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FGF14 Regulates the Intrinsic Excitability of Cerebellar Purkinje Neurons

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Abstract

A missense mutation in the fibroblast growth factor 14 (*FGF14*) gene underlies SCA27, an autosomal dominant spinocerebellar ataxia in humans. Mice with a targeted disruption of the *Fgf14* locus (*Fgf14*^{−/−}) develop ataxia resembling human SCA27. We tested the hypothesis that loss of FGF14 affects the firing properties of Purkinje neurons, which play an important role in motor control and coordination. Current clamp recordings from Purkinje neurons in cerebellar slices revealed attenuated spontaneous firing in *Fgf14*^{−/−} neurons. Unlike in the wild type animals, more than 80% of *Fgf14*^{−/−} Purkinje neurons were quiescent and failed to fire repetitively in response to depolarizing current injections. Immunohistochemical examination revealed reduced expression of Nav1.6 protein in *Fgf14*^{−/−} Purkinje neurons. Together, these observations suggest that FGF14 is required for normal Nav1.6 expression in Purkinje neurons, and that the loss of FGF14 impairs spontaneous and repetitive firing in Purkinje neurons by altering the expression of Nav1.6 channels.

Keywords

Spinocerebellar ataxia; Intracellular fibroblast growth factor 14 (iFGF14); Purkinje neurons; Nav 1.6; SCA27

Introduction

The autosomal dominant spinocerebellar ataxias (SCAs) are heterogeneous neurological disorders characterized by progressive cerebellar ataxia that also often present with paroxysmal dyskinesia, tremor, abnormal eye movements, and/or cognitive impairment (Schols et al., 2004; Taroni and DiDonato, 2004; Zoghbi, 2000; Zoghbi and Orr, 2000). The unexpected findings of ataxia, dystonia and tremor in mice with a targeted disruption in the *Fgf14* locus (Wang et al., 2002) suggested a role for FGF14 in regulation of motor function, and led directly to the identification of a mutation in *FGF14* in a family with a new type of progressive spinocerebellar ataxia now referred to as SCA27 (Van Swieten et al., 2003). Affected

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individuals in this family possess a missense mutation (F145S) in the *FGF14* gene on chromosome 13q34 (Brusse et al., 2005; Van Swieten et al., 2003). Interestingly, a single base pair deletion, leading to a frameshift mutation (D163fsX12) in *FGF14* has also been identified in an individual patient presenting with ataxia and mild mental retardation (Dalski et al., 2005; Soong and Paulson, 2007).

FGF14 belongs to the intracellular fibroblast growth factor subfamily (iFGF) that also includes FGFs 11–14 (Itoh and Ornitz, 2008), proteins that are widely expressed in the nervous system (Smallwood et al., 1996; Wang et al., 2000; Yamamoto et al., 1998). Unlike the other members of the FGF family, iFGFs are not secreted and do not interact with classical tyrosine kinase FGF receptors (Itoh and Ornitz, 2008; Olsen et al., 2003; Ornitz and Itoh, 2001; Smallwood et al., 1996). Members of the iFGF subfamily, however, have been shown to colocalize with voltage-gated sodium (Nav) channels, to interact with the C-termini of Nav channel pore-forming (α) subunits (Goldfarb et al., 2007; Laezza et al., 2007; Liu et al., 2001; Liu et al., 2003; Lou et al., 2005; Wittmack et al., 2004), and to modulate hippocampal and granule cell excitability (Goldfarb et al., 2007; Laezza et al., 2007; Wozniak et al., 2007; Xiao et al., 2007).

Purkinje neurons are the sole output of the cerebellum, critical for motor regulation and coordination (Burgess et al., 1995; Grusser-Cornehls and Baurle, 2001; Koeppen, 2005; Kohrman et al., 1996; Levin et al., 2006; Sausbier et al., 2004; Trudeau et al., 2006). A distinctive feature of mature Purkinje neurons is the robust expression of Nav1.6-encoded sodium channels (Afshari et al., 2004; Krzemien et al., 2000; Raman et al., 1997), the channel that underlies the “resurgent” sodium current that is critical for sustaining the characteristic high frequency firing of these cells (Raman et al., 1997). Mutations in human *SCN8A*, which encodes Nav1.6, are associated with cerebellar atrophy and ataxia (Trudeau et al., 2006). In addition, mice in which *Scn8a* has been disrupted or that lack Nav1.6 specifically in Purkinje cells display cerebellar ataxia and impaired Purkinje cell firing (Kohrman et al., 1996; Levin et al., 2006; Raman et al., 1997). These observations suggested that the ataxia in SCA27 individuals and in *Fgf14*^{-/-} mice reflects impaired Purkinje cell firing due to alterations in Nav channel expression and/or functioning. The experiments here were designed to explore this hypothesis directly.

Materials and Methods

Cerebellar slice recordings

Whole-cell recordings were obtained from Purkinje neurons in 300 μ m parasagittal cerebellar slices prepared from 25–30 day old wild type (WT) and *Fgf14*^{-/-}C57BL6 mice. Vibratome sections were cut in ice-cold solution containing (in mM): 87 NaCl, 2.5 KCl, 25 NaHCO₃, 1 NaH₂PO₄, 0.5 CaCl₂, 7 MgCl₂, 75 sucrose and 10 glucose, bubbled with 5% CO₂/95% O₂. Slices were incubated at room temperature in artificial CSF (ACSF), containing in mM: 125 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂ and 10 glucose, bubbled with 5% CO₂/95% O₂.

Purkinje neurons were visualized with infrared differential interference contrast (IR-DIC) optics on a Nikon upright microscope. Borosilicate glass patch pipettes (with resistances of 3–6 M Ω) were filled with internal recording solution containing (in mM): 140 K Gluconate, 2 MgCl₂, 1 CaCl₂, 10 EGTA, 2 MgATP, 10 HEPES. Whole-cell recordings were made in ACSF at room temperature 1–5 hours after slice preparation using an Axopatch 200B amplifier, Digidata 1322A interface and pClamp-9 software (Molecular Devices, Union City, CA, USA). Although non-bridge amplifiers can distort action potentials (Magistretti et al., 1996), studies utilizing the Axopatch amplifier showed no significant distortion of Purkinje neuron action potentials (Edgerton and Reinhart, 2003). Series resistance was monitored but not

compensated; cells were rejected if the series resistance exceeded 30 MΩ. Firing properties were examined in current clamp mode, and excitatory postsynaptic currents (EPSCs) were recorded in voltage-clamp mode at a holding potential of -70 mV. EPSCs from selected Purkinje neurons were evoked by applying square wave current pulses via a tungsten bipolar electrode to the molecular layer ~ 100 μ m from the Purkinje cell of interest. In some experiments, 5 μ M 6,7-dinitroquinoxaline-2,3-dione (DNQX), 10 μ M $\pm$ 3,3-(2-carboxypiperazine-4-yl)-propyl-1-phosphate (CPP) and 100 μ M picrotoxin were included in the ACSF. Analog current and voltage traces were digitized at 10 kHz.

Histology

WT and *Fgf14*^{-/-} C57BL6 mice were anesthetized with pentobarbital (60mg/kg,i.p.), transcardially perfused with a vascular rinse containing 0.9% NaCl, followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were dissected, postfixed in the same solution overnight at 4°C, and cryoprotected in 30% sucrose in 0.1 M phosphate buffered saline (PBS). After embedding in O. C. T. (Canemco and Morivac, Quebec), 14 μ m serial sagittal cryostat sections were collected in PBS.

For immunohistochemistry, free-floating tissue sections were washed in PBS and subsequently blocked in a solution containing 7.5% goat serum (Sigma) and 0.25% Triton X-100 (TX-100; Sigma) in PBS. Sections were then incubated at 4°C for 15 hr with a mouse anti-FGF14 or anti-Nav 1.6 (NeuroMab) monoclonal antibody diluted 1:1000, or with a polyclonal anti-calbindin (0–28K) antibody (Swant, Switzerland), diluted 1:5000, in PBS containing 1% goat serum and 0.25% TX-100. Sections were washed three times for 5 minutes at room temperature in PBS and subsequently incubated with an Alexa 488-conjugated goat anti-mouse or an Alexa 594 goat anti-rabbit secondary antibody (Molecular Probes), diluted 1:100, in PBS containing 1% goat serum and 0.25% TX-100. After rinsing in PBS, sections were mounted and examined using a Zeiss Axioskop 40 or Apo Tome microscope.

For *in situ* hybridization, a 550 bp *Fgf14* specific RNA probe (Wang et al., 2000) was labeled with digoxigenin following the manufacturer's protocol (Roche Diagnostics, IN). Free-floating brain sections (30 μ m) were washed twice in PBS, and treated with freshly prepared 10 μ g/ml proteinase K (Invitrogen, Carlsbad, CA) at 37°C. After acetylation, sections were incubated in hybridization buffer containing 0.2 μ g/ml of the digoxigenin-labeled *Fgf14* riboprobe at 43°C overnight. Hybridized sections were washed by successive immersions in 4x sodium chloride citrate buffer (SCC; containing 150 mM NaCl, 15 mM sodium citrate; pH 7.0) at room temperature; 2x SCC containing 50% formamide at 50°C for 30 min; 2x SCC at 37°C for 10 min; 2x SCC, containing 20 μ g/ml RNase A, at 37°C for 30 min; 2x SCC at 37°C for 20 min; and 0.1x SCC at room temperature for 10 min. Controls hybridized without primary probe showed no signal. The hybridization signals were detected with digoxigenin detection reagents (Roche Diagnostics, Indianapolis, IN). After rinsing in PBS, sections were mounted and analyzed with a Zeiss Axioskop microscope.

Data Analyses and Statistics

Current and voltage-clamp data were compiled and analyzed using Clampfit Version 9 (Axon) and Origin 7 (Origin Lab, Northampton, MA). All data are presented as means $\pm$ SEM. Statistical differences between WT and *Fgf14*^{-/-} neurons were examined using the Students *t* test; where appropriate, *p* values are reported in the text.

Results

Expression of *Fgf14* in Purkinje Neurons

Although it has previously been reported that *Fgf14* expression is evident in the granule cell layer in the (mouse) cerebellum, the resolution was insufficient to identify expression in specific cell types (Wang et al., 2002). To determine if *Fgf14* is expressed in Purkinje neurons, high resolution *in situ* hybridization was performed on thick sections of wild type (WT) mouse cerebellum. These experiments revealed robust expression of the *Fgf14* transcript in Purkinje neurons and moderate expression in granule cells, as well as in other interneurons of the granule cell layer (Figure 1A). In addition, immunohistochemical analysis revealed robust expression of the FGF14 protein in WT Purkinje and granule cells (Figure 1B), whereas FGF14 is undetectable in *Fgf14*^{-/-} cerebellum (Figure 1B).

Lack of Spontaneous and Tonic Firing in *Fgf14*^{-/-} Purkinje Neurons

The finding of robust expression of FGF14 in Purkinje neurons suggested that this protein might play a role in regulating the excitability of these cells and that the loss of FGF14 function in these cells could contribute to the ataxic phenotype observed in *Fgf14*^{-/-} mice and humans with *FGF14* mutations. To explore this hypothesis directly, whole-cell recordings were obtained from Purkinje neurons in acute parasagittal cerebellar slices prepared from 25–30 day old WT and *Fgf14*^{-/-} mice. As reported previously (Raman et al., 1997), WT Purkinje neurons are characterized by spontaneous repetitive firing (Figure 2). Most (10/14; 71%) of the WT Purkinje neurons examined displayed sustained tonic firing (Figure 2A). Three (of 14, 21%) of the cells fired episodic bursts of action potentials followed by quiescent periods (Figure 2B). One of the (14, 7 %) WT Purkinje neurons studied was quiescent and did not display spontaneous tonic or burst firing. In marked contrast with WT Purkinje neurons, most (14 of 17, 82%) of the *Fgf14*^{-/-} Purkinje neurons did not fire spontaneously and had a resting membrane potential of -60.1 ± 1.1 mV (Figure 2C). Three (3 of 17, 18%) of the *Fgf14*^{-/-} Purkinje neurons exhibited sustained tonic firing (Figure 2D), and the firing properties of these cells were indistinguishable from tonic firing WT cells (Figure 2A). The main difference between the WT and *Fgf14*^{-/-} Purkinje neurons, therefore, appeared to be the relative proportions of spontaneously active and “silent” Purkinje neurons (Figure 2D).

In WT Purkinje neurons that display sustained tonic firing, small hyperpolarizing current injections stopped repetitive firing (Figure 3A), and injections of depolarizing currents induced higher frequency sustained repetitive firing (Figure 3B,C). In the WT Purkinje neurons with intermittent bursting and in the single “silent” WT cell, small depolarizing current injections converted these cells to sustained tonic firing. All WT Purkinje neurons, therefore, either displayed tonic firing at rest or could be converted to a tonic firing pattern by depolarizing current injections. In addition, the frequency of repetitive firing in WT cells increased when the amplitudes of the depolarizing current injections were increased (Figure 3B, C). The mean $\pm$ SEM (n = 14) maximal firing frequency of WT Purkinje neurons recorded in response to 300 pA current injections was 58 ± 6 Hz (Figure 3F).

In *Fgf14*^{-/-} Purkinje neurons, the responses to depolarizing current injections were quite distinct. Prolonged injections of depolarizing currents into “silent” *Fgf14*^{-/-} Purkinje neurons resulted in the firing of one or a few action potentials (Figure 3D, E), but these cells did not sustain tonic repetitive firing regardless of the amplitudes of the injected currents. The mean $\pm$ SEM current threshold to evoke a spike in these “silent” *Fgf14*^{-/-} cells was 160 ± 30 pA (n = 14). The properties of the small subset (3 of 17) of *Fgf14*^{-/-} Purkinje neurons that displayed spontaneous tonic repetitive firing were similar to WT Purkinje neurons in that the firing frequencies of these cells also increased when the amplitudes of the depolarizing current injections were increased, and the range of repetitive firing frequencies was similar to that

determined in WT cells (Figure 3F). The maximal firing frequency in the non-silent *Fgf14*^{-/-} Purkinje neurons was 47 ± 24 Hz (Figure 3F).

Individual Action Potential Waveforms are Similar in WT and *Fgf14*^{-/-} Purkinje Neurons

Examination of the waveforms and the properties of individual action potentials in WT and *Fgf14*^{-/-} Purkinje neurons revealed no apparent differences (Figure 4 A, B). The voltage trajectories (dV/dt) versus time (Figure 4 C, D), as well as phase plots of dV/dt versus voltage (Figure 4 E, F), of individual action potentials in WT and *Fgf14*^{-/-} Purkinje neurons were similar. There were also no significant differences in the voltage thresholds for action potential generation (Figure 4G) or in the durations of individual action potentials in WT and *Fgf14*^{-/-} Purkinje neurons (Figure 4H). In addition, the input resistances of WT and *Fgf14*^{-/-} Purkinje neurons were not significantly different (Figure 4H).

Evoked EPSCs are Indistinguishable in WT and *Fgf14*^{-/-} Purkinje Neurons

Decreased excitability and impaired ability to sustain repetitive firing in response to depolarizing current injections was recently reported in *Fgf14*^{-/-} cerebellar granule cells (Goldfarb et al., 2007). It was also reported in this study that the properties of voltage-gated (Nav) currents in *Fgf14*^{-/-} cerebellar granule cells are altered. These observations might be interpreted to suggest the possibility that the firing properties of *Fgf14*^{-/-} Purkinje neurons are altered indirectly due to reduced excitatory inputs from *Fgf14*^{-/-} granule cells. To explore this hypothesis directly, paired excitatory postsynaptic currents (EPSCs) were evoked in WT and *Fgf14*^{-/-} Purkinje neurons by parallel fiber stimulation; stimuli were presented at an interval of 50 ms. The amplitudes of the first and the second EPSCs evoked were examined and the extent of paired pulse facilitation was determined. As illustrated in Figure 5, the amplitudes of the evoked EPSCs, as well as the paired pulse facilitation ratios (PPFR), in WT (n = 7) and *Fgf14*^{-/-} (n = 14) Purkinje neurons were not significantly different (Figure 5C). To further examine whether altered granule cell synaptic input could contribute to reduced Purkinje firing, recordings were obtained from *Fgf14*^{-/-} Purkinje neurons in the presence of 100 μ M Picrotoxin, 10 μ M $\pm$ CPP, and 5 μ M DNQX to block GABA, NMDA and AMPA/Kainate postsynaptic receptors, respectively. The resting and active membrane properties of *Fgf14*^{-/-} Purkinje neurons (n = 3) examined in the presence and absence of these blockers of postsynaptic receptors were indistinguishable (data not shown). The findings here that (most) *Fgf14*^{-/-} Purkinje neurons lack the ability to sustain tonic repetitive firing, therefore, does not appear to reflect altered synaptic inputs but rather reflects alterations in the intrinsic membrane properties of these cells.

Nav1.6 Expression is Reduced in *Fgf14*^{-/-} Purkinje Neurons

Previous studies in heterologous cells and in isolated hippocampal neurons *in vitro* have revealed that FGF14 affects the densities and the biophysical properties of Nav channels (Laezza et al., 2007; Liu et al., 2001; Liu et al., 2003; Lou et al., 2005; Wittmack et al., 2004). The observations here that repetitive firing is attenuated in *Fgf14*^{-/-} Purkinje neurons (Figures 2 and 3), but that action potential thresholds and waveforms in *Fgf14*^{-/-} and WT Purkinje neurons are similar (Figure 4), suggested that the loss of FGF14 likely reflects reduced expression (rather than alterations in the properties) of Nav1.6 channels which underlie the high frequency firing characteristic of these cells (Afshari et al., 2004; Raman et al., 1997). Interestingly, it has been reported that the selective elimination of Nav 1.6 in Purkinje neurons produces a phenotype (Levin et al., 2006) that is remarkably similar to that observed here in *Fgf14*^{-/-} Purkinje neurons. To determine the functional consequences of the loss of FGF14 on Nav1.6 expression, immunohistochemical experiments were performed with a specific anti-Nav1.6 antibody. In addition to morphological features, Purkinje cells were identified by Calbindin expression. As illustrated in Figure 6, although these experiments revealed robust

expression of Nav1.6 in the cell bodies and processes of calbindin-positive WT Purkinje neurons, Nav 1.6 expression was reduced in *Fgf14*^{-/-} Purkinje neurons. Calbindin staining in WT and *Fgf14*^{-/-} Purkinje neurons, in contrast, was similar (Figure 6). These observations suggest the interesting hypothesis that, in the absence of FGF14, Nav1.6 expression is reduced in Purkinje neurons, which results in impaired firing in these cells and underlies the ataxic phenotype that is prominent in *Fgf14*^{-/-} mice.

Discussion

Spontaneous and Evoked Repetitive Firing are Attenuated in *Fgf14*^{-/-} Purkinje Neurons

Purkinje neurons are the sole output of the cerebellar cortex, and Purkinje cell dysfunction or degeneration has been linked to cerebellar ataxias in human and in animal models (Burgess et al., 1995; Grusser-Cornehls and Baurle, 2001; Koeppen, 2005; Kohrman et al., 1996; Levin et al., 2006; Sausbier et al., 2004; Trudeau et al., 2006). Mature Purkinje neurons express predominantly Nav 1.6-encoded Nav channels, which generate resurgent Nav currents during action potential repolarization, enabling sustained, high frequency repetitive firing (Afshari et al., 2004; Raman et al., 1997). Mice (*Scn8a*^{tg}) in which the gene (*Scn8a*) encoding Nav1.6 has been disrupted throughout the nervous system develop early onset ataxia with tremor, and cellular electrophysiological studies revealed impaired repetitive firing in Purkinje neurons (Raman et al., 1997). In addition, mice (*Scn8a-KO*) harboring a Purkinje neuron specific disruption of *Scn8a* display a mildly ataxic gait (Levin et al., 2006) that resembles that observed in mice with Purkinje cell degeneration (Grusser-Cornehls and Baurle, 2001). In contrast, no motor impairment was evident in mice harboring granule cell specific deletion of *Scn8a* (Levin et al., 2006). Taken together, these observations suggest that the disruption of Nav1.6 function in Purkinje neurons cells has greater impact on motor control and coordination compared to Nav1.6 disruption in granule cells. Purkinje neurons of *Scn8a-KO* and *Fgf14*^{-/-} mice exhibit impaired spontaneous tonic repetitive firing even in response to depolarizing current injections. The loss of Nav1.6 in Purkinje neurons in combination with the previously described alteration in granule cell physiology (Goldfarb et al., 2007) probably accounts for the ataxic phenotype of the *Fgf14*^{-/-} mice similar to mice with a knockout of *Scn8a* in both Purkinje and granule cells. Although a majority of *Fgf14*^{-/-} Purkinje neurons exhibited impaired firing, a small subset of neurons retained the pattern of sustained tonic firing seen in the WT neurons. It has previously been demonstrated that the expression of Nav channel isoforms changes during development with Nav1.6 being the major Nav channel expressed in mature Purkinje neurons (Shah et al., 2001). The observations here may be related to having performed experiments during a phase of development in which some Purkinje neurons retained a more immature pattern of Nav channel expression. Alternatively, the phenotypic differences in cell properties may represent heterogeneity in patterns of Nav channel expression (Shah et al., 2001), or possibly heterogeneities in FGF14 expression, in different areas of the cerebellum.

These combined observations also support the hypothesis that the attenuation of Purkinje neuron repetitive firing contributes importantly to the ataxic phenotype seen in *Fgf14*^{-/-} mice, as well as in SCA27 afflicted individuals, harboring the FGF14^{F145S} mutation, which was shown recently to act as a dominant negative that disrupts the function of wild type FGF14 (Laezza et al., 2007). It is tempting to further speculate that the ataxia phenotype in humans with a deletion mutation (D163fsx12) that produces a truncated FGF14 protein (Brusse et al., 2005), may also result from alterations in the firing properties of Purkinje neurons. Further experiments aimed at exploring this hypothesis directly will be of considerable interest.

Nav1.6 Expression is Reduced in *Fgf14*^{-/-} Purkinje Neurons

Several previous studies have demonstrated that FGF14 and the other intracellular FGFs interact directly with the pore forming α subunits of neuronal voltage-gated sodium (Nav)

channels and regulate the cell surface expression of Nav channel α subunits (Goldfarb et al., 2007; Laezza et al., 2007; Lou et al., 2005). The results presented here demonstrate that the loss of FGF14 is associated with reduced expression of Nav1.6 in cerebellar Purkinje neurons. These observations, together with the phenotypic similarities between the *Fgf14*^{-/-} mice and both the *Scn8a*^{tg} and the Purkinje cell specific *Scn8a-KO* mice suggest that reduced expression of *Scn8a*-encoded Nav1.6 channels may be a common pathogenic mechanism underlying impaired Purkinje neuron repetitive firing and cerebellar ataxia.

It seems reasonable to speculate that the direct modulation of the cell surface expression, distribution and/or localization of Nav1.6-encoded channels in Purkinje neurons, accounts for the observed impairment of spontaneous and tonic repetitive firing in these cells. Further experiments aimed at exploring the molecular mechanisms underlying the observed reduction in Nav1.6 expression will be needed to distinguish among these possibilities. Understanding the etiology of the resulting disruption in physiology and patterns of Purkinje neuron firing may lead to identification of novel therapeutic agents that can potentially restore normal firing as has been demonstrated in the Nav1.6 deficient *Scn8a*^{tg} mice (Grieco and Raman, 2004). Delineating the role of FGF14 in the regulation of cerebellar Nav channel expression could provide novel insights into the mechanisms underlying inherited ataxias, and may lead to new therapeutic targets for treating these progressive disorders.

Relationship to Previous Studies

It was recently reported that cerebellar granule cells in mice (*Fgf12*^{-/-}/*Fgf14*^{-/-}) lacking both FGF12 and FGF14 express Nav channels that inactivate more rapidly and at more negative membrane potentials, and that recover from inactivation more slowly than WT granule cell Nav channels (Goldfarb et al., 2007). In addition, the *Fgf12*^{-/-}/*Fgf14*^{-/-} mice have more dramatic motor deficits than *Fgf14*^{-/-} mice (Goldfarb et al., 2007). Electrophysiological experiments revealed that *Fgf14*^{-/-} granule cells were not able to sustain repetitive firing of action potentials in response to prolonged membrane depolarizations, in spite of the fact that input resistances, whole-cell membrane capacitances, resting membrane potentials, action potential thresholds and afterhyperpolarizations in *Fgf14*^{-/-} granule cells were shown to be quite similar to WT granule cells (Goldfarb et al., 2007). The results presented here, however, suggest that impaired Purkinje cell firing will also be prominent and likely underlies the ataxic phenotype in *Fgf12*^{-/-}/*Fgf14*^{-/-}, as in *Fgf14*^{-/-} mice. The observed reduction in the firing of *Fgf14*^{-/-} cerebellar granule neurons (Goldfarb et al., 2007), may also be a reflection of altered Nav channel expression in these cells. However, loss of Nav1.6 protein from axon initial segments was not detected in granule cells from *Fgf12*^{-/-}/*Fgf14*^{-/-} mice. One possible explanation is that FGF14 deficiency causes a reduction in granule cell Nav1.6 protein levels sufficient to produce impaired granule cell firing, but that the Nav1.6 protein level reduction is not detectable by immunohistochemistry. Reduced excitability of cerebellar granule neurons, which project excitatory synaptic afferents to Purkinje cells, might be expected to further reduce the excitability of Purkinje neurons in *Fgf14*^{-/-} mice. The experiments here, however, demonstrate that granule cell stimulated EPSC and paired pulse facilitation are similar in WT and *Fgf14*^{-/-} Purkinje neurons. Nevertheless, further studies will be needed to determine if the selective loss of FGF14 in Purkinje neurons is sufficient to produce ataxia.

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Figure 1.

Fgf14/FGF14 expression in adult mouse Purkinje neurons. (A) High resolution thick section *in situ* hybridization revealed robust expression of *Fgf14* mRNA in the Purkinje (p) cell layer, moderate expression in the granule (g) cell layer and low expression in the molecular (m) layer of the adult mouse cerebellum. At higher magnification (A'), *Fgf14* mRNA is evident in Purkinje neuron cell bodies (arrow). Immunohistochemical experiments using a monoclonal anti-FGF14 antibody demonstrated FGF14 immunoreactivity in wild type (B), but not in *Fgf14*^{-/-} (C), adult mouse cerebellar Purkinje (arrows) and granule cells. Scale bar corresponds to 1 mm in (A), 125 μm in (A') and 250 μm in (B) and (C).

Figure 2.

Spontaneous firing is attenuated in *Fgf14*^{-/-} Purkinje neurons. Whole-cell current clamp recordings were obtained from visually identified Purkinje neurons in acute cerebellar slices prepared from 25–30 day old wild-type (WT) and *Fgf14*^{-/-} mice as described in **Materials and Methods**. Representative recordings from WT Purkinje neurons displaying (A) sustained tonic firing (observed in ~70% of WT cells) and (B) episodic bursting (observed in ~20% of WT cells) are shown; note the compressed time-base in (B) to illustrate several seconds of quiescence preceding and following the burst of tonic firing. In contrast, most (~80%) *Fgf14*^{-/-} Purkinje neurons were quiescent (C). The other (~20%; 3 of 17) *Fgf14*^{-/-} Purkinje neurons exhibited sustained tonic firing, and the repetitive firing properties of these cells appeared indistinguishable from WT tonic firing Purkinje neurons. (D) Histogram showing percentages of spontaneously firing and silent WT and *Fgf14*^{-/-} Purkinje neurons.

Figure 3.

Repetitive firing in response to depolarizing current injections is impaired in *Fgf14*^{-/-} Purkinje neurons. Current clamp recordings were obtained as from visually identified Purkinje neurons in acute cerebellar slices from 25–30 day old WT and *Fgf14*^{-/-} mice. (A) Spontaneous firing (gray) in WT Purkinje neurons stops in response to hyperpolarizing current injection (black). (B, C) In response to depolarizing current injection, repetitive firing is increased in WT Purkinje neurons (B), and the rate of firing is increased (C) with the amplitude of the depolarizing current injection. (D) In contrast, depolarizing current injection in a quiescent *Fgf14*^{-/-} Purkinje neuron (gray) evoked only a single spike (black). (E) Multiple spikes could be elicited in quiescent *Fgf14*^{-/-} Purkinje neurons by increasing the amplitude of the depolarizing current injected, but sustained high frequency firing was not observed. (F) Mean $\pm$ SEM spikes evoked in response to 1 sec depolarizing current injections in tonic firing WT (filled squares; n = 14), “silent” *Fgf14*^{-/-} (filled triangles, n = 14) and tonic firing *Fgf14*^{-/-} (filled circles; n = 3) Purkinje neurons are plotted as a function of injected current amplitude.

Figure 4.

Waveforms and properties of individual action potentials are similar in WT and *Fgf14*^{-/-} Purkinje neurons. Representative action potential waveforms, recorded from WT (**A**) and *Fgf14*^{-/-} (**B**) Purkinje neurons in cerebellar slices prepared from 25–30 day old WT and *Fgf14*^{-/-} mice, are displayed. Plots of dV/dt plots versus time (**C**, **D**) and phase plots of dV/dt versus voltage (**E**, **F**) in WT (**C**, **E**) and *Fgf14*^{-/-} (**D**, **F**) Purkinje neurons revealed the similarities in the properties of evoked action potentials. (**G**) Cumulative spike threshold data, derived from phase plots such as those illustrated in panels **E** and **F**, revealed no significant difference in the voltage thresholds for individual action potentials in WT (n = 13) and *Fgf14*^{-/-} (n = 16) Purkinje neurons. (**H**) In addition, there were no significant differences in the mean spike widths or input resistances of WT (n = 13) and *Fgf14*^{-/-} (n = 17) Purkinje neurons.

Figure 5.

Paired pulse facilitation of granule cell synaptic inputs is similar in WT and *Fgf14*^{-/-} Purkinje neurons. Whole-cell voltage-clamp recordings were obtained from visually identified Purkinje neurons in acute cerebellar slices from 25–30 day old WT and *Fgf14*^{-/-} mice as described in **Materials and Methods**. Pairs of excitatory postsynaptic currents (EPSCs) were evoked by bipolar stimulation of parallel fibers approximately 100 μ m from the Purkinje neurons at an interstimulus interval of 50 ms. Representative recordings of pairs of evoked EPSCs from WT (A) and *Fgf14*^{-/-} (B) Purkinje neurons at a holding potential -70 mV are illustrated. (C) The mean $\pm$ SEM amplitudes of evoked EPSCs and the paired-pulse facilitation ratios (PPFR) in WT (n = 7) and *Fgf14*^{-/-} (n = 14) are not significantly different.

Figure 6.

Expression of Nav1.6 is reduced in *Fgf14*^{-/-} Purkinje neurons. Confocal images were obtained from WT (A–C) and *Fgf14*^{-/-} (D–F) cerebellar sections stained with primary antibodies against Calbindin and Nav 1.6, as described in **Materials and Methods**. Calbindin immunoreactivity is evident in the cell bodies of Purkinje neurons in the Purkinje (p) cell layer and in the dendrites of these cells in the molecular (m) layer in WT (A) and in *Fgf14*^{-/-} (D) cerebellar sections. The expression of Nav 1.6, which is readily detected in calbindin-positive Purkinje neurons in WT sections (B) is less evident in *Fgf14*^{-/-} Purkinje neuron cell bodies and dendrites (E). Merged images suggest that Nav 1.6 is mainly expressed in Purkinje neurons (C) and that Nav 1.6 expression is decreased in FGF14 deficient (F) Purkinje neurons. Scale bar corresponds to 125 μ m.