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Increased Noise Level of Purkinje Cell Activities Minimizes Impact of Their Modulation during Sensorimotor Control

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Summary

While firing rate is well established as a relevant parameter for encoding information exchanged between neurons, the significance of other parameters is more conjectural. Here, we show that regularity of neuronal spike activities affects sensorimotor processing in *tottering* mutants, which suffer from a mutation in P/Q-type voltage-gated calcium channels. While the modulation amplitude of the simple spike firing rate of their floccular Purkinje cells during optokinetic stimulation is indistinguishable from that of wild-types, the regularity of their firing is markedly disrupted. The gain and phase values of *tottering's* compensatory eye movements are indistinguishable from those of flocculectomized wild-types or from *totterings* with the flocculus treated with P/Q-type calcium channel blockers. Moreover, normal eye movements can be evoked in *tottering* when the flocculus is electrically stimulated with regular spike trains mimicking the firing pattern of normal simple spikes. This study demonstrates the importance of regularity of firing in Purkinje cells for neuronal information processing.

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Introduction

Modulation of firing rate is typically the only parameter of neural activity that is considered when assessing the influence of that activity on sensorimotor behavior. Modulation-behavior relationships have been demonstrated in many experimental contexts, including lower and higher sensory and motor systems (Frazor et al., 2004; Li et al., 1999). Theoreticians have pointed out the impact of noise levels (i.e., regularity of firing) in neuronal network models (Mar et al., 1999; Steinmetz et al., 2001; Tiesinga et al., 2002), but it remains to be demonstrated experimentally whether a change in noise without a change in spike modulation can alter sensorimotor behavior. We looked for such a dissociation between neuronal firing rate modulation and neuronal noise in the context of calcium channelopathies, because various mutations in voltage-gated calcium channels result in both cell physiological and behavioral aberrations (Cao et al., 2004; Llinas et al., 1989; Mintz et al., 1992; Ophoff et al., 1996; Qian and Noebels, 2000; Stahl, 2002). We focused on the *tottering* mutant (*tg*), which suffers from a point mutation in CACNA1A, the gene encoding the $\alpha 1_a$-subunit of the P- and Q-type voltage-gated calcium channel (Bourinet et al., 1999; Fletcher et al., 1996). The mutation affects the extracellular membrane domain of the pore-forming loop. The cerebellar Purkinje cells of these mutants show a complex combination of cellular abnormalities, including a reduction in Ca^{2+} channel current density (Wakamori et al., 1998), a reduction in the amplitude of the parallel fiber-Purkinje cell EPSC (Matsushita et al., 2002), and an increased susceptibility to inhibitory modulation by GABAergic interneurons (Zhou et al., 2003). The general importance of these deficits for motor behavior is suggested by the fact that mutations in the $\alpha 1_a$-subunit of the P- and Q-type channels are associated with motor coordination problems in both mice (Campbell et al., 1999; Fletcher et al., 1996; Green and Sidman, 1962; Stahl, 2004) and humans (Ducros et al., 1999; Ophoff et al., 1996; Zhuchenko et al., 1997). We therefore set out experiments in *tg* mutants and controls to systematically investigate the relationship between motor performance and the modulation and regularity of Purkinje cell simple and complex spike firing rates. The flocculus of the vestibulocerebellum, which controls compensatory eye movements, was used as a model system to determine these correlations quantitatively. In this system, relationships among sensory input, motor output, and intermediate Purkinje cell activities can be rigorously defined (De Zeeuw et al., 1995; Goossens et al., 2004; Stahl and Simpson, 1995a; Stahl and Simpson, 1995b; Simpson et al., 1996).

Results

Mutation in P/Q-Type Calcium Channel Leads to Abnormal Sensorimotor Behavior

The *tg* mutants under investigation showed general ataxic behavior during locomotion, as described pre-

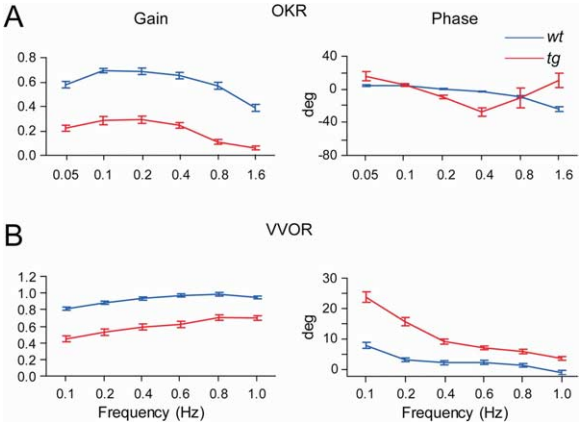

Figure 1. Compensatory Eye Movements in the Presence of Vision Are Impaired in *tg* Mice

Gain values (left panels) of *tg* mutants are decreased during the optokinetic reflex (OKR) (A) and vestibuloocular reflex in the light (VVOR) (B). Phase curves (right column) of the *tg* mutants only show significant changes during VVOR. Blue lines and red lines indicate data of wild-types (wt; n = 13) and mutants (*tg*; n = 9), respectively. Error bars indicate the mean ± SEM.

viously (e.g., Campbell et al., 1999). To quantify motor dysfunction, we determined gain and phase of eye movements during sinusoidal optokinetic and vestibular stimulation at frequencies varying from 0.05 Hz to 1.6 Hz (Figure 1). We studied nine *tg* mutants and thirteen wild-type littermates. During the optokinetic reflex (OKR), the gain values of *tg* mice ranged from 0.06 to 0.29 over the tested frequency band, while those in wild-types ranged from 0.39 to 0.69; these values were significantly different ($p << 0.001$, repeated measures ANOVA) (Figure 1A). During vestibular stimulation in the light (VVOR), the gain values ranged from 0.44 to 0.70 in *tg* mice and from 0.81 to 0.99 in wild-types; these gain versus frequency relationships also differed significantly ($p << 0.001$, repeated measures ANOVA) (Figure 1B). During the vestibuloocular reflex in the dark (VOR), the gain values in *tg* mutants were hardly different from those in wild-type littermates (data not shown). The phase versus frequency curves of *tg* mutants differed significantly from those in wild-types during VVOR ($p < 0.001$, repeated measures ANOVA), but not during OKR ($p = 0.5$, repeated measures ANOVA). These data indicate that compensatory eye movements driven in part or in totality by vision (i.e., VVOR and OKR) are markedly abnormal in *tg* mutants.

Purkinje Cells with Abnormal P/Q-Type Calcium Channels Fire Irregularly, but Modulate Normally

To find out whether the motor deficits in *tg* mice can be caused by abnormal firing of their Purkinje cells, we recorded Purkinje cell simple spike and complex spike activity at rest (i.e., spontaneous activity) and during optokinetic stimulation. Extracellular recordings of Purkinje cells in alert *tg* mutants (n = 86 cells, 22 animals) and wild-type mice (n = 79 cells, 14 animals) during spontaneous activity in the light did not reveal gross anomalies in amplitude, shape, or duration of their simple or complex spikes. However, the firing pattern of the simple spikes was much more irregular in *tg* mutants than in wild-types (Figure 2A). This difference held true equally for all cerebellar regions from which we recorded, i.e., the flocculus as well as extraflocculus regions including crus I and II, paramedian lobule, and paraflocculus. The coefficient of variance (CV) (for details, see Experimental Procedures) of the spontaneous simple spike activities in the flocculus of *tg* mice (2.57 ± 0.25) did not differ ($p = 0.4$, Student's t test) from that in the extraflocculus regions (2.18 ± 0.19), indicating that the abnormal firing pattern was a general phenomenon. After pooling Purkinje cells from all regions, the average CV of spontaneous activity in *tg* was almost four times higher than that of wild-types (2.25 ± 0.16 versus 0.63 ± 0.07; $p << 0.001$, Student's t test). The increase in irregularity in *tg* mice was also reflected in a shorter climbing fiber pause (defined by the period between the start of the complex spike and the start of the first simple spike after this event). In *tg* and wild-types, the pause was 13.6 ± 0.55 ms and 16.4 ± 0.07 ms, respectively ($p < 0.05$, Student's t test). The reduction in pause length cannot be explained by a higher firing frequency of the simple spikes in *tg*, because the average firing frequency was in fact slightly lower than in wild-type (varying from 52 spikes/s to 65 spikes/s, respectively).

In order for the irregularity to account for abnormalities of motor behavior, the irregularity must be present during movements as well as when the animal is at rest. We therefore investigated the activities of floccular Purkinje cells in *tg* mutants (n = 25 cells, 10 animals) and wild-types (n = 14 cells, five animals) that made compensatory eye movements in response to sinusoidal optokinetic stimulation (Figure 2B). During this behavior, as at rest, the simple spikes' activities in the *tg* proved to be more irregular, both during the excitation (on-phase) and suppression (off-phase) halves of each stimulus cycle. A raster diagram generated from a typical *tg* and wild-type neuron, shown in Figure 2C, demonstrates qualitatively the greater irregularity of the *tg* neuron. To quantify the irregularity, we calculated the autocorrelation and correlation coefficients of both the binned simple spike counts, using a bin size of 50 ms (Figures 2D and 2E), and the individual interspike intervals (Figure 2F). In both calculations, the correlation coefficients were much lower in *tg* than in controls ($p < 0.001$ in both analyses, ANOVA). Moreover, while the correlation coefficient in wild-type increased with increasing stimulus frequency, that of the *tg* mutants did not (Figure 2E). In addition, the average autocorrelation of the interspike intervals in wild-types during modulation was significantly higher than that during spontaneous activity ($p < 0.001$; ANOVA), while that in the *tg* mutants was not ($p = 0.4$, ANOVA). Apart from the greater irregularity of the interspike intervals, there was a greater variability in the modulation depth from cycle to cycle (trial-to-trial variability). We calculated the standard deviation of the difference between the number of spikes during the on- and off-phases of OKR at all frequencies (i.e., 0.05 Hz, 0.1 Hz, 0.2 Hz, 0.4 Hz, and 0.8 Hz, all with a peak velocity of 8°/s) and compared these standard deviations for *tg* and wild-types. The standard deviations were significantly increased in *tg* mutants ($p < 0.001$, nonorthogonal ANOVA), while the mean difference in spikes between the on-phase and

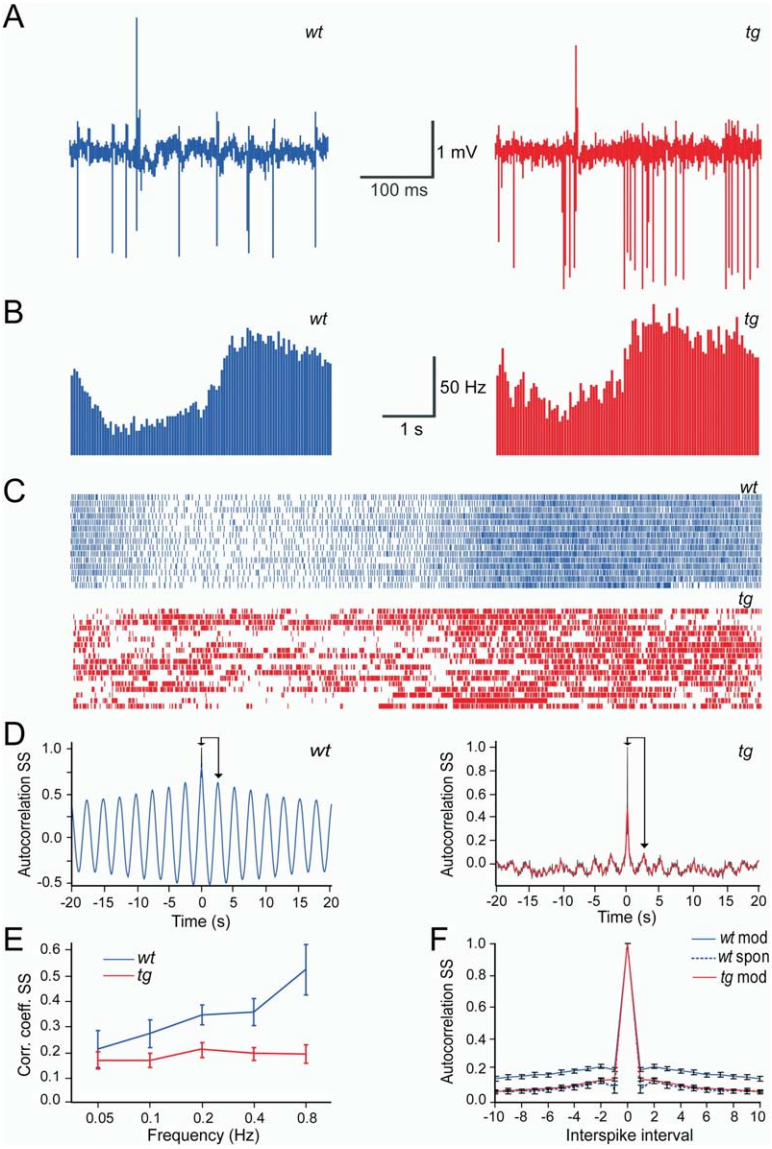

Figure 2. Simple Spikes of Purkinje Cells in *tg* Mutants Modulate Normally during Optokinetic Stimulation, but Fire Irregularly

(A) Extracellular recordings of spontaneous Purkinje cell activities of a wt (left panel) and a *tg* mouse (right panel). Note the increased irregularity of the simple spikes in *tg*. Complex spikes are positive and simple spikes are negative. (B) PSTHs show that the modulation of the simple spike activities of a floccular Purkinje cell during optokinetic stimulation in *tg* is indistinguishable from that in wt (for population data, see Figure 3A). (C) Raster plots of the same spikes that are presented in the PSTHs in (B) demonstrate the qualitatively obvious nature of the greater irregularity of *tg* Purkinje cells. Each small stripe represents a single spike, and each row of stripes contains spike data for a complete stimulus cycle. (D) Autocorrelations during modulation show that the predictability of simple spikes in wt is much better than that in *tg* (stimulus at 0.4 Hz and 8°/s; bin size, 50 ms). Unfiltered traces are indicated in black, and low-pass-filtered traces are indicated in blue for wt and in red for *tg*. Arrows indicate values used for calculation of correlation coefficients (see Experimental Procedures). (E) Correlation coefficients between the number of spikes in the center 50 ms bin and the number of spikes in the neighboring bins during optokinetic stimulation in *tg* mice are lower than those of wild-types, and they are not influenced by stimulus frequency, in contrast to those of wild-types. (F) Autocorrelation of simple spike interspike intervals in wt during optokinetic modulation (continuous blue line, wt mod) is significantly higher than that in wt during spontaneous activity (dotted blue line, wt spon) as well as higher than that in *tg* during modulation (red line, *tg* mod). Error bars indicate the mean ± SEM.

off-phase did not differ between *tg* and wild-type littermates (p = 0.7, nonorthogonal ANOVA). Although modulation depth was more variable in *tg* than in controls, its average value was comparable; sine waves fitted to the simple spike responses to the optokinetic stimulation showed that the average modulation amplitudes did not differ (p = 0.7, nonorthogonal ANOVA) (Figure 3A). Together, these data demonstrate that Purkinje cells in *tg* mice fire much more irregularly during modulation than those in wild-types and that the predictive power of a single interspike interval of a modulating Purkinje cell is greater in wild-type than in *tg* mice.

A factor that could potentially confound the comparison of simple spike regularity in *tg* and wild-types would be the presence of modulation related to fast phases, either if *tg* and wild-types generated different numbers of fast phases, or if the modulation associated with each fast phase differed. We eliminated both possibilities by compiling saccade-triggered averages of the simple spike data (Figure 3B). These triggered averages showed no significant peaks in firing frequency before or after saccades, neither in wild-types (n = 51 cells, 12 animals) nor in *tg* mutants (n = 55 cells, 13 animals) (p = 0.9 and p = 0.2, respectively, Kolmogorov-Smirnov test). Since there was no saccade-associated modulation, differences in the occurrence of saccades or activity associated with each saccade could not have influenced the assessments of regularity during OKR. Taken together, we conclude from our floccular Purkinje cell recordings during optokinetic responses that a change in regularity of simple spike activities, but not in modulation amplitude or saccade-related activity, may explain the abnormal eye movement behavior of *tg* mutants.

Output of Modulating Purkinje Cells in Vestibulocerebellum of *tg* Mutants Is Not Functional

Even though OKR and VVOR gain values, as well as the regularity of simple spike responses during these

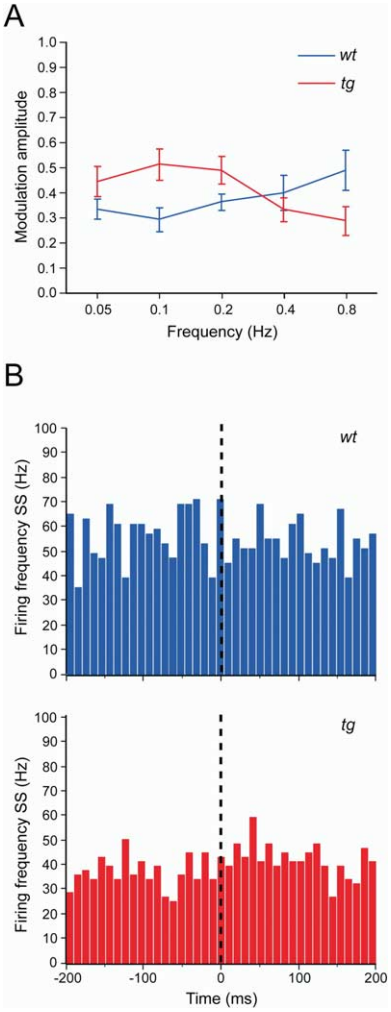

Figure 3. Modulation-Related and Saccade-Related Simple Spike Activities of Floccular Purkinje Cells in *tg* Are Indistinguishable from Those in wt

(A) Average modulation amplitude of simple spike activities of all Purkinje cells that modulate optimally around the vertical axis; no significant difference was observed between wt and *tg* mice. (B) Histograms of average simple spike activities occurring around the onset of fast phases, which are superimposed at moment zero and indicated by vertical dotted lines (bin size, 10 ms). Note that there was no significant correlation between spike data and saccadic eye movements, either for wt (n = 51) or for *tg* (n = 55). Error bars indicate the mean ± SEM.

movements, are affected in *tg* mutants, the modulating Purkinje cells in their flocculus may still contribute, although less effectively than in wild-types, to increase the gain of their compensatory eye movements (De Zeeuw et al., 2003). To determine the extent to which signal processing in the flocculus of the *tg* contributes to compensatory eye movements under vision, we ablated the flocculus of *tg* (n = 6) and wild-type (n = 3) mice and evaluated the differences between the prelesion and postlesion gain values (Figures 4A and 4B). In *tg* mutants, we did not observe any significant decrease in OKR or VVOR gain values after the lesions ($p = 0.1$ and $p = 0.4$, respectively, ANOVA). In contrast, the gains did decrease significantly in wild-types during OKR and VVOR ($p < 0.03$ and $p < 0.05$, respectively, ANOVA) (see also Koekkoek et al., 1997). For both OKR and VVOR, we found that the absolute gain values in the wild-types after the lesions did not differ from those in the *tg* mutants before the lesions ($p = 0.3$ and $p = 0.2$, respectively, ANOVA). Both wild-type and *tg* VOR gain values did not change after the lesion ($p = 0.1$ and $p = 0.9$, respectively, ANOVA; data not shown). The completeness of the lesions was verified by silver staining to reveal the degenerating fibers in the floccular peduncle (Figures 4C and 4D) and by reconstructing the core of the lesions in each animal (Figures 4E and 4F). These data indicate that the output of the flocculus in *tg* mutants does not contribute significantly to the gain of the optokinetic reflex or visually enhanced vestibuloocular reflex.

Connections Downstream of Cerebellar Cortex Are Functionally Intact

The recording and lesion experiments described above suggest that the floccular output is functionally absent in *tg* due to irregular simple spike activities. However, an alternative or additional explanation could be that the floccular output is blocked downstream, at the synapses between Purkinje cells and their targets, or possibly at more distal locations, such as the neuromuscular junctions with the eye muscles (Plomp et al., 2000). To find out whether such a downstream blockade contributes to the behavioral deficits, we investigated whether saccadic eye movements are impaired in *tg* mutants. Saccades require the highest levels of muscular force and would presumably be slow if there was a functionally meaningful defect of neuromuscular transmission (Zee and Leigh, 1983). Saccades manifest an orderly relationship between both their duration and their peak velocity relative to amplitude (i.e., main sequence), and in mice, this relationship is linear over a broad range of amplitudes (Stahl, 2004). Figure 5A shows typical examples of the main sequences for fast phases of optokinetic nystagmus of a *tg* and a wild-type mouse. The average slopes of duration relative to amplitude (1.2 ± 0.2 ms/deg) as well as the offsets of the linear fits (15.3 ± 4.5 ms) in *tg* mice (n = 6) did not differ from those in wild-types (n = 5) (0.9 ± 0.2 ms/deg and 19.6 ± 3.3 ms, respectively) ($p = 0.2$ and $p = 0.3$, Student's t test). Moreover, the slope (39.6 ± 1.8 rad/s/deg) and offset (7.4 ± 1.4 rad/s) of the peak velocity to amplitude fits in *tg* did not differ either ($p = 0.2$ and $p = 0.9$, respectively, Student's t test) from those in wild-types (36.7 ± 0.8 rad/s/deg and 7.3 ± 1.6 rad/s, respectively). These data suggest that the presence of abnormal P/Q-type calcium channels at synapses within the vestibular and ocular motor nuclei, as well as at neuromuscular junctions, does not limit the development of muscular force, at least on the short timescales associated with saccades (Robinson, 1970).

We also tested the integrity of postfloccular pathways by electrical stimulation of the flocculus. The short latencies of the eye movements that could be evoked by electrical stimulation of the floccular vertical axis zone with a 100 Hz pulse train of short duration (200 ms) supported the observation described above.

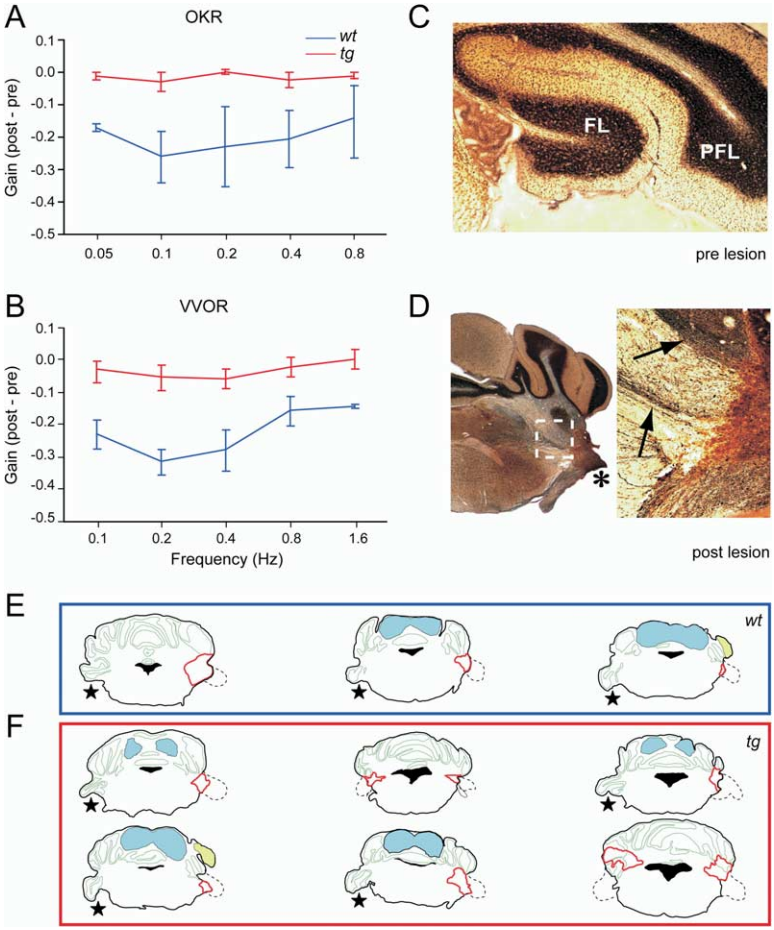

Figure 4. Flocculus of *tg* Mutant Does Not Contribute to Compensatory Eye Movements

(A and B) Gain values of *tg* mutants during OKR (A) and VVOR (B) are not decreased by ablation of their flocculus, whereas those in wild-types are. (C and D) A silver-stained section of an intact flocculus and paraflocculus of a *tg* mouse in which no lesion was placed (C) and a silver-stained section of degenerated fibers in the floccular peduncle of a *tg* mouse in which the flocculus was ablated (D). Asterisk indicates the location of the lesion. Inset in low-magnification panel on the left corresponds to high-magnification panel on the right. Arrows indicate borders of the floccular peduncle with bundles of silver-stained fibers. (E and F) Reconstructions of core of lesions of floccular complex in three wt (E) and six *tg* mice (F). Dashed lines indicate core of the lesions; red lines indicate area with abundant degenerated fibers as visualized by silver staining; light blue and yellow indicate colliculi and cerebral cortex, respectively; and stars indicate intact floccular complex on the contralateral side. Note that two *tg* mice received a bilateral ablation of the flocculus. Drawing of lesion at the middle bottom panel in (F) corresponds to panels (C) and (D). Error bars indicate the mean ± SEM.

The average latency in *tg* mice (12.1 ± 1.2 ms; n = 7) did not differ (p = 0.4, Student's t test) from that in wild-types (10.5 ± 0.7 ms; n = 7) (Figure 5B). Moreover, the stimulus thresholds that were necessary to evoke these short-latency, temporally directed eye movements were the same in both types of animals (varying from 10 μA to 18 μA in both groups). Since CACNA1A mutations have been associated with a more rapid run-down of neurotransmitter release (Plomp et al., 2000), the possibility remained that *tg* mutants suffer from a functionally significant neuromuscular blockade that only becomes apparent during periods of activation longer than those associated with saccades or 200 ms pulse trains. To investigate this possibility, we also tested pulse trains up to 1500 ms (Figure 5C). Independent of the duration of the stimulation, the dynamics of the movements elicited in the ipsilateral eye in *tg* mutants (n = 4) were comparable to those in wild-types (n = 4), and in both *tg* mutants and wild-types, these movements never stopped before the stimulus stopped. Moreover, the average time constants of the curves fitted to the responses in *tg* mutants did not differ significantly from those in wild-types, either for the long stimulations or for the shorter stimulation protocols (600 ms, p = 0.5; 1000 ms, p = 0.9; 1500 ms, p = 0.4; Student's t tests). These results argue against the presence of a defect in signal transmission downstream of the flocculus, and thereby they suggest that the reduced visual compensatory eye movement performance in *tg* should not be attributed to such a barrier.

Olivary Connections Upstream of Cerebellar Cortex Are Functionally Intact

Since P/Q-type calcium channels are also moderately expressed in the inferior olive neurons (Fletcher et al., 1996; Hillman et al., 1991; Stea et al., 1994; Westenbroek et al., 1995), the increased irregularity in simple spike activities may also be imposed by changes upstream of the flocculus, that is, in climbing fiber activities generated in the olive (De Zeeuw et al., 1998). This speculation is plausible because sudden increases or decreases of climbing fiber activities decrease and increase the simple spike frequency, respectively (Montarolo et al., 1982). We therefore evaluated the normality of complex spike activities (Figure 6). During spontaneous activity, the mean firing frequency and CV of complex spikes in *tg* mice (n = 86 cells, 22 animals) equaled 0.98 ± 0.04 and 0.78 ± 0.02 spikes/s, respectively, while those in wild-types (n = 79 cells, 14 animals) equaled 0.90 ± 0.03 and 0.81 ± 0.02 spikes/s (p = 0.1 and p = 0.3, respectively; Student's t test). Autocorrelograms of complex spikes in *tg* mutants recorded during optokinetic stimulation (n = 25 cells, 10 animals) did not differ from those in wild-types (n = 14 cells, five animals),

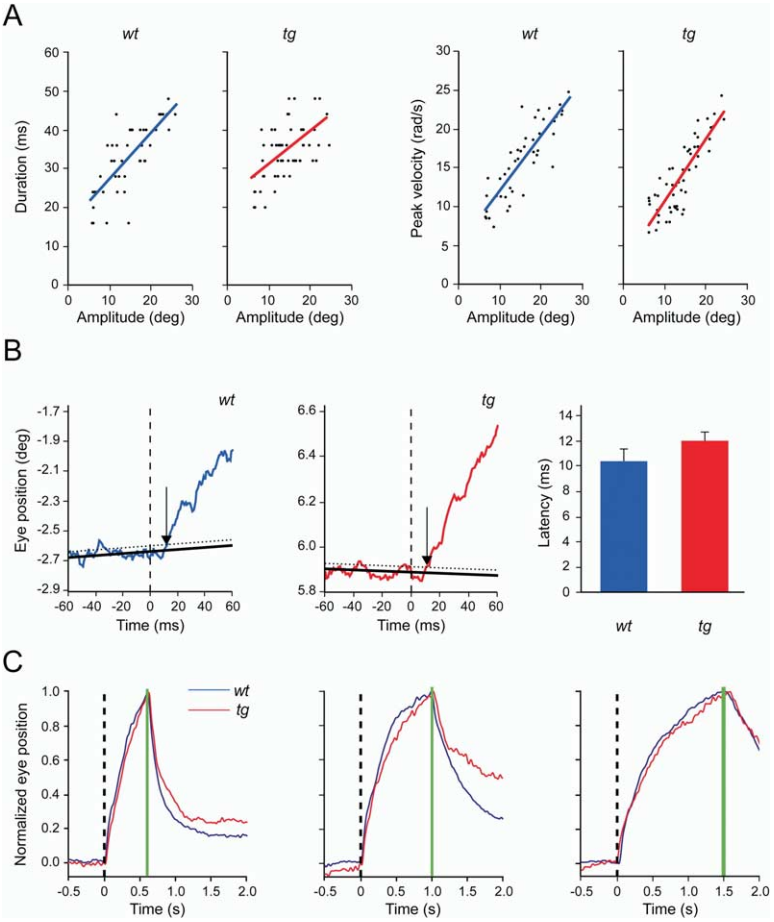

Figure 5. Oculomotor Connections Downstream of the Flocculus Appear Functionally Intact

(A) Typical examples of the dynamics of saccadic eye movements in *tg* (red) and wt (blue). Data show representative linear fits to the duration relative to amplitude (left panels) and peak velocity relative to amplitude (right panels). The average main sequence parameters of *tg* are not different from those in wild-type littermates (see text for numbers). (B) Eye movements evoked by electrical stimulation in the flocculus show normal latencies in *tg* mutants. The left and middle panels show eye position traces before and after the onset (dashed line) of electrical stimulation in wt and *tg*, respectively. Horizontally oriented black straight lines indicate results of linear regression of the 200 ms period prior to the onset of the stimulus with (dotted line) and without 1 SD (bold line). Arrows indicate crossings of eye position traces and SD lines, which are used to determine response latency. The right panel indicates that the average latency of the eye movements following electrical stimulation of the flocculus in seven wt mice is indistinguishable from that in seven *tg* mice. (C) Left, middle, and right panels show normalized eye positions following 600 ms, 1000 ms, and 1500 ms stimulus trains, respectively (for all paradigms, n = 4, for both wild-types and *tg* mutants). Dashed and green lines indicate onset and end of the electrical stimulus, respectively. The finding that the eyes in *tg* mutants did not drift back during the application of the stimulus suggests that the neurotransmitter stores in its oculomotor synapses and neuromuscular junctions are not depleted during the stimulus. Error bars indicate the mean ± SEM.

either with regard to the analyses using binned spike counts (Figures 6A and 6B) or in the analyses of the individual interspike intervals ($p = 0.4$ and $p = 0.1$, respectively; ANOVA) (Figure 6C). To further assess the integrative properties of the olivary neurons and its inputs from the accessory optic system (Simpson et al., 1996), we also investigated the preference of their climbing fiber responses for particular axes of optokinetic stimulation, i.e., their spatial tuning (Figure 6D). In addition to the neurons that modulated optimally by rotations about the vertical axis, we also identified numerous neurons preferring a horizontal axis directed 135° ipsilateral to the azimuth. The bimodal vector distribution and the resulting vectors of the spatial tuning curves in *tg* mutants did not differ from those in wild-types, and they resembled those described for other animals, such as rabbits and pigeons (De Zeeuw et al., 1994; Graf et al., 1988; Wylie et al., 1995). Moreover, the complex spike activities were always in counterphase with the simple spike activities, independent of the spatial axis used for stimulation. We conclude that both the spontaneous activities and integrative properties of the olivary neurons that ultimately determine the complex spike output of the floccular Purkinje cells are not affected by the *tg* mutation and that the simple spike irregularities are therefore not an indirect consequence of the expression of mutant P/Q-type calcium channels in the inferior olive.

Acute Blockade of P/Q-Type Calcium Channels in Flocculus of Wild-Types Partially Mimics Behavioral Phenotype of *tg*

The data presented above suggest that the connections downstream and upstream of the cerebellar cortex are sufficiently intact in the *tg* mutants to allow functional synaptic transmission, but it remains to be demonstrated whether dysfunctional P/Q-type calcium channels in the cerebellar cortex alone are sufficient to induce the eye movement abnormalities. Based on electrophysiological observations gathered in vitro, abnormal Purkinje cell activities in mutants could potentially arise from direct effects of the CACNA1A mutation on dendritic and presynaptic P/Q-type calcium currents. In addition, the P/Q-type calcium channel dysfunction could trigger compensatory changes (e.g., developmental changes in circuitry, channel distribution, and/or channel accessory protein composition) that play out over longer timescales (Wakamori et al., 1998). We therefore examined whether the ocular motor abnormalities and simple spike irregularity in *tg* could be approximated by an acute pharmacological manipulation in P/Q-type calcium channel function in wild-types

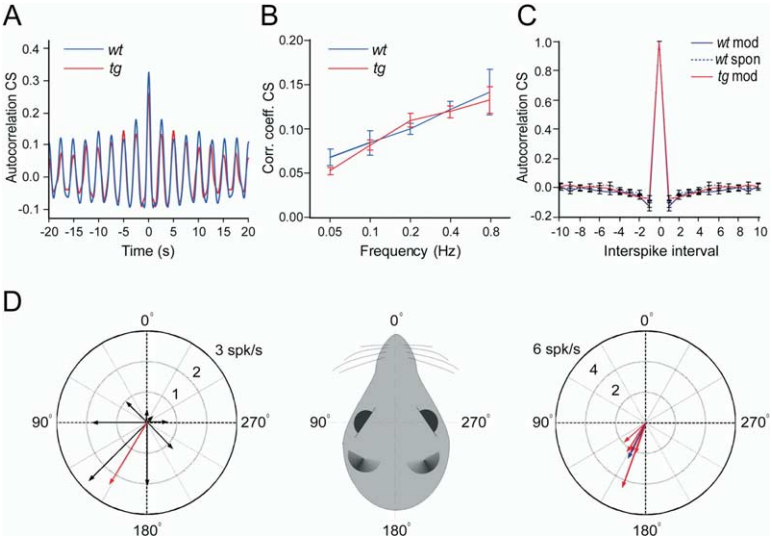

Figure 6. Olivary Connections Upstream of the Flocculus of *tg* Mutants Are Functionally Intact

(A) Autocorrelations show that there is no difference between wt and *tg* data in the predictability of complex spike activities (CS) during optokinetic modulation (stimulus frequency, 0.4 Hz; bin size, 50 ms). Blue and red lines indicate low-pass-filtered autocorrelations for wt and *tg*, respectively. (B) Correlation coefficients of wt and *tg* CS activities during modulation are equally dependent on stimulus frequency. (C) Autocorrelation of CS interspike intervals in wt during modulation did not differ from that in wt during spontaneous activity nor from that in *tg* mice during modulation. Note that all CS data presented in (A–C) show opposite results to the simple spike data presented in Figures 2D–2F. (D) Tuning curves of climbing fiber responses of floccular Purkinje cells are normal in *tg* mutants. Left panel shows an example of the depth of modulation of the climbing fiber responses of a single cell for eight different horizontal axes in space. Lengths of arrows indicate the depth of modulation, and the red arrow indicates the vector for optimal modulation (close to 135° azimuth). Right panel shows these vectors for five different cells (blue arrow indicates the average vector). Error bars indicate the mean ± SEM.

(Figure 7). In wild-types (n = 6), injections of 100 nM ω-Agatoxin IVA into the flocculus resulted in a significant decrease of the OKR gain values over the entire frequency range ($p < 0.001$, ANOVA) (Figure 7A). Injections of the vehicle solution alone (n = 6) had no significant effect. Injections of the P/Q-type calcium channel blocker also caused a significant reduction in the gain of the VVOR ($p < 0.02$, ANOVA), but the differences in the results following vehicle injections were relatively small at the higher frequencies. The phase lead during VVOR was significantly enhanced ($p < 0.001$, ANOVA; data not shown), whereas the phase lag during OKR was not affected ($p = 0.1$, ANOVA). In *tg* mutants (n = 6), the injections did not produce any significant effect ($p = 0.5$ and $p = 0.6$ for OKR and VVOR gain values, respectively; ANOVA). These data indicate that acute local application of P/Q-type calcium channel blockers to the flocculus of wild-type mice can be sufficient to mimic the eye movement performance of *tg* mice during OKR and VVOR.

Following juxtacellular application of ω-Agatoxin IVA in both the floccular and nonfloccular regions of the cerebellar cortex, three categories of Purkinje cell activity were observed: a type in which simple spike activity intermittently ceased for long periods (seconds) while complex spike activity continued (n = 14); a second type in which both simple spike and complex spike activity continued throughout the recordings (n = 13); and a third type in which simple spikes continued, but complex spike activity ceased (n = 3). All cells in the second category exhibited an unambiguous pause in simple spike activity following each complex spike throughout the recordings, confirming that the recording was obtained from a well-isolated, single-unit Purkinje cell (Simpson et al., 1996) (Figure 7B). Analysis of this second response category showed that the average simple spike frequency increased from 59 ± 11 spikes/s to 83 ± 16 spikes/s and that their average CV tended to change concomitantly following injection of ω-Agatoxin IVA as compared to injection of the vehicle alone (Figure 7C). However, none of the average changes in simple spike responses was statistically significant when we considered this category alone ($p = 0.1$ and $p = 0.3$ for differences in CV and firing frequency, respectively; ANOVA). Yet, when we pooled the simple spike data sets of all three categories of cells, the change in CV, but not in firing frequency, was significant ($p < 0.02$ and $p = 0.4$, respectively; ANOVA). In contrast, the average change in CV of the complex spike activities after application of ω-Agatoxin IVA was not significant ($p = 0.2$, ANOVA). Thus, even though one cannot rule out the possibility that some of the cells of the first and third categories were not a pure single-unit recording (for technical reasons, see Simpson et al., 1996), the data are at least consistent with the possibility that reduction in P/Q-type calcium currents is sufficient to create the irregular firing rates, which our other data suggest are responsible for deficits in motor performance.

Discussion

The potential impact of noise on signal processing has been recognized by theoretical neuroscientists, but experimental evidence of a change in noise resulting in altered sensorimotor behavior has been lacking. Here we provide such evidence, showing that a mutation in P/Q-type voltage-gated calcium channels leads to irregular simple spike activities of Purkinje cells without any change in other activity parameters (most importantly, the firing rate modulation) that could explain the deficiencies we observed in types of eye movement normally mediated by the cerebellum. The data support the idea that regularity of firing influences sensorimotor processing. Because mutations in CACNA1A are associated with ataxia in humans, the current data raise the

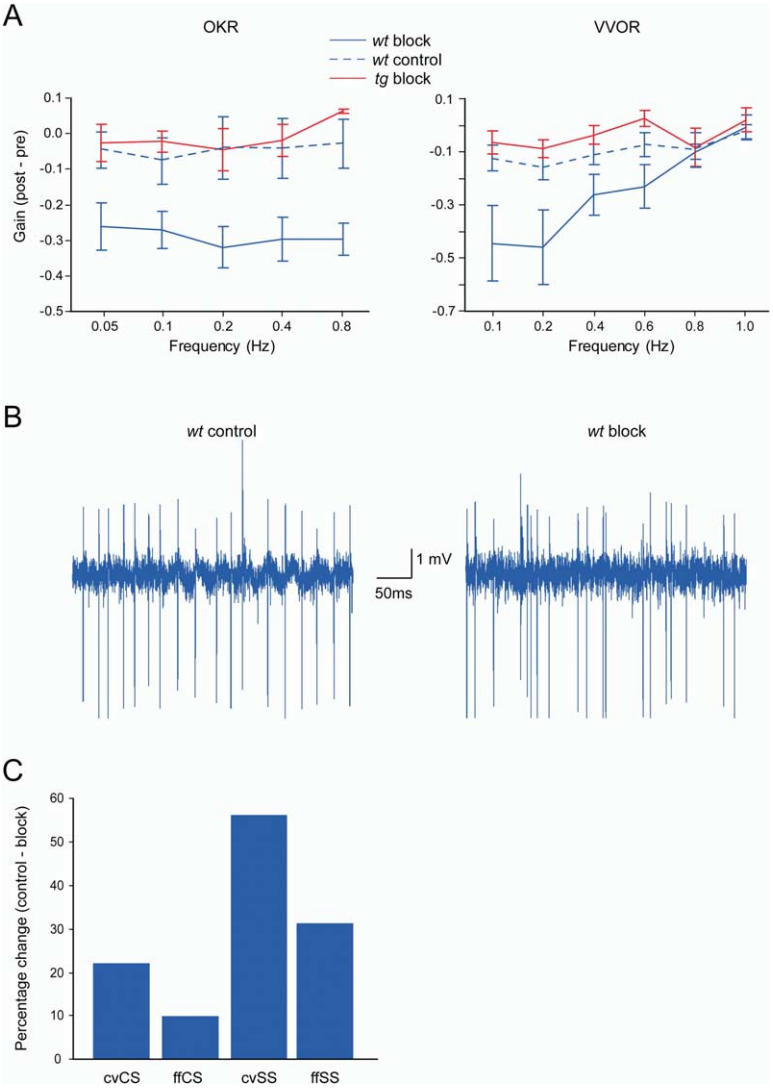

Figure 7. Phenotype of *tg* Mutant Can Be Largely Mimicked by Local Injection of Selective P/Q-Type Calcium Channel Blocker

(A) OKR and VVOR gain values in wt (n = 6) decreased after injection of ω-Agatoxin IVA into the flocculus (continuous blue line, wt block), but not after injection of the solution vehicle (dashed blue lines, wt control; n = 6). Injection of the blocker into the flocculus in *tg* mice (red line, *tg* block; n = 6) resulted in no change in OKR gain values. (B) Extracellular recordings of spontaneous Purkinje cell activities after juxtacellular application of the vehicle (control, left panel) or ω-Agatoxin (block, right panel) in the cerebellum of wild-types. Note the increased irregularity of the simple spikes in the right panel (complex spikes go up, and simple spikes go down). (C) Histograms showing the differences in CV and firing frequency of complex spikes and simple spikes in wild-type mice (cvCS, ffCS, cvSS, and ffSS, respectively) following juxtacellular application of ω-Agatoxin IVA as compared to the control injection with the vehicle alone. Error bars indicate the mean ± SEM.

possibility that alterations in noise levels of neuronal signals could be sufficient to produce the behavioral manifestations of certain neurological diseases.

Cellular Basis of Irregular Simple Spike Firing Pattern

The increased irregularity of simple spike firing in *tg* could arise from several sources, including alterations of the signals carried by mossy fiber inputs, alterations of signals carried by climbing fibers, or abnormalities of neurons in the cerebellar cortex that alter their response to mossy and climbing fiber inputs. The first possibility appears unlikely in the case of the flocculus, since in situ hybridization and immunocytochemical studies indicate that P/Q-type calcium channels are relatively rare in regions of the brain giving rise to mossy fiber inputs to the vestibulocerebellum (Craig et al., 1998; Fletcher et al., 1996; Stea et al., 1994; Westenbroek et al., 1995). The second possibility is more plausible, as P/Q-type calcium channels are expressed throughout the inferior olive. However, we were able to refute this possibility by demonstrating that complex spike activity was normal in terms of waveform, firing frequency, regularity, preferred stimulus axis, and phase with respect to simple spikes. Thus, simple spike irregularities in *tg* are most likely the result of the third source, i.e., an abnormal response due to the alteration of synaptic and dendritic properties of the cerebellar cortical neurons that are affected by the mutated P/Q-type calcium channels.

Because P/Q-type calcium channels are heavily expressed throughout all layers of the cerebellar cortex (Fletcher et al., 1996; Hillman et al., 1991; Stea et al., 1994; Westenbroek et al., 1995), there are several sites and types of electrophysiological abnormalities that could contribute to the simple spike irregularity. First, alterations in P/Q-type calcium channel current density and microscopic and macroscopic current magnitudes are likely to affect the intrinsic excitability of Purkinje cells and granule cells (Randall and Tsien, 1995; Wakamori et al., 1998; Zhang et al., 1993). Second, the synaptic connection between the granule and Purkinje

cells is likely weakened in that the amplitude of their EPSC evoked by parallel fiber stimulation is smaller (Matsushita et al., 2002). Third, even though the climbing fiber-mediated EPSC is normal (Matsushita et al., 2002), our results indicated that the length of the climbing fiber pause is slightly reduced. Pause duration, which probably largely reflects the activity of calcium-dependent potassium channels (Schmolesky et al., 2002; Sausbier et al., 2004), may influence simple spike activities even after firing resumes (Sato et al., 1993). Fourth, and finally, the P/Q alteration may trigger indirect, compensatory mechanisms in the cerebellar cortex of *tg*. For example, there may be a shift in the reliance of neurotransmitter release mechanisms from P/Q-type calcium channels to N-type channels, as has been shown in the forebrain and hippocampus (Cao et al., 2004; Leenders et al., 2002; Qian and Noebels, 2000). Because an increased reliance on N-type channels leads to an increased susceptibility to inhibitory modulation by G protein-coupled receptors (Zhou et al., 2003), and because a single action potential of a cerebellar inhibitory interneuron is able to delay action potentials in Purkinje cells (Hausser and Clark, 1997), such an increased susceptibility would be predicted to prolong the silent periods that basket cells and stellate cells can impose on Purkinje cells. Thus, a combination of multiple defects of different types at various synapses in the cerebellar cortex may well explain the enormously enhanced irregularity of the simple spike response in *tg* mutants.

Behavioral Consequences of Irregular Simple Spike Activities

Our behavioral and neuronal observations that gain and phase values during compensatory eye movements under vision are affected and that simple spike activities in their flocculus are irregular while their modulation amplitude is normal raise the possibility that irregular simple spike activities of Purkinje cells are one of the prime causes of ataxia in *tg* mutants. This notion is in line with the fact that the density of P/Q-type calcium channels in the cerebellum far exceeds that of other brain structures involved in ocular motor behavior (Fletcher et al., 1996; Hillman et al., 1991; Stea et al., 1994; Westenbroek et al., 1995). However, these studies cannot exclude the possibility that P/Q-type calcium channels play key roles at restricted sites in tissues with overall low expression levels or the possibility that the P/Q mutation had developmental effects on circuits outside the regions of high-density P/Q expression in the adult brain. It remained therefore possible that the alterations in visually driven eye movements reflect derangements of extrafloccular circuits. To overcome this potential confound, we addressed the integrity of signal flow from the flocculus to the eye muscle by investigating the dynamics of saccadic eye movements in *tg* mutants as well as their eye movements following electrical stimulation in the flocculus. The duration and velocity relative to amplitude profiles of their saccadic eye movements were normal, supporting the functional integrity of the neuromuscular junction. In this respect the phenotype of *tg* mutants diverges from that in *rocker* mice, which suffer from a different mutation in the same $\alpha 1_a$-subunit of the P/Q-type calcium channel and show deficits in both slow and fast phases of their eye movements (Stahl, 2004). Thus, strengthened by the specificity of the behavioral phenotype, the experiments involving saccadic eye movements indeed suggest that neurons of the oculomotor nuclei in *tg* mice function normally. In addition, we demonstrated that the eye movements evoked by prolonged floccular stimulation appeared normal in *tg* mice, indicating that rapid depletion of neurotransmitters at synapses or neuromuscular junctions from flocculus to eye muscles is unlikely to contribute to the ocular motor abnormalities, despite such depletion having been observed at selected synapses outside the ocular motor system (see also Plomp et al., 2000; Qian and Noebels, 2000; Cao et al., 2004). Finally, we also demonstrated that gain and phase values of the vestibuloocular reflex in the dark were hardly affected. Thus, all synaptic connections in the oculomotor pathway downstream of the flocculus appear, at least at the systems level, functionally intact. These data, however, do not allow us to conclude that there are no changes at these synaptic inputs at the cell physiological level (Qian and Noebels, 2000). In fact, the total of cellular compensations may be such that the overall synaptic strengths in these connections are sufficiently preserved despite the shift away from the P/Q-type predominance mentioned above (Cao et al., 2004).

If Purkinje cell irregularities in *tg* cause complete loss-of-function of the cerebellar cortex, ablations of their flocculus should not further decrease the gain values of their compensatory eye movements, and their gain values with an intact flocculus should be similar to those of flocculectomized wild-types. On the other hand, if aberrations in neurons downstream of the cerebellar cortex contribute to ataxia in *tg* mutants, ablations of their flocculus should further decrease the gain values of their compensatory eye movements, and their gain values with an intact flocculus do not need to be similar to those of wild-types with an ablated flocculus. The outcome of our flocculectomy experiments in wild-types and *tg* mutants confirmed the first hypothesis and contradicted the second. Finally, if the abnormalities in the cerebellar cortex of the *tg* mutant are indeed sufficient to cause the ocular motor deficits, one expects that local blockade of P/Q-type calcium channels in some portion of the wild-type flocculus might be sufficient to qualitatively reproduce some of the electrophysiological and behavioral abnormalities seen in *tg*. This prediction was also largely upheld. The relatively minor, but present, discrepancies between the phenotype of *tg* mutants and that of wild-types treated with the P/Q-type calcium channel blocker ω-Agatoxin IVA could be due to the long-term secondary compensations that occur in the mutant, or to the more profound, but also restricted, region of the pharmacological blockade in wild-types. Taken together, the data from our eye movement recordings combined with those from our electrophysiological recordings, electrical stimulations, lesions, and pharmacological blockage experiments are consistent with the speculation that the *tg* mutation in the $\alpha 1_a$-subunit of the P- and Q-type voltage-gated calcium channel leads to irregular simple spike firing patterns and that these irregular simple spike

activities are sufficient to contribute to deficits in motor performance.

Functional Implications

Our data obtained in *tg* mutants suggest that the noise level of neuronal firing patterns can be altered without any impact on their modulation depth and that such a change is sufficient to alter motor behavior. This possibility is strengthened by our analyses of simple spike autocorrelations. Not only was the regularity of wild-types greater than that of *tg* mice when recorded in the absence of eye movements, during compensatory eye movements the regularity increased in normal animals, while it remained unchanged in the mutants. Together, the data suggest that the average firing rates and modulation amplitudes are not the sole determinants of cerebellar output. It has been recognized by information theoreticians that an optimal level of noise (i.e., irregularity) can be important for signal processing (Bialek et al., 1991; Mar et al., 1999; Steinmetz et al., 2001; Tiesinga et al., 2002). For example, models by Rieke and Chacron indicate that, on the one hand, noise will increase the trial-to-trial variability of a neural response to repeated presentations of a stimulus, but on the other hand, it will also increase the variability of the spike train and thereby potentially lead to increased information capacity (Chacron et al., 2003; Rieke et al., 1997). Here, we showed that a mutation in the $\alpha 1_a$-subunit of the P/Q-type voltage-gated calcium channel can lead to an increased noise level in the simple spike activities of Purkinje cells in that both the variability in interspike intervals and the trial-to-trial variability are increased. In principle, both forms of irregularity may affect signal transmission at the synaptic input from the Purkinje cells to their target neurons in the cerebellar and vestibular nuclei and thereby contribute equally well to deficits in motor performance. In fact, one may hypothesize that, due to both forms of irregularity, too many of the simple spikes in *tg* mutants fall outside the temporal window that is formed by the effective range of spike frequencies that can be handled by the propagation properties of the Purkinje cell axons and/or by the properties of their synapses onto their target neurons in the cerebellar and vestibular nuclei. Such a notion would be in line with recent findings by Monsivais and Hausser (P. Monsivais and M. Hausser, 2003, Soc. Neurosci., abstract), who showed that propagation failures in Purkinje cell axons during climbing fiber bursts can be correlated with a frequency limit of about 252 Hz, which is less than the maximum frequency one can observe during a short burst of simple spikes in *tg* (even though the average firing frequency is not above normal). Moreover, the effective range of simple spike activities may not only be determined by properties of the Purkinje cell axons or their synaptic terminals onto their target neurons in the cerebellar and vestibular nuclei, but also by changes in intrinsic excitability of these neurons (Aizenman and Linden, 2000; Nelson et al., 2003).

The gross motor deficits of *tg* resemble those seen in human patients with the CACNA1A-related disorders—familial hemiplegic migraine, episodic ataxia type II, and spinocerebellar ataxia type VI (Baloh et al., 1997; Ducros et al., 1999; Harno et al., 2003; Ophoff et al., 1996; Zhuchenko et al., 1997). To the extent that the function of the flocculus in eye movements parallels the role of the extrafloccular cerebellum in limb and trunk motor control, and assuming that attenuated compensatory eye movements are analogous to limb and truncal ataxia, then irregular Purkinje cell activities could lie at the root of the ataxia found in these patients. It should be noted, however, that the most severe forms of ataxia in these patients appear in association with Purkinje cell degeneration (Gomez et al., 1997; Buttner et al., 1998), so that irregular Purkinje cell activities could only explain the milder types of incoordination seen in the first years of the disease. By suggesting a physiological mechanism that could account for motor incoordination, the current data raise the possibility of designing therapeutic neurostimulation protocols for patients in the early stages of ataxia that result from similar mutations in their P/Q-type calcium channel (Ophoff et al., 1996). The fact that we were able to evoke eye movements by electrical stimulation in the flocculus of *tg* mutants further supports this possibility. The significance of noise levels in firing patterns is probably not restricted to the CACNA1A diseases or to the neurons of the cerebellum. For example, Huxter et al. (2003) recently showed in hippocampal pyramidal cells that the time of firing and firing rate are dissociable and can encode independent variables (respectively, the animal's location within, and speed of movement through, a place field). Thus, proper modulation of neuronal activities is essential, but the absolute moment in time at which individual spikes occur can also influence signal processing under both pathological and normal circumstances.

Experimental Procedures

Animal Preparation

Data were collected from 54 *tg* mice and 54 wild-type littermates (C57BL/6J background; The Jackson Laboratory, Bar Harbor, ME), which were prepared for chronic experiments (Goossens et al., 2001; van Alphen et al., 2001). All preparations were done with approval of the European Communities Council Directive (86/609/EEC).

Optokinetic and Vestibular Stimulation

The OKR was assessed by rotating a planetarium sinusoidally about both vertical and horizontal axes at various frequencies (0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 Hz) and a fixed peak velocity (8°/s) (Stahl et al., 2000; van Alphen et al., 2001). The (V)VOR was assessed by rotating the animal sinusoidally around the vertical axis at various frequencies (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 Hz) and fixed amplitude (10°) in the dark or light. Stimuli were controlled and monitored by a 1401plus unit (CED, Cambridge, UK).

Eye Movement Recordings

The position of the left eye was measured using a video method operating at 240 samples/s (ETL-200, ISCAN, Burlington, MA) (Stahl, 2004; Stahl et al., 2000; van Alphen et al., 2001). For baseline compensatory eye movements (see Figure 1), the eye position signal was resampled at 500 Hz (1401plus unit; CED). Data were stored for offline analysis using Spike 2 and MATLAB (Mathworks Inc., Natick, MA).

Single-Cell Recordings

Extracellular Purkinje cell activities were recorded from either the flocculus or nonfloccular regions in the hemisphere of the left cerebellar cortex (Goossens et al., 2004). The cells were recorded either

during spontaneous activity in the light or during optokinetic stimulation (see above). Signals were amplified, filtered, digitized, and stored for offline analysis. Purkinje cells were identified by their brief pause in simple spike activity following each complex spike. Once a floccular Purkinje cell was isolated, the preferred axis of rotation was determined by rotating the planetarium around the vertical axis or a horizontal axis at 135° azimuth, ipsilateral to the side of recording. In a limited number of cells, a wider range of axes was tested to allow for the construction of a spatial tuning curve.

Floccular Lesions and Histology

After locating the left flocculus by recording its typical complex spike response to optokinetic stimulation, we lesioned the flocculus by suction (n = 6 *tg* and 3 wild-type animals). Eye movement recordings were done for 2 days prelesion, averaged, and subsequently compared to data collected on day 3 postlesion. To check the extent of the lesions, the animals were anesthetized and perfused, and subsequently Nissl and silver staining was applied to cerebellar and brainstem tissue (Jaarsma et al., 1992; Nadler and Evenson, 1983).

Electrical Stimulation

Custom-made urethane-insulated tungsten electrodes were used to electrically stimulate the left floccular peduncle. Regular trains of pulses (200 ms, 400 ms, 600 ms, 800 ms, 1000 ms, and 1500 ms trains; 80 μs pulse duration; 100 Hz pulse frequency with various stimulation intensities) were used to evoke eye movements in the ipsilateral eye. Each paradigm was applied to at least four wild-types and four *tg* mutants. Averages of >10 stimulus responses per paradigm were used to analyze the latencies and waveforms of the evoked eye movements. Latencies were calculated using averages of the short stimulus paradigms following linear regression analysis (Van der Steen et al., 1994). To quantify eye movement waveforms, we averaged responses to 600 ms, 1000 ms, and 1500 ms pulse trains, normalized them, and fitted them iteratively by a single exponential function of the form $y = 1 - e^{(-t/\tau)}$. The resulting time constants were tested for significance.

Injections of ω-Agatoxin IVA

The border of the left flocculus was identified using the electrophysiogical recordings described above. Subsequently, the recording electrode was replaced by a borosilicate glass electrode filled with 100 nM ω-Agatoxin IVA (diluted in 0.9% saline; Alomone Labs, Jerusalem, Israel) (Knight et al., 2002). Approximately 10 μl of the ω-Agatoxin IVA solution were injected by pressure at multiple sites evenly distributed over the entire flocculus. Compensatory eye movements were measured before the localization of the flocculus and 3 days after the injection of the blocker in six wild-type mice. For controls, we injected the vehicle (saline) in six wild-types and the ω-Agatoxin IVA solution in six *tg* mice. Juxtacellular recordings of Purkinje cell activities were made with the use of multiple barrel electrodes following iontophoretic injections of ω-Agatoxin IVA solution as described by Shields et al. (2005).

Data Analysis

Offline analysis of eye movements and neuronal firing rates was performed in MATLAB (Mathworks) (Goossens et al., 2004). Gain and phase values were determined by fitting sine functions to the slow-phase eye velocity traces. Simple spikes and complex spikes were discriminated using custom-made routines based on cluster analysis (Goossens et al., 2004). Simple spike Per-Stimulus Time Histograms (PSTHs) (100 bins per cycle) were compiled at each stimulus frequency and fit by a sine function. Neuronal amplitude of modulation was calculated by dividing the amplitude of the fitted sine wave by its offset. The phase of the simple spike activity relative to the eye velocity (θ) was calculated from the difference of the phase of the sinusoidal fits to firing rate and eye velocity (De Zeeuw et al., 1995; Stahl and Simpson, 1995b). CV values of spontaneous spike activities were calculated by dividing the standard deviations of the interspike interval lengths by their means. Autocorrelograms of spiking data were constructed using custom-made routines in MATLAB. A fixed bin size of 50 ms and a 7th order low-pass filter were used to calculate the correlogram coefficient of the spiking data (ratio of the amplitude of the first off-center peak in the filtered data and the amplitude of the center peak of the raw data). Autocorrelograms of interspike intervals were created by correlating the duration of each interval with the duration of its neighbors. While the CV values and autocorrelations were used to determine the regularity of the interspike interval, the trial-to-trial variability was determined by averaging the standard deviation of the mean difference between the number of spikes in the on-phase and that in the off-phase over the cycles. With respect to the relation between firing rate and saccadic eye movements, epochs containing quick phases were analyzed separately. Saccades were detected with a velocity threshold of 25°/s (for details see Van der Steen and Bruno, 1995). More than 40 saccades per animal were used to calculate the main sequence parameters. Data are presented as mean ± SEM.

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