surgeries and was done by imaging through the transparent bone over the cerebellar cortex (Tohmi et al. 2006). In the initial surgery, a headholder system consisting of a restraining bar was attached to the frontal skull with three to four stainless steel screws embedded in cement. After a 1-wk recovery, the second surgery exposed the skull over Crus I, II, and PML by removing only the soft tissues. A watertight chamber around the exposed skull was constructed and covered with petroleum jelly. An analgesic (buprenorphine, 0.1 mg/kg, ip, b.i.d.) and antibiotic (penicillin 10,000 IU/kg, administered intramuscularly, q.d.) were given after both surgeries for 3–5 days. Data collection was initiated the day after the second surgery and continued for ≤5 days.

We also assessed the effects of L-type Ca<sup>2+</sup> channel agonists and antagonists on the oscillations in the awake tg mouse. The challenge was to gain access to the cerebellar cortex in the awake animal. In preliminary experiments, we found that removal of the bone to expose several folia of the cerebellar cortex (as in the anesthetized experiments) invariably resulted in the cerebellar cortex swelling, pushing through the craniotomy, and eventual vascular compromise of the cerebellar cortex. However, if on the day of imaging, only a small opening was made in the chamber under anesthesia that exposed approximately 50–75% of Crus II this problem could be minimized. The mouse was allowed to recover from the anesthesia and optical imaging was performed. After imaging for a period of about 60 min (baseline period), FPL (50 μM) was added to the bath over the site of the small craniotomy followed by imaging for another 60 min. Finally, diltiazem (50 μM) was added to the bath and the imaging resumed for 60 min.

Optical imaging

Using a modified Nikon epifluorescence microscope fitted with a ×4 objective, images were acquired with a Quantix cooled charge-coupled device camera with 12-bit digitization at a final resolution of 256 × 256 pixels (~10 × 10 μm per pixel) (Roper Scientific, Tucson, AZ). A 100-W mercury–xenon lamp (Hamamatsu Photonics, Shizuoka, Japan) with a DC-controlled power supply (Opti Quip, Highland Mills, NY) was used as the light source. Imaging flavoprotein autofluorescence used a band-pass excitation filter (455 ± 35 nm), an extended reflectance dichroic mirror (500 nm), and a >515-nm long-pass emission filter (Reinert et al. 2004). The imaging procedures for the awake mouse were similar to those for the anesthetized mouse experiments with the following additional steps. Using the restraining bar, the head was fixed and the body loosely fit into a plastic tube attached to the stereotaxic frame to prevent excessive body movement. The optical chamber was cleaned and filled with Ringer solution prior to placing the mouse on the imaging station. Caffeine (10–15 mg/kg, ip) was used to facilitate an attack of dystonia (Fureman et al. 2002).

Each image series consisted of 600 sequential frames, with an exposure time of 200 ms for each frame. The first 20 frames were used as control for baseline fluorescence. In some experiments, surface stimulation was delivered to the cortex after the first 20 frames. Surface stimulation consisted of a short train (10 pulses) at either 100 or 10 Hz, with a pulse width of 100 μs and amplitude of 100–300 μA. The optical responses evoked by PF stimulation were quantified as the change in fluorescence above background (ΔF/F) by defining a 15-pixel-wide region of interest along the entire evoked beam as detailed in previous publications (Gao et al. 2003; Reinert et al. 2004).

Neural and EMG data acquisition

Single-neuron firing was recorded with single-core quartz-platinum/tungsten electrodes (tetrode, 0.5–1.5 MΩ; Thomas Recording) using conventional electrophysiological techniques (Gao et al. 2006). The recording electrode was introduced caudally and used the imaging data as reference to ensure that the position of the recording electrode remained on or off the oscillation domains. The presence of spontaneous complex spikes and simple spikes was used to identify PCs. All other cells were classified as unidentified cerebellar neurons. The single-unit recording data were digitized (25 kHz) on-line and stored for additional off-line analysis including discrimination and firing rate histograms of the spike discharge in 1-ms bins (Spike2, Cambridge Electronic Design, Cambridge, UK). The locations of the cell recordings were mapped in relation to the optical signal.

The field potentials evoked by PF stimulation were recorded in the molecular layer using glass microelectrodes (2 M NaCl, 2–5 MΩ) as detailed in previous studies (Gao et al. 2003; Reinert et al. 2004). The field potential recordings were digitized at 25 kHz and the average of the responses to 16 stimuli at 1.0 Hz generated. Surface stimulation results in a classic triphasic potential (positive/negative/positive, P/N/P) “on beam” that reflects the action potential conducted along the parallel fibers, followed by a slower negativity (N<sub>2</sub>) that reflects the synaptic depolarization of Purkinje cells (Eccles et al. 1967). The
P₁/N₁ component was used as a measure of the presynaptic component and N₂ the postsynaptic component.

Bipolar intramuscular EMG recordings were obtained from ipsilateral hamstring and/or perioral whisker pad muscles using pairs of insulated fine wires (50 μm) exposed 1 mm at the tips. The EMG signals were band-pass filtered on-line (10 Hz to 3 kHz) and amplified, then digitized and stored at 6,250 samples/s (Spike2).

Optical analysis of oscillations

In each frame of an image series, the image intensity values were low-pass filtered using a 3 × 3-pixel mean filter centered in the current pixel. The optical signal at a pixel consists of the filtered intensity values across all frames within an image series. The optical signal for each pixel was linearly detrended and transformed into the frequency domain using a 2,048-point fast Fourier transform (FFT) algorithm. The power spectral distribution of the optical signals was computed using a 50% Hanning window with 25% overlap. At each pixel, the frequency at which the maximal power was reached was defined as the dominant frequency. The frequency and power maps of each series were created by assigning to each pixel a value equal to the dominant frequency and maximal power, respectively. The phase of the dominant Fourier coefficient was used to create the phase-shift maps. The frequency histograms presented in the figures were binned to 0.0098-Hz resolution and normalized to the total number of bins.

The frequency and power maps were used to quantify the oscillations and determine the effects of various manipulations on the oscillations in the anesthetized animals. Similarly, in the awake animals the frequency and power maps were used to quantify the oscillations in relation to the motor attacks and EMG activity. Frequency histograms of the maximal power at each pixel were used to determine the frequency bands of interest and to compare tg mice versus wild-type controls and dystonic versus baseline periods (see Figs. 1J and 5C). Using the frequency bands of interest, the next analyses were based on defining regions of high-amplitude oscillations, referred to as high power domains. The first step was to determine a common power threshold for all image series obtained during an experiment. The power threshold was defined as the mean power of all pixels during the control image series with frequencies in the band of interest (as defined earlier for anesthetized and awake animals). In the anesthetized animals, the control image series were those recorded before applying a drug. In the awake animals, the baseline image series were those recorded during periods without dystonia. All pixels above the power threshold, with frequencies in the band of interest, were conserved and the remaining pixels were set to 0. The conserved pixels were either in contiguous domains or isolated

![Image of optical analysis of oscillations](http://jn.physiology.org/)

**Fig. 1.** Spontaneous, low-frequency oscillations in the cerebellar cortex of the anesthetized tg mouse. A: sequential images of the cerebellar cortex show spontaneous oscillations in an anesthetized tg mouse. Large-amplitude oscillations are present in the paramedian lobule (PML) and lower-amplitude oscillations in Crus I and II. Each pseudocolored image shows the difference in fluorescence level relative to the background fluorescence (ΔF/F). Time from image acquisition onset indicated in the top right corner of each image. B: time course of ΔF/F obtained from 3 regions of interest (ROIs) indicated in the first image of A (colored boxes of 20 × 20 pixels). C–F: pixel-based spectral analysis shows the frequency (C), phase shift (D), power (E), and high-power domain maps (F) for the same experiment. Each map is superimposed on a background image of the cerebellar cortex. G–I: frequency (G), power (H), and high-power domain (I) maps from a wild-type (WT) mouse. Note that the frequency (G) and power maps (H) appear almost identical due to the very low power of the frequency band of interest, but in fact are not the same. J: distribution of frequencies shown as percentage of all pixels in anesthetized tg (n = 18) and WT (n = 9) mice.
pixels. Only the former were considered of interest. Therefore for the second step a domain area threshold of 400 contiguous pixels (0.04 mm²) was selected to eliminate isolated pixels and very small domains of contiguous pixels. All pixels not included in a domain of ≥400 pixels were set to 0. Also, if a high domain was located on a blood vessel, the pixels were set to 0. All null pixels were excluded from the maps. Therefore the autofluorescence signal in the high power domains reflects activity primarily within the cerebellar cortical neuropil.

If a map yielded no high power domains, the average domain power and the average domain area were set to 0. Finally, for the statistical analysis, the average domain power and the average domain area were normalized to the maximum respective values recorded during an experiment.

Although the power threshold and domain area were chosen empirically, a range of power thresholds (0.0, 0.5, and 1 SD above the mean) and domain areas (100–1,000 contiguous pixels) were examined and yielded similar results. The decision to use mean power for the threshold was to be widely inclusive of high-amplitude oscillations. Similarly, the domain area of 400 pixels retained small regions with well-defined oscillations.

To determine whether the area and power of the high power domains differed with the various drugs or in the dystonic versus baseline periods, a within-subjects repeated-measures analysis using a Friedman nonparametric test was performed on the rank order of the normalized average domain area and normalized average power data.

**Neural and EMG data analysis**

The continuous EMG recordings were divided in temporal windows of the same duration as the optical recordings and then rectified and linearly detrended. The analysis in the frequency domain is the same as that performed on the optical signals, except for the number of points in the FFT, which was set to 2²². This algorithm yields a frequency resolution close to that obtained in the optical data analysis. To perform coherence analysis between EMG and optical signals, the EMG signals were down-sampled from 6,250 to 5 Hz. Each down-sampled signal was then normalized to the maximum value.

The coherence (|R<sub>OptEMG</sub>(λ)|²) between the optical signal at each pixel (f<sub>Opt</sub>) and the EMG signal (f<sub>EMG</sub>) was evaluated by

\[ |R_{OptEMG}(\lambda)|^2 = \frac{|f_{OptEMG}(\lambda)|^2}{f_{OptEMG}(\lambda)f_{EMGEMG}(\lambda)} \]

\[ f_{OptEMG}(\lambda) = \frac{1}{2\pi LT} \sum_{i=1}^{L} d_{Opt}(\lambda, t) d_{EMG}(\lambda, t) \]

in which λ is a value in the frequency domain, L is the number of nonoverlapping windows, T is the time duration of a window, and d represents the Fourier coefficients (d is the complex conjugate of d) obtained by transforming the optical and the down-sampled EMG signals in the lth window through a 2,048-point FFT algorithm. The threshold for coherence significance was obtained by the formula 1 − (1 − α)²⁴⁴, where α is the level of confidence and L is the number of nonoverlapping windows used. In the present study 10 nonoverlapping windows were used and the level of confidence was set to 0.95. The resulting threshold to reach a significant coherence is 0.283 (Rosenberg et al. 1989). Coherence maps were constructed for each image series by assigning the coherence value to each pixel within a high power domain. All other pixels were excluded. For each animal, the coherence maps were averaged during baseline and dystonic periods, taking into account the overlaps of high power domains in different maps (i.e., the average of the coherence in overlapping high power domains was normalized so that overlapping and nonoverlapping regions contributed equally). For each of the behavior periods the average coherence and the relative percentage of nonnull pixels above the coherence significance threshold (0.283) were determined. For these analyses the data from the coherence maps based on EMG recordings from the hamstring and face were combined. Repeated-measures ANOVA analyses were performed to assess the differences in the average coherence and percentage of significant coherence between baseline and dystonic periods.

The continuous extracellular recordings were also divided into temporal windows of the same duration as the image series (124 s). The spike discrimination was performed using Spike2. The spike train data were transformed to a continuous discharge rate using fractional interspike intervals with down-sampling to 5 Hz (Taira et al. 1996). The spectral content was obtained using a 2,048-point FFT algorithm. A cell was classified as having low-frequency oscillations if the highest peak in the spectrum (excluding 0) was in the frequency band of interest for anesthetized animals (0.039–0.078 Hz).

All the data processing was performed using programs written in Matlab (The MathWorks, Natick, MA). The statistical analysis was performed using SAS (SAS Institute, Cary, NC). In the text and figures all results are reported as means ± SE.

**RESULTS**

Low-frequency oscillations characterized by cycles of large increases and decreases in fluorescence were observed in the cerebellar cortex of the tg mouse (Fig. 1, A and B). Oscillations were found in all folia imaged, including Crus I and II, PML, lobulus simplex, and lobules VII and VIII (Figs. 1C and 2A; see METHODS). The magnitude of the autofluorescence changes associated with the oscillations (Fig. 1B) are comparable to those evoked by direct electrical stimulation of the PFs (Fig. 2C), implying large fluctuations in cell activity (Reinert et al. 2004).

Analysis and quantification of the oscillations were based on spectral analyses of the autofluorescence signal. The oscillations in the optical signal were characterized by determining the frequency with the maximum power at each pixel location. Using these values, frequency, power, and phase-shift maps were constructed. Contiguous regions of oscillations in phase and within a narrow range of frequencies are evident in the frequency and phase maps (Fig. 1, C and D). Conversely, different cortical regions oscillate at varying mean frequencies and different regions are not necessarily in phase. The frequency histograms from anesthetized tg and wild-type mice show a different distribution of frequencies. The largest differences were between 0.039 and 0.078 Hz (Fig. 1J, Student’s t-test, P = 0.02), which was defined as the frequency band of interest.

The power maps based on the frequency band reveal a wide range of amplitude in the oscillations (Fig. 1E). In this study we focused on analyzing and quantifying the larger-amplitude oscillations because the low-amplitude oscillations were highly variable within and across experiments. Therefore, high power domains were defined as regions of ≥400 contiguous pixels (area of 0.04 mm²) with frequencies within this frequency band and greater than average power (Fig. 1F). High power domains were observed in 78% (14/18) of the anesthetized tg mice. The average area and power of the high power domains were 0.34 ± 0.04 mm² and 1,123 ± 147, respectively, in the anesthetized tg mice (n = 18). In wild-type animals (n = 9), oscillations at these frequencies occurred only at isolated pixels and high power domains were never found (Fig. 1, G–J). Based on the presence of high power domains, the low-frequency oscillations occurred most frequently in PML and Crus II (37
and 34%, respectively, of total observations), less frequently in the vermis and Crus I (24 and 18%), and occasionally in lobulus simplex (LS, 5%).

The oscillations were spontaneous and transient. The oscillations develop, increase in amplitude, and subside over 30–120 min (Fig. 2A). At the beginning of this series of images, there were oscillations in both Crus II and SL. Over a period of about 60 min, the oscillations in SL expanded in size and spread into both Crus I and Crus II. Subsequently, the oscillations contracted to a few small regions. This sequence of development, expansion, and contraction of the oscillations was observed in 72% (13/18) of the anesthetized tg mice studied.

There are several reports that PF–PC synaptic transmission is altered in the cerebellar slice from the tg mouse (Matsushita et al. 2002; Zhou et al. 2003). Therefore we used flavoprotein imaging to assess the optical response to PF stimulation in the intact preparations. Stimulation of the PFs evokes a beam-like increase in fluorescence (Fig. 2B) that primarily reflects activation of PCs and inhibitory interneurons (Gao et al. 2006; Reinert et al. 2004). This evoked response was decreased in tg mice compared with wild-type. During time periods in which high power domains were not present, the response amplitude evoked by surface stimulation was reduced by 47 ± 4% (P = 0.0006, Student’s t-test, n = 5) in the tg mice (Fig. 2, B and C). Interestingly, when high power domains were present, the response to PF stimulation was suppressed by >90% (P = 0.0004, Student’s t-test, n = 5), irrespective of stimulation strength (Fig. 2C).

Low-frequency oscillations were also common in the spontaneous firing of 88 cerebellar cortical neurons recorded in nine anesthetized tg mice. This is illustrated in Fig. 3, A–C for the discharge of two PCs and one unidentified cerebellar neuron. The firing of each neuron exhibited oscillations within the frequency range observed in the optical recordings, as shown in the power spectrum of the firing. A cell was defined as having low-frequency oscillations if the highest peak in the spectrum (excluding 0 Hz) from the firing discharge occurred within the frequency band identified from the optical imaging (0.039–0.078 Hz). Using this criterion, 91% of PCs (42/46) and 74% (31/42) of unidentified cerebellar neurons exhibited low-frequency oscillations (Fig. 3D). Therefore the oscillations in the autofluorescence signal are not merely a metabolic phenomenon and the oscillations are widely reflected in the firing of cerebellar cortical neurons.

A major question is whether the oscillations are intrinsic to the cerebellar cortex or are due to abnormal afferent input. Blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors with 50 μM DNQX (a potent and competitive antagonist of non-N-methyl-D-aspartate, ionotropic glutamate receptors), the dominant ionotropic glutamate receptors at PF–PC and climbing fiber–PC synapses (Konnerth et al. 1990; Perkel et al. 1990), did not significantly affect the occurrence (χ² = 0.61, P = 0.43), average area [F(1.4) = 0.39, P = 0.59], or power [F(1.4) = 0.09, P = 0.77] of the high power domains in tg mice (Fig. 4, A and B). The effectiveness of DNQX on blocking PF–PC synaptic transmission in vivo was verified by testing the effects on the field potentials evoked by PF stimulation. Application of DNQX produced a complete block of the postsynaptic component N₂ (< 0.16 ± 0.01 mV during baseline and 0.00 ± 0.00 mV with DNQX) with no effect on the presynaptic component P₁/N₁ (0.37 ± 0.02 and 0.36 ± 0.04 mV). This observation is in agreement with our previous studies in vivo showing that a similar antagonist, CNQX, blocks the postsynaptic responses to PF stimulation in the molecular layer and the postsynaptic responses evoked by peripheral inputs in the granular layer (Chen et al. 1996, 2001; Reinert et al. 2004). Furthermore, stimulation of the PFs (including stimulation of PFs that intersected high power domains) over a wide range of frequencies (0.05–100 Hz) and
train durations (1–100 s) did not initiate oscillations or modify ongoing oscillations. In six tg mice, PF stimulation did not significantly affect the occurrence ($\chi^2 = 0.88, P = 0.64$), power [$F(2,5) = 0.7, P = 0.52$], or size [$F(2,5) = 0.19, P = 0.83$] of the high power domains. These results demonstrate that the oscillations in high power domains are not dependent on AMPA-mediated glutamatergic synaptic transmission. Nor are the oscillations initiated or affected by PF stimulation. We conclude that the oscillations are primarily intrinsic to the cerebellar cortex.

Because L-type Ca\(^{2+}\) channels are essential for the expression of the episodic dystonia in tg mice (Campbell and Hess 1999), we tested whether the oscillations are Ca\(^{2+}\) and L-type Ca\(^{2+}\) channel dependent. Removal of Ca\(^{2+}\) from the bathing solution significantly reduced the occurrence ($\chi^2 = 8.97, P = 0.003$), area [$F(1,4) = 25.85, P = 0.01$], and power [$F(1,4) = 15.19, P = 0.02$] of the oscillations (Fig. 4, C and D). Blocking L-type Ca\(^{2+}\) channels with diltiazem also reduced the occurrence ($\chi^2 = 10.4, P = 0.001$), area [$F(1,3) = 10.33, P = 0.049$], and power [$F(1,3) = 10.33, P = 0.049$] (Fig. 4, E and F). Conversely, addition of the L-type Ca\(^{2+}\) channel agonist FPL (50 \(\mu\)M) to the bath resulted in an increase in the occurrence ($\chi^2 = 4.99, P = 0.003$), power [$F(1,4) = 17.5, P = 0.014$], and area [$F(1,4) = 9.1, P = 0.039$] of the oscillations (Fig. 4, G and H). The application of FPL also induced oscillations in wild-type littermates ($n = 8$). This is not unexpected because systemically injected L-type Ca\(^{2+}\) agonists induce dystonia in both wild-type and tg mice (Campbell and Hess 1999; Jinnah et al. 2000). Although the average area of the high power domains of these oscillations was not statistically different in wild-type (0.27 ± 0.03 mm\(^2\)) and tg (0.49 ± 0.09 mm\(^2\)) mice [$F(1,11) = 1.12, P = 0.313$], the power was lower in wild-type (601 ± 216) than that in the tg mice (5,590 ± 1,388) [$F(1,11) = 6.56, P = 0.026$]. These results demonstrate that L-type Ca\(^{2+}\) channels play a major role in the generation of the oscillations.

In the tg mouse, the FPL was added to the bath when the amplitude of the oscillations was small, partially accounting for the relatively smaller area and power in the control period (Fig. 4, G and H). To test whether the effects of FPL were due to random occurrence of oscillations that followed a period of weak oscillations an additional analysis was performed. In the anesthetized tg mice without application of FPL, the periods with spontaneous oscillations were divided in half. The animals that showed strong oscillations in the first half (mean of area and power greater than the mean area and power of the baseline periods in the mice with FPL application) were discarded. For the remaining animals ($n = 11$) there were no significant differences in area [$F(1,10) = 0.02, P = 0.88$] or power [$F(1,10) = 0.02, P = 0.88$] of the oscillations between the first and second halves. Therefore the oscillations observed after FPL application were due to the application of this L-type Ca\(^{2+}\) channel agonist.

Low-frequency oscillations are present in the cerebellar cortex of awake tg mice (Fig. 5A). This was determined by taking advantage of the transparent bone over the cerebellar cortex and the ability to image the flavoprotein signal transcranially (Tohmi et al. 2006). Comparing the frequency histograms in the baseline (i.e., nondystonic periods) versus during dystonic periods in awake animals ($n = 7$), the frequency band of interest was determined to be 0.078 to 0.313 Hz (Fig. 5C). Student’s $t$-test, $P = 0.0001$. The oscillation frequencies were higher and spanned a greater range than those in the anesthetized animals (compare Figs. 5C and 1J), likely reflecting the effects of anesthetics on cerebellar cortical and PC excitability (Sato et al. 1993). High power domains defined by this frequency band were observed both during baseline (average area 0.11 ± 0.02 mm\(^2\), average power 547 ± 74) and dystonic

FIG. 3. Oscillating activity in the firing of single cells in the anesthetized tg mice. A–C: on the left are examples of the spontaneous firing rate of 2 Purkinje cells (A and B) and an unidentified cerebellar cell (C). On the right are the corresponding power spectra. The vertical bars on the $y$-axis denote the power. D: number of neurons with and without low-frequency oscillations in their spontaneous firing. Neurons were defined as possessing oscillations if the peak power in the firing was between 0.039 and 0.078 Hz, as shown for the example cells in A–C.
periods (average area $0.61 \pm 0.08 \text{ mm}^2$, average power $1.316 \pm 124$). In the awake $tg$ mouse, caffeine not only triggers the motor attacks but also accentuates the oscillations. The occurrence ($\chi^2 = 46.6, P = 0.0001$), area $[F(1,6) = 13, P = 0.01]$, and power $[F(1,6) = 9.51, P = 0.02]$ of the high power domains increased significantly during the dystonic episodes induced by caffeine (Fig. 5B).

In awake wild-type mice ($n = 8$), only very weak oscillations (average area $0.012 \pm 0.006 \text{ mm}^2$, average power $11 \pm 5$) were observed. It is worth noting that the average area and power of the oscillations in the wild-type mice were an order-of-magnitude smaller than those observed in the $tg$ mice. The occurrence ($\chi^2 = 15.82, P = 0.0001$), area $[F(1,13) = 8.24, P = 0.01]$, and power $[F(1,13) = 8.24, P = 0.01]$ in the wild-type mouse were significantly different from those in the $tg$ mouse. These marked differences in the oscillations also exclude potential confounds in the awake preparation, such as the use of buprenorphine, because this analgesic was used in both the $tg$ and wild-type mice.

To test for coupling between the oscillations in the cerebellar cortex and the motor attacks, hindlimb and/or face EMG activity were recorded simultaneously with the cerebellar optical imaging in awake $tg$ mice (see Fig. 6A, inset). During the periods of dystonia there were bursts of EMG activity at the same low frequencies as those observed in the cerebellar cortex (Fig. 6A). The power spectrum of the EMG activity was determined from 11 hamstring and 11 whisker pad recordings in 12 $tg$ mice and the power spectra were averaged across baseline and dystonic periods (Fig. 6B). As expected, during the dystonic movements there was a general increase in the EMG power. The largest differences in power were observed between $0.077$ and $0.28$ Hz (Fig. 6B, Student’s $t$-test, $P = 3.5 \times 10^{-17}$), similar to the frequency range found for the optical recordings in the cerebellar cortex (Fig. 5C).

Next, we calculated the coherence between the EMG activity and the optical signals within the high power domains to generate a coherence map. Coherence magnitude increased during the dystonic periods, as shown in the example coherence maps from an experiment in which both the hamstring and whisker pad EMG activity were recorded (Fig. 6, C and D). In six $tg$ mice in which simultaneous EMG and optical recordings were obtained, the average coherence increased from $0.17 \pm 0.02$ during the baseline periods to $0.31 \pm 0.05$ during dystonic periods $[F(1,5) = 6.98, P = 0.046]$; Fig. 6E). The average coherence magnitude exceeded the level required for significance (0.283 for the 95% confidence level). Furthermore, the percentage area of the high power domains with significant coherence increased from $17 \pm 5$ to $50 \pm 11% [F(1,5) = 8.53, P = 0.03$; Fig. 6E]. Therefore during the attacks of dystonia the cerebellar cortical oscillations increase and are coupled with the abnormal EMG activity.

The final experiment tested whether L-type Ca$^{2+}$ channels are involved in the oscillations in the awake $tg$ mouse. A small craniotomy was made in the bone over Crus II under anesthesia. The mice ($n = 4$) were allowed to recover from the anesthesia followed by imaging in the awake animal before, during, and after the application of FPL and diltiazem (see METHODS). Adding FPL (50 $\mu$M) to the bath produced low-

![Fig. 4. Roles of AMPA receptors, extracellular Ca$^{2+}$, and L-type Ca$^{2+}$ channel agents on the cerebellar oscillations in anesthetized $tg$ mice. A and B: high power domain maps (A) and average normalized area and power (B) before and after bath application of DNQX (50 $\mu$M) in 4 $tg$ mice. C–F: similar maps and plots for normal Ringer’s (Control) and Ca$^{2+}$-free Ringer’s (0 Ca$^{2+}$ and 2 mM EGTA) in 6 $tg$ mice (C and D) and before and after diltiazem (50 $\mu$M) in 4 $tg$ mice (E and F). G and H: high power domain maps and area and power averages in normal Ringer’s and with the addition of FPL (50 $\mu$M) to the bath in 5 $tg$ mice.](http://jn.physiology.org/DownloadDownloaded from http://jn.physiology.org by 10.220.33.2 on April 12, 2017)
frequency oscillations (Fig. 7A) and the frequency range of the oscillations was similar to that evoked by caffeine (Fig. 5C). During FPL application the power in these low-frequency oscillations (using the frequency range defined for awake animals of 0.078 to 0.313 Hz) was significantly greater than that observed in the baseline period (Student’s $t$-test, $P < 0.002$). The subsequent addition of diltiazem (50 μM) to the bath completely suppressed the oscillations (Fig. 7A). The two drugs had significant effects on both the area and power ($F(2,6) = 14.5$, $P = 0.005$ and $F(2,6) = 13.4$, $P = 0.006$, respectively). A post hoc comparison with Bonferroni correction showed that the FPL produced a significant ($P < 0.05$) increase in the area and power compared with the baseline and diltiazem produced a significant suppression of the FPL-induced oscillations (Fig. 7B). There were no significant differences in the area and power in a comparison of the mice at baseline and after addition of diltiazem. Although systemic administration of an L-type Ca$^{2+}$ channel agonist evokes an attack of dystonia (Campbell and Hess 1999), the application of FPL to Crus II did not evoke abnormal movements. However, this is not unexpected because only a small and specific area of the cerebellar cortex was exposed to the drug. For the induction of dystonia most likely a larger area of the cerebellar cortex needs to be exposed to the FPL.

**DISCUSSION**

Taking advantage of the capacity of flavoprotein optical imaging to monitor the circuitry of the cerebellar cortex, this

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** Cerebellar oscillations in awake tg mouse and their relationship with episodes of dystonia. A: example high power domain maps in the awake tg mice during baseline and dystonic periods. B: average normalized area and power of the oscillations (means ± SE, $n = 7$ tg mice). Both measures increase significantly during dystonia (*). C: frequency histograms from the optical recordings during the baseline and dystonia periods ($n = 7$ tg mice).

![Figure 6](http://jn.physiology.org/)

**FIG. 6.** Oscillations in the electromyographic (EMG) activity and relation to the optical signals in the cerebellar cortex. A: power spectrum frequency distributions from a single mouse for the optical (bars) and hamstring EMG recordings (lines) during dystonia (red) and baseline (blue) periods. Inset shows regular, low-frequency bursts in the hamstring EMG during dystonia. B: average power spectrum in the EMG activity in awake tg mice during baseline (blue) and dystonia (red) periods. The data from the hamstring and whisker pad recordings were combined in this plot. C and D: examples of coherence maps between the optical activity within high power domains and the EMG activity for the hamstring (C) and whisker pad (D). Arrow pointing to the scale bar denotes coherence significance level ($P < 0.05$). Data are from same experiment shown in A. E: average area with significance coherence and average coherence magnitude during baseline and dystonic periods; both are significantly increased during the dystonia (*).
had oscillations at the same low frequencies. That the firing of the vast majority of cerebellar cortical neurons
neuronal activity is demonstrated in this study by the finding close coupling between the optical signal and the underlying
an increase in activity results in an increase in fluorescence
ative metabolism and closely reflects neuronal activity in which
awake animal. The flavoprotein signal is a measure of oxida-
space and time, the use of optical imaging proved invaluable to
mouse. Given the variable nature of the oscillations in both
Diltiazem resulted in a significant reduction in the FPL-induced oscilla-
in an increase both area and power compared with the baseline (*).
oscillations in the awake tg mouse. The flavoprotein signal is a measure of oxida-
mental and has been attributed to vasomotor activity (Mayhew
-oscillations in the cerebellar cortex of the
tg mouse, low-frequency oscillations between 0.039 and 0.078 Hz were observed in the various folia
oscillations within a region of a folium were in phase
frequency band of the oscillations is more than an order
study uncovered the existence of transient, very low frequency,
Ca2+-dependent oscillations in the cerebellar cortex of the tg
variable nature of the oscillations in both space and time, the use of optical imaging proved invaluable to
detect and characterize the oscillations in the anesthetized and
animal. The flavoprotein signal is a measure of oxidative
metabolism and closely reflects neuronal activity in which
an increase in activity results in an increase in fluorescence
(Gao et al. 2006; Reinitert et al. 2004; Shibuki et al. 2003). The close coupling between the optical signal and the underlying
neuronal activity is demonstrated in this study by the finding that the firing of the vast majority of cerebellar cortical neurons
had oscillations at the same low frequencies.
In the anesthetized tg mouse, low-frequency oscillations
between 0.039 and 0.078 Hz were observed in the various folia
oscillations within a region of a folium were in phase and
within a narrow range of frequencies, although the amplitudes varied greatly in different regions. The oscillations can
develop into high power domains and propagate to neighboring regions. The discharge of the majority of cerebellar cortical neurons, both PCs and unidentified cerebellar neurons, were oscillating within the same frequency band. Similar oscillations were imaged in the cerebellar cortex of the awake tg mouse and the oscillations increased during the dystonic episodes. The limb and face EMG activity have oscillations at similar frequencies during the episodes of dystonia and the EMG activity becomes coherent with the oscillations in the cerebellar cortex. These previously unreported oscillations in the optical signal and single-cell firing provide new informa-
tion into the abnormalities in cerebellar cortical function in the 
tg mouse, adding to the previously described reduction in PF–PC synaptic transmission, increase in GABAergic synaptic efficacy, and increased variability in PC discharge (Hoebeek et 
The frequency band of the oscillations is more than an order
of magnitude lower than expected for cardiovascular or respira-
atory movements. In the anesthetized animal, the heart rate
was typically about 8 Hz (~500 beats/min) and the respiratory
rate about 2 Hz (~120 strokes/min). The latter was clearly
detectable in the spectral analyses (data not shown). Similar
heart and respiratory rates are found in awake animals (Desai
et al. 1997; Fairchild 1972). A pervasive 0.1-Hz signal has
been described for the intrinsic hemodynamic reflectance signal and has been attributed to vasomotor activity (Mayhew
et al. 1996). However, there is no evidence for a 0.1-Hz
oscillation in the flavoprotein signal in wild-type mice (Fig. 1,
G–I). Also, the hemodynamic reflectance signal is of a funda-
mentally different origin than flavoprotein autofluorescence and
is imaged at different wavelengths using reflectance. Hemodynamics contributes little, if any, to the flavoprotein signal
(Husson et al. 2007; Reinitert et al. 2004; Shibuki et al. 2003).
Also, several properties of the low-frequency oscillations in the
tg mouse are not consistent with a systemically generated
vasomotor signal, particularly the loss of the response to PF
stimulation when high power domains were evident.
The evidence suggests that the oscillations are likely gener-
ated within the cerebellar cortex. Blocking AMPA-mediated
afferent inputs to the cerebellar cortex did not affect the
oscillations. DNQX and similar AMPA antagonists block the
vast majority of the PF and climbing fiber input to PCs (Dunbar
et al. 2004; Konnerth et al. 1990; Reinitert et al. 2004) as well
as a large fraction of the mossy fiber input to granule cells
(Konnerth et al. 1990). Also, PF stimulation did not initiate
oscillations or alter ongoing oscillations. These observations,
coupled with the fact that the oscillations occur in anesthetized
mice in which sensory feedback from the periphery is greatly
reduced, suggest that it is unlikely that the oscillations are due
to peripheral feedback generated during dystonic attacks. Fi-
nally, topical application of the L-type Ca2+ channel agonist
FPL increased the area and power of the high power domains
in both the anesthetized and awake mice. This provides the
most direct evidence that the oscillations can be initiated and
generated within the cerebellar cortex. Our results do not
address the question of whether neurons outside of the cere-
bellar cortex also exhibit low-frequency oscillations. P/Q-type
Ca2+ channels are also expressed in noncerebellar neurons,
including in the neocortex, hippocampus, and spinal cord (Stea
et al. 1994; Svoboda et al. 1997; Westenbroek et al. 1995), and
it remains to be determined whether in the tg mouse these
regions have similar low-frequency oscillations.
The reduction in the optical response to PF stimulation when the high power domains were not present is consistent with
previous observations that PF–PC synaptic transmission is
decreased in the tg mouse (Matsushita et al. 2002) and/or that
PF–PC synaptic transmission is more susceptible to inhibition
mediated by γ-aminobutyric acid (GABA) type B receptors
(Zhou et al. 2003). We did not attempt to distinguish between
these two possibilities. However, the extent of the reduction in
glutamatergic transmission at PF–PC synapses remains to be
clarified because the loss of presynaptic P/Q-type Ca\(^{2+}\) channels can be compensated to some degree by an increase of N-type Ca\(^{2+}\) channels (Zhou et al. 2003). The evoked response to PF stimulation is almost completely lost (<90%) when high power domains are present. Because the optical signal represents the overall energy metabolism of the cells and the reduction/oxidation (redox) state of the flavoproteins (Reinert et al. 2004, 2007; Shibuki et al. 2003), the simplest explanation is that the large-amplitude oscillations in the flavoprotein signal mask the optical response evoked by PF stimulation. Other possibilities include changes in the flavoproteins (e.g., density) or changes in the activation of the mitochondria due to alterations in Ca\(^{2+}\) dynamics in the tg mouse. Irrespective of the state of PF–PC synaptic transmission, these large oscillations in the redox state of the tg cerebellar cortex are highly abnormal.

The cellular mechanisms underlying the oscillations need elucidation. Calcium entry through up-regulated L-type Ca\(^{2+}\) channels on PCs is involved because oscillations are reduced by blocking these channels and augmented by opening them. Similar oscillations occur in immature PCs in which L-type Ca\(^{2+}\) channels are dominant (Liljelund et al. 2000), further implicating L-type Ca\(^{2+}\) channels. We can only speculate, but one plausible mechanism is the interplay between Ca\(^{2+}\) entering through the L-type Ca\(^{2+}\) channels and Ca\(^{2+}\) released from inositol-1,4,5-trisphosphate and ryanodine-sensitive intracellular stores in PCs (Berridge 1998; Finch and Augustine 1998; Kano et al. 1995). Oscillations at low frequencies involving intracellular Ca\(^{2+}\) stores occur in both neuronal and nonneuronal systems (Berridge 1998; Tsien and Tsien 1990). This permissive mechanism also offers an explanation for the role of caffeine in triggering an attack. Caffeine could initiate oscillations by mobilizing Ca\(^{2+}\) from PC intracellular stores (Carter et al. 2002; Kano et al. 1995), which in turn results in the depolarization of the cell membrane and opening of L-type Ca\(^{2+}\) channels. However, the role that the reduction in P/Q-type Ca\(^{2+}\) channel conductance plays in the oscillations is unclear. One possibility is that the loss of P/Q-type Ca\(^{2+}\) channel function simply results in the overexpression of L-type Ca\(^{2+}\) channels and changes in the dynamics of Ca\(^{2+}\) influx. Another possible mechanism is that the decrease in PF–PC synaptic transmission effectively isolates PCs from their normal PF input and without that normal input the oscillations are no longer generated. Clearly, studies are needed of the cellular mechanisms responsible for the oscillations.

The mechanisms by which the oscillations increase in power and expand into neighboring regions also need to be investigated. One possibility is that the oscillations propagate via intracortical inhibitory circuitry, including molecular layer inhibitory interneurons, Purkinje cell axon collaterals, or Golgi cells (Eccles et al. 1967; Ito 1984). Molecular layer interneurons are electrotonically coupled sufficiently to generate synchronous firing (Mann-Metzer and Yarom 1999). It has been shown that electrotonically coupled inhibitory neurons could synchronize oscillatory activity in neural networks (Bartos et al. 2002; Kopell and Ermentrout 2004). Moreover, GABAergic transmission is increased in the tg mouse (Zhou et al. 2003) and increased inhibition can lead to synchronization of PCs (Cheron et al. 2004). Another potential pathway for the propagation is the electrotonically coupled Bergmann glia (Muller et al. 1996) because these cells can alter PC excitability (Araque and Perea 2004).

The differences in the frequencies observed between anesthetized and unanesthetized tg mice are likely due to effects of the anesthetics. Application of FPL in the awake tg mouse results in oscillations in a frequency range similar to that observed with systemic caffeine injection. Conversely, in the anesthetized mouse FPL results in an increase in the oscillations within the frequency band in which the spontaneous oscillations occur. In various cell types including neurons, the frequency of the Ca\(^{2+}\) oscillations is dependent on both the Ca\(^{2+}\) fluxes across the cell membrane and the release/loading dynamics of the intracellular stores (Berridge 2005). Anesthetics, especially the ketamine used in the present study, have substantial effects on the excitability of the cerebellar cortex, including PCs (Sato et al. 1993; Schenewille et al. 2006; Servais and Cheron 2005). The up-regulated L-type Ca\(^{2+}\) channels are also likely to contribute to the differences in the oscillation frequencies, given that the recovery and restoration time constants of these channels are highly dependent on the depolarization level (Harasztosi et al. 1999). Therefore by altering Ca\(^{2+}\) conductance, anesthetics could alter the frequency of the oscillations.

We hypothesize that the low-frequency oscillations in the cerebellar cortex play a major role in the motor attacks in the tg mouse. Both the cerebellar oscillations and the motor attacks are transient and can be triggered by caffeine (Fureman et al. 2002). The synchronization and propagation of the oscillations occur over 30–120 min, consistent with the time course of the motor attacks (Green and Sidman 1962; Noebels and Sidman 1979). Also, the propagation of the oscillations across the cerebellar cortex is consistent with the spread of the motor attacks, beginning in the hindlimbs and then progressing to the forelimbs and face. The oscillations were most frequently observed in two adjacent folia, Crus II and PML, and this finding is suggestive of a consistent initiation site. However, we note that this inference is based on imaging in a limited number of folia. Low-frequency bursts are present in the EMG activity of hindlimb and face muscles during the attacks of dystonia and the coherence between the cerebellar cortical flavoprotein signals and EMG activity increases. The results also demonstrate that L-type Ca\(^{2+}\) channel agonists induce both the oscillations in the cerebellar cortex (Figs. 4 and 7) and the episodic dystonia (Campbell and Hess 1998). Conversely, L-type Ca\(^{2+}\) channel blockers suppress the oscillations and decrease the duration of the motor attacks (Campbell and Hess 1999; Fureman et al. 2002). Together these findings suggest these low-frequency oscillations are integral to the pathological processes that underlie the motor attacks in the tg mouse.

A model by which the cerebellar cortical oscillations contribute to the episodic dystonia is that these intrinsically generated oscillations greatly alter PC output. This abnormal output is likely transmitted downstream to the cerebellar nuclei (Campbell and Hess 1998). The synchronization of neuronal activity over a region of the cerebellar cortex is likely amplified at the level of the cerebellar nuclei due to the extensive convergence of PCs on nuclear neurons (Ito 1984). There is also a preliminary report of abnormalities in synaptic transmission between PCs and cerebellar nuclear neurons and in the firing properties of nuclear neurons in thetg mouse (Hoebeck et al. 2007). The targets of this abnormal cerebellar output,
including the red nucleus, motor thalamus, and motor cortex, will be extensively modulated. The motor attacks increase expression of the immediate early transcription factor c-fos in the cerebellar cortex and in cerebellar output pathways, including the deep cerebellar nuclei, red nucleus, and the ventrolateral thalamic nuclei (Campbell and Hess 1998). In turn, these motor structures, through descending pathways to the spinal cord and brain stem motor systems, can influence the excitability of motoneurons and muscle activation.

Finally, it is not known whether low-frequency oscillations occur in the cerebellum of EA2 patients or contribute to their movement disorder. However, there is increasing evidence for the involvement of the cerebellum in the generation of dystonic movements (Jinnah and Hess 2006; Raike et al. 2005). Some dystonia patients have cerebellar structural abnormalities and hypermetabolic signals (Delmaire et al. 2007; LeDoux et al. 2003; Odergren et al. 1998; Preibisch et al. 2001). In animal models, both the leaner mouse (a Cacna1a mutant) and the Ca\textsubscript{2.1} null mutant exhibit dystonic movements (Fletcher et al. 2001; Green and Sidman 1962). In both of these models, Ca\textsuperscript{2+} currents in Purkinje cells are either reduced (leaner) or eliminated (null). Dysfunctional cerebellar output is a major contributor to the generalized dystonia of the dystonic rat and lesions of the cerebellum mitigate the dystonia (LeDoux et al. 1993, 1995). Cerebellar abnormalities in this rat model include decreased GABAergic activity in the cerebellar nuclei and defective climbing fiber innervation of PCs (Beales et al. 1990; Brown and Lorden 1989; Lorden et al. 1985; Stratton et al. 1988). The oscillations in the cerebellar cortex of the ig mouse provide another example of abnormal cerebellar activity in a dystonic syndrome.

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References


Matsushita K, Wakamori M, Rhyu IJ, Arii T, Oda S, Mori Y, Imoto K.

Noebels JL.

Mintz IM, Sabatini BL, Regehr WG.

Mintz IM, Adams ME, Bean BP.


Moral changes and channelopathies of the central nervous system.


