



Lack of Cav3.1 channels causes severe motor coordination defects and an age-dependent cerebellar atrophy in a genetic model of essential tremor

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ABSTRACT

T-type Ca²⁺ channels have been implicated in tremorogenesis and motor coordination. The $\alpha 1$ subunit of the Cav3.1 T-type Ca²⁺ channel is highly expressed in motor pathways in the brain, but knockout of the Cav3.1 gene ($\alpha 1G^{-/-}$) *per se* causes no motor defects in mice. Thus, the role of Cav3.1 channels in motor control remains obscure *in vivo*. Here, we investigated the effect of the Cav3.1 knockout in the null genetic background of $\alpha 1$ GABA_A receptor ($\alpha 1^{-/-}$) by generating the double mutants ($\alpha 1^{-/-}/\alpha 1G^{-/-}$). $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice showed severer motor abnormalities than $\alpha 1^{-/-}$ mice as measured by potentiated tremor activities at 20 Hz and impaired motor learning. Propranolol, an anti-ET drug that is known to reduce the pathologic tremor in $\alpha 1^{-/-}$ mice, was not effective for suppressing the potentiated tremor in $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice. In addition, $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice showed an age-dependent loss of cerebellar Purkinje neurons. These results suggest that $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice are a novel mouse model for a distinct subtype of ET in human and that Cav3.1 T-type Ca²⁺ channels play a role in motor coordination under pathological conditions.

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1. Introduction

Essential tremor (ET) is a common [1], complex neurologic disorder with a broad spectrum of clinical characteristics such as age of onset, primarily affected body region, and rate of progression [2,3]. Diverse neural circuits and pathophysiological mechanisms likely contribute to the complexity of ET symptoms although supporting evidences remain to be elucidated.

The search for the mechanisms underlying ET has been facilitated by the use of harmaline, a plant-derived metabolite, known to be increased in the blood of ET patients [4]. When administered to human and rodents [5,6], harmaline causes essential-like tremor. Studies revealed that harmaline induces tremor rhythms at around 10 Hz through the olivocerebellar pathway [7,8] by increasing the amplitude of subthreshold oscillations in the inferior olive neurons, which are regulated by T-type Ca²⁺ currents [9]. And among the three T-type Ca²⁺ channel isoforms (Cav3.1, Cav3.2, and Cav3.3), null mutation or shRNA-mediated knockdown of Cav3.1 ($\alpha 1G^{-/-}$) in the inferior olive significantly attenuated the harmaline-induced tremor [10], suggesting that Cav3.1 is the tremor-producing isoform in the harmaline model of ET.

Recently, a novel genetic model of ET has been reported; mice lacking the GABA_A receptor $\alpha 1$ subunit ($\alpha 1^{-/-}$) show several behavioral and pharmacological characteristics of human ET [11]. The loss of GABA inhibition [12] and subsequent adaptation and reorganization of GABAergic circuits in the brain regions [13–15] may underlie the genetic tremor in $\alpha 1^{-/-}$ mice, but it remains unknown whether T-type Ca²⁺ channels are involved in the tremorogenesis. To address this issue, we investigated the role of Cav3.1 T-type Ca²⁺ channels in a genetic ET model by generating double knockout mice ($\alpha 1^{-/-}/\alpha 1G^{-/-}$) and found that markedly increased rather than decreased tremor was observed. The pathologic tremor in $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice was symptomatically similar as but pharmacologically different from that of $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice, indicative of a mouse model for novel type of ET. Our data further support the idea that Cav3.1 T-type Ca²⁺ channels are involved in modulating motor function under pathological condition.

2. Materials and methods

2.1. Animals

GABA_A receptor $\alpha 1$ heterozygous ($\alpha 1^{+/-}$) mice with a C57BL/6J genetic background were obtained from the laboratory of Dr. Gregg E. Homanics and utilized for the production of wild-type ($\alpha 1^{+/+}$) and homozygous mutants ($\alpha 1^{-/-}$). Double heterozygotes were

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produced ($\alpha 1^{+/-}/\alpha 1G^{+/-}$) and crossed to generate double mutants ($\alpha 1^{-/-}/\alpha 1G^{-/-}$) and their littermate controls ($\alpha 1^{-/-}/\alpha 1G^{+/+}$, $\alpha 1^{+/+}/\alpha 1G^{+/+}$). The genotypes were confirmed by PCR analysis, as previously described [16,17]. All the experiments below were performed using 4–8 month-old mice. Animal care and handling were carried out following the institutional guidelines of the Animal Care and Use Committee of KAIST. Mice were housed in a 12-h light/dark cycle (7 AM–7 PM) with free access to water and food.

2.2. Tremor measurement and analysis

The tremor recording platform was an opaque metal cylinder (diameter 10 cm \times height 15 cm) mounted with an accelerometer (DC response accelerometer, Model No. 3711D1FA3G, Piezotronics) and suspended by two elastic wires inside a soundproof chamber [10]. A video camera was placed on top of the platform and the behaviour of each mouse was monitored during the recording session.

After acclimatization to the soundproof chamber for 1 h, each mouse was placed on the recording platform and tremulous activity was measured for 10 min. Electrical signals from the accelerometer were sampled at 1 kHz and digitized with the MiniDigi 1A (Axon Instrument). Raw traces and tremor strength for individual 2-min epochs were analyzed using Clampfit 9.2 (Axon Instrument) and Fourier transformation algorithm (MATLAB 7.0; MathWorks), respectively.

2.3. Rotarod test

Mice ($n=7$ for $\alpha 1^{-/-}/\alpha 1G^{-/-}$, $n=5$ for $\alpha 1^{-/-}/\alpha 1G^{+/+}$) were placed on an accelerating rotating rod (3 cm in diameter) apparatus (Model No. 47600, Ugo Basile) that accelerated from 5 to 40 rpm over a period of 5 min. Each mouse was given four trials per day for four consecutive days, and each trial lasted a maximum of 5 min with a 30-min inter-trial interval. The latency for each mouse to fall off the rod was recorded.

2.4. Pharmacological analysis

Propranolol (10 and 20 mg/kg, Sigma–Aldrich) and ethanol (0.5 and 1 g/kg, OCI Company Ltd.) were dissolved in saline. The dose of each drug was determined based on previous study [11] and prepared fresh each day. Vehicles and drugs were administered intraperitoneally in a 10 ml/kg injection volume 1 h before tremor measurement. A separate group of mice was used to test each dose.

2.5. Immunohistochemical staining and counting of Purkinje cells

$\alpha 1^{+/+}/\alpha 1G^{+/+}$, $\alpha 1^{-/-}/\alpha 1G^{+/+}$, and $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice ($n=3-5$ per genotype) were perfused with 4% formaldehyde in PBS at 4 and 8 month of age, and the brains were extracted and postfixed for 8 h at 4 °C. Cerebellar paraffin blocks were prepared using a Tissue Processor (Leica) and cut sagittally at 10 μ m using a rotary microtome (Leica). Following standard deparaffinization procedures, the sections were antigen-retrieved by incubating at 90 °C for 50 min in 10 mM sodium citrate buffer with 0.05% Tween 20, pH 6.0. After rinsing the sections in distilled water (D.W.) for 5 min, immunoperoxidase staining was performed using the VECTASTAIN® Elite ABC kit according to the manufacturer's instructions. In brief, the sections were incubated with a 0.3% H_2O_2 solution in D.W. for 30 min, rinsed in 0.02% Tween 20 in PBS, and incubated for 30 min with 0.3% Triton®X-100, 5% horse serum, and 5% goat serum in PBS. After washing in 0.02% Tween 20 in PBS, the sections were incubated for 1 h with anti-calbindin D-28 K polyclonal antibody (1:50, Millipore) diluted in 0.3% Triton®X-100 in PBS. The sections were washed in 0.02% Tween

20 in PBS, incubated with biotinylated secondary antibody for 30 min, washed, and incubated with VECTASTAIN® Elite ABC reagent for 30 min. Sections were then washed and further incubated with peroxidase substrate solution (3,3'-diaminobenzidine tetrahydrochloride hydrate, Aldrich®) for 2 min. After washing in D.W., the sections were briefly immersed in xylene, air dried, and coverslipped with Shandon Synthetic Mountant (Thermo). All procedures were performed at room temperature. Calbindin-positive Purkinje cells were observed under a confocal microscope (LSM510, Carl Zeiss) using a 20 \times objective.

Nucleated Purkinje cells in the anterior lobe (lobule I–III) of the cerebellar cortex in every fifth sections were counted blind to genotypes using ImageJ software, and Purkinje cell linear density was calculated by dividing the number of cells by the linear distance of the Purkinje cell layer per section.

2.6. Statistical analysis

All data are presented as the mean \pm SEM and subjected to ANOVAs with appropriate post hoc tests. This analysis was performed using SigmaStat 3.1 software package.

3. Results

$\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice were born at the normal Mendelian ratio and showed normal growth and survival, but they were strikingly distinguishable from their wild-type ($\alpha 1^{+/+}/\alpha 1G^{+/+}$) and $\alpha 1^{-/-}/\alpha 1G^{+/+}$ littermates by outstanding tremulous activity. The double mutants shared several tremor-related features with $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice; they exhibited tremor when freely moved or suspended by tails, but not when relaxed, and all four limbs were equally affected. For a more quantitative analysis, the motion activity displayed by the three genotypic groups of mice was assessed and compared using an accelerometric transducer (Fig. 1). All three groups of mice showed 3–5 Hz activity reflecting basal locomotion, without quantitative differences ($p>0.05$, Fig. 1B). $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice exhibited tremor at 20–25 Hz as reported previously [11], but the tremor was evident during only the first 2-min period (Fig. 1C). In $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice, the 20–25 Hz tremor was increased 3- to 15-fold as compared to that of $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice and persisted throughout the recording session (Fig. 1C).

Based on the fact that ET impairs normal motor performance in patients [18,19], rotarod testing was used to measure motor coordination (Fig. 2). The rotarod performance of $\alpha 1^{+/+}/\alpha 1G^{+/+}$ mice reached the maximum level (5 min) since the second day (data not shown). Compared with $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice, $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice demonstrated a significantly lower score on the third (51.8 ± 10.2 and 25.6 ± 5.2 s, respectively) and fourth (70.8 ± 2.7 and 35.4 ± 4.8 s, respectively; $*p<0.05$) day. Furthermore, $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice did not show motor learning at all, as measured by the increase in latency to fall from the rotating rod as the number of trials increased, while $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice did ($*p<0.001$). Taken together, these results suggest that the loss of $Ca_v3.1$ channels in $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice leads to a more pronounced, persistent, and severe form of essential-like tremor.

Next, anti-ET drugs were used to determine whether the 20–25 Hz pathologic tremor in $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice had the pharmacological properties of ET. Propranolol, a first-line medication for ET, and ethanol, a more potent anti-ET drug, markedly reduced tremor amplitudes in $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice [11]. However, $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice still showed tremor even after administration of 20 mg/kg of propranolol (Fig. 3A and B), a dose two times higher than necessary to suppress the tremor in $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice [11]. On the contrary, ethanol effectively ameliorated the tremor by $\sim 90\%$ at a non-sedative dose of 1 g/kg (Fig. 3A and B). Thus, unlike the $\alpha 1^{-/-}$

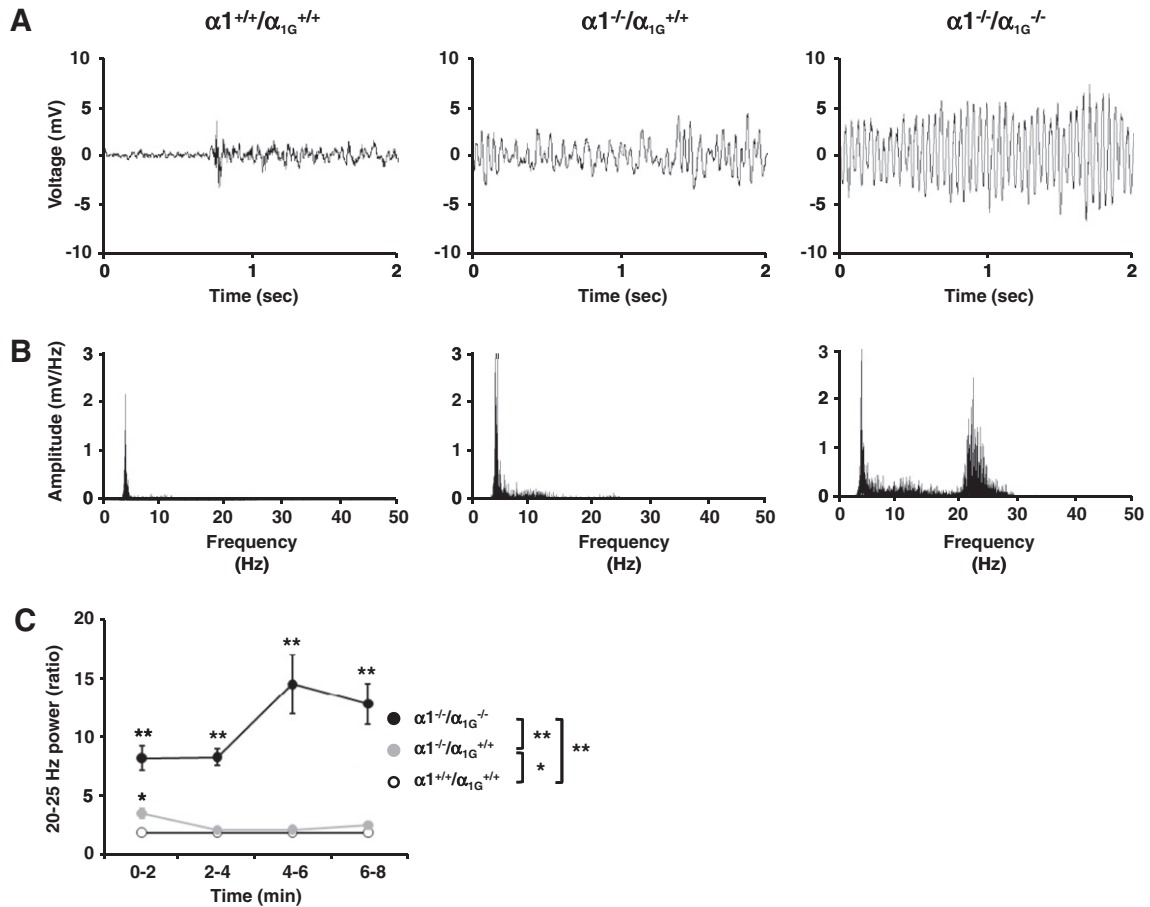


Fig. 1. Tremor measurement and analysis. Representative voltage tracings (A) and peak amplitudes (B) of tremulous activity in the three genotypes ($n = 3$ for $\alpha 1^{+/+}/\alpha 1G^{+/+}$ and $\alpha 1^{-/-}/\alpha 1G^{-/-}$, $n = 4$ for $\alpha 1^{-/-}/\alpha 1G^{+/+}$). (C) Relative changes in tremor strength. Data represent tremor strength (20–25 Hz; see Section 2) for each 2-min epochs in $\alpha 1^{-/-}/\alpha 1G^{+/+}$ (gray circle) and $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice (black circle), respectively, normalized by that in $\alpha 1^{+/+}/\alpha 1G^{+/+}$ mice (white circle; * $p < 0.05$, ** $p < 0.001$, two-way ANOVA). Error bars indicate SEM.

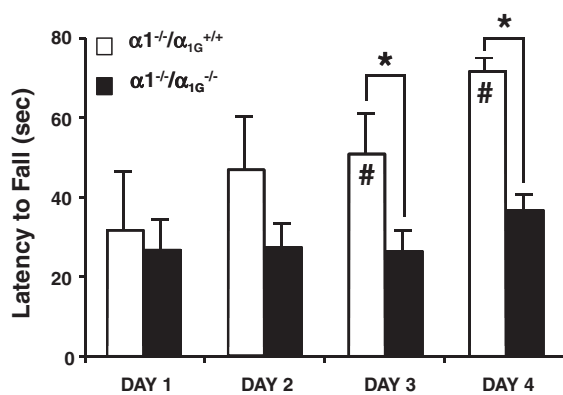


Fig. 2. Comparison of rotarod performance between the two mutants. Data represent the mean latency to fall (in seconds) \pm SEM for each trial over four successive days (* $p < 0.05$, # $p < 0.001$ versus day 1, two-way ANOVA with repeated measures).

$\alpha 1G^{+/+}$ mice, our double knockout mice differentially respond to a subset of ET medications.

Several studies have shown that ET is accompanied by pathologic changes in the cerebellum; such as a loss of cerebellar Purkinje cells (PCs) [20–22]. In addition, PC degeneration has been shown to be involved in tremor in rodents [23,24]. However,

$\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice did not show any deficits in the number or morphology of PCs [11]. To understand the etiology of the severer essential-like tremor in $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice, histological analysis of the cerebellum in the three genotypic groups of mice was performed. No significant differences in the number (21.0 ± 0.2 for wild-type, 22.7 ± 0.4 for $\alpha 1^{-/-}/\alpha 1G^{+/+}$, and 22.3 ± 1.5 for $\alpha 1^{-/-}/\alpha 1G^{-/-}$), location or morphology of calbindin-stained PCs between genotypes at 4-months of age were observed (Fig. 4A). However, a mild decrease in PCs was detected in 8-month-old $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice (Fig. 4A and B). Given the early onset of tremulous symptoms in our mutant mice (<3-week of age), these results show that the observed PC degeneration, rather than playing a causative role, is a parallel manifestation or a by-product of the persistent, pathologic tremor in $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice.

4. Discussion

Our study provides the first *in vivo* evidence of the role of T-type Ca^{2+} channels in motor control. Though $\alpha 1G^{-/-}$ mice showed no defects in normal motor performance [10], knockout of the $\text{Ca}_v3.1$ gene in a GABA_A receptor $\alpha 1$ subunit-null genetic background leads to severe motor disturbances (Figs. 1 and 2) that even lead to an age-dependent loss of cerebellar Purkinje cells (Fig. 4). Thus, it is likely that the role of $\text{Ca}_v3.1$ channels in motor control is more pronounced under pathological conditions.

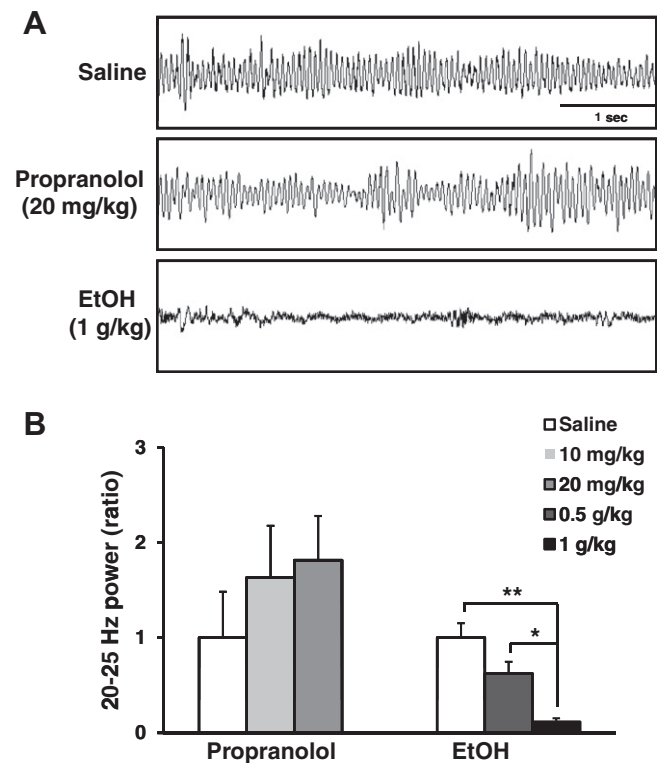


Fig. 3. Responses of $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice to anti-tremor agents. (A and B) Motion activity in $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice ($n=3-4$ per treatment) was measured after administration of vehicle (saline), propranolol (10 and 20 mg/kg), or ethanol (0.5 and 1 g/kg). Representative voltage tracings (A) and relative tremor strength (B) from vehicle- or drug-treated $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice are shown. (B) Data represent the changes of mean tremor amplitudes (20–25 Hz) \pm SEM for 100-s epochs in drug treatment relative to that in vehicle treatment (* $p < 0.05$, ** $p < 0.001$, one-way ANOVA).

ET, once known as a monosymptomatic disorder, has nowadays been recognized as a highly heterogeneous entity. The genetic [25] and clinical [26] variability of ET thus infer diverse and complex tremorogenic mechanisms. In an attempt to better understand the etiology of ET, here we extend the scope of our previous study using harmaline model [10] to the $\alpha 1$ -null genetic model [11], thereby generating double knockout mice ($\alpha 1^{-/-}/\alpha 1G^{-/-}$). Considering our previous results that $\alpha 1G^{-/-}$ mice showed no overt motor defects and were resistant to the harmaline-induced tremor [10], no alterations or suppression of the genetic tremor was anticipated. Rather, however, severer tremor was produced from the double knockout mice. Compared to $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice, the $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice exhibited a pathologic tremor with increased amplitudes followed by exacerbated motor disturbances (Figs. 1 and 2).

It cannot convincingly be answered why the pathologic tremor in $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice was enhanced, instead of being intact or abolished, by $\text{Ca}_v3.1$ knockout. Given that the tremor frequency of $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice precisely overlap with that of $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice (20–25 Hz), present results may not be due to a non-specific increase of tremor susceptibility but rather suggest a possibility of the two ET models having different tremorogenic origins whereat the role of $\text{Ca}_v3.1$ channels differs. Harmaline tremor has been known to be induced in inferior olive neurons [10,27,28]. But the $\alpha 1$ subunit of the GABA_A receptor is not expressed in the inferior olive [29] and the loss of GABA_A receptor binding sites in $\alpha 1^{-/-}$ mice was pronounced in the major motor pathways from the brainstem to the thalamus and the basal ganglia [12], suggesting a non-olivocerebellar origin of the tremor observed in

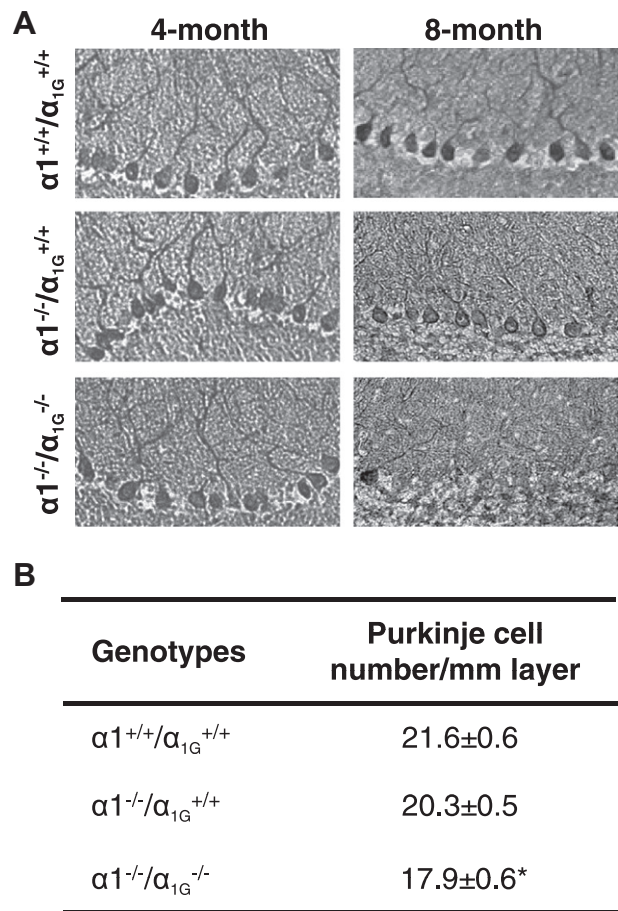


Fig. 4. Age-dependent change of Purkinje layer integrity. (A) Calbindin-stained Purkinje cells in the cerebellum of 4- or 8-month-old mice from the three genotypic groups. Images are magnified by 400 \times . (B) Purkinje cell linear density in 8-month-old $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice and littermate controls was calculated. Data are presented as the mean cell number per mm layer \pm SEM (* $p < 0.05$, one-way ANOVA).

$\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice. In addition, $\text{Ca}_v3.1$ channels are highly expressed in the motor pathway [30,31], thus likely contribute to modulate the pathologic tremor. Further studies using region-specific Cre drivers and $\alpha 1$ -floxed mice ($\alpha 1^{F/F}$) [32], along with $\text{Ca}_v3.1$ -specific shRNA, will be required to accurately explain present results and to support our hypothesis regarding tremor origins.

Our double mutants, as well as $\alpha 1^{-/-}/\alpha 1G^{+/+}$ mice, share common behavioral and pharmacological features with ET patients; $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice showed a persistent, pathologic tremor with postural and kinetic components and ethanol-responsiveness (Fig. 3). Furthermore, the prominent pathological changes in ET patients were also detected in $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice (Fig. 4). The only difference between them was that propranolol was not efficacious in reducing tremor amplitudes in $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice (Fig. 3). However, Deuschl et al. reported the identification of a subgroup among ET patients who showed no improvement of the tremor when given propranolol [33]. Thus, our double knockout model likely represents a distinct subtype of ET that is refractory to the β -blocker.

In conclusion, our study suggests that the $\text{Ca}_v3.1$ T-type Ca^{2+} channel plays a role in modulating motor function under pathological condition and thus, the treatment of ET by antagonizing T-type Ca^{2+} channels [34] should be considered with caution, due to possible contraindications. In addition, our $\alpha 1^{-/-}/\alpha 1G^{-/-}$ mice can be used as a novel genetic model for studying ET and for screening drug candidates.

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References

- [1] E.D. Louis, J.J. Ferreira, How common is the most common adult movement disorder? Update on the worldwide prevalence of essential tremor, *Movement Disorders* 25 (2010) 534–541.
- [2] J.P. Hubble, K.L. Busenbark, R. Pahwa, K. Lyons, W.C. Koller, Clinical expression of essential tremor: Effects of gender and age, *Movement Disorders* 12 (1997) 969–972.
- [3] E.D. Louis, B. Ford, L.F. Barnes, Clinical subtypes of essential tremor, *Archives of Neurology* 57 (2000) 1194–1198.
- [4] E.D. Louis, W. Zheng, E.C. Jurewicz, D. Watner, J. Chen, P. Factor-Litvak, M. Parides, Elevation of blood beta-carboline alkaloids in essential tremor, *Neurology* 59 (2002) 1940–1944.
- [5] H.H. Pennes, P.H. Hoch, Psychotomimetics clinical and theoretical considerations: harmine, Win-2299 and nalline, *American Journal of Psychiatry* 113 (1957) 887–892.
- [6] G.L. Henderson, D.E. Woolley, Ontogenesis of drug-induced tremor in the rat, *Journal of Pharmacology and Experimental Therapeutics* 175 (1970) 113–120.
- [7] C.D. Montigny, Y. Lamarre, Rhythmic activity induced by harmaline in Olivo-Cerebello-Bulbar system of cat, *Brain Research* 53 (1973) 81–95.
- [8] R. Llinas, R.A. Volkind, Olivo-Cerebellar system - functional properties as revealed by harmaline-induced tremor, *Experimental Brain Research* 18 (1973) 69–87.
- [9] I. Lampl, Y. Yarom, Subthreshold oscillations and resonant behavior: Two manifestations of the same mechanism, *Neuroscience* 78 (1997) 325–341.
- [10] Y.G. Park, H.Y. Park, C.J. Lee, S. Choi, S. Jo, H. Choi, Y.H. Kim, H.S. Shin, R.R. Llinas, D. Kim, Ca(V)3.1 is a tremor rhythm pacemaker in the inferior olive, *Proceedings of the National Academy of Sciences of the United States of America* 107 (2010) 10731–10736.
- [11] J.E. Kralic, H.E. Criswell, J.L. Osterman, T.K. O'Buckley, M.E. Wilkie, D.B. Matthews, K. Hamre, G.R. Breese, G.E. Homanics, A.L. Morrow, Genetic essential tremor in gamma-aminobutyric acidA receptor alpha1 subunit knockout mice, *Journal of Clinical Investigation* 115 (2005) 774–779.
- [12] J.E. Kralic, E.R. Korpi, T.K. O'Buckley, G.E. Homanics, A.L. Morrow, Molecular and pharmacological characterization of GABA(A) receptor alpha1 subunit knockout mice, *Journal of Pharmacology and Experimental Therapeutics* 302 (2002) 1037–1045.
- [13] J.E. Kralic, C. Sidler, F. Parpan, G.E. Homanics, A.L. Morrow, J.M. Fritschy, Compensatory alteration of inhibitory synaptic circuits in cerebellum and thalamus of gamma-aminobutyric acid type A receptor alpha 1 subunit knockout mice, *Journal of Comparative Neurology* 495 (2006) 408–421.
- [14] W. Ogris, R. Lehner, K. Fuchs, B. Furtmuller, H. Hoger, G.E. Homanics, W. Sieghart, Investigation of the abundance and subunit composition of GABA(A) receptor subtypes in the cerebellum of alpha 1-subunit-deficient mice, *Journal of Neurochemistry* 96 (2006) 136–147.
- [15] E.M. Schneider Gasser, V. Duveau, G.A. Prenosil, J.M. Fritschy, Reorganization of GABAergic circuits maintains GABA(A) receptor-mediated transmission onto CA1 interneurons in alpha 1-subunit-null mice, *European Journal of Neuroscience* 25 (2007) 3287–3304.
- [16] D. Kim, I. Song, S. Keum, T. Lee, M.J. Jeong, S.S. Kim, M.W. McEnery, H.S. Shin, Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha1G T-type Ca²⁺ channels, *Neuron* 31 (2001) 35–45.
- [17] P.I. Ortinski, C.Y. Lu, K. Takagaki, Z.Y. Fu, S. Vicini, Expression of distinct alpha subunits of GABA(A) receptor regulates inhibitory synaptic strength, *Journal of Neurophysiology* 92 (2004) 1718–1727.
- [18] H. Stolze, G. Petersen, J. Raethjen, R. Wenzelburger, G. Deuschl, The gait disorder of advanced essential tremor, *Brain* 124 (2001) 2278–2286.
- [19] M. Kronenburger, J. Konczak, W. Ziegler, P. Buderath, B. Frank, V.A. Coenen, K. Kiening, P. Reinacher, J. Noth, D. Timmann, Balance and Motor Speech Impairment in Essential Tremor, *Cerebellum* 8 (2009) 389–398.
- [20] E.D. Louis, J.P.G. Vonsattel, L.S. Honig, A. Lawton, C. Moskowitz, B. Ford, S. Frucht, Essential tremor associated with pathologic changes in the cerebellum, *Archives of Neurology* 63 (2006) 1189–1193.
- [21] E.D. Louis, P.L. Faust, J.P.G. Vonsattel, L.S. Honig, A. Rajput, C.A. Robinson, R. Pahwa, K.E. Lyons, G.W. Ross, S. Borden, C.B. Moskowitz, A. Lawton, N. Hernandez, Neuropathological changes in essential tremor: 33 cases compared with 21 controls, *Brain* 130 (2007) 3297–3307.
- [22] J.E. Axelrad, E.D. Louis, L.S. Honig, I. Flores, G.W. Ross, R. Pahwa, K.E. Lyons, P.L. Faust, J.P.G. Vonsattel, Reduced Purkinje cell number in essential tremor, *Archives of Neurology* 65 (2008) 101–107.
- [23] E. Ohearn, M.E. Molliver, Degeneration of purkinje-cells in parasagittal zones of the cerebellar vermis after treatment with ibogaine or harmaline, *Neuroscience* 55 (1993) 303–310.
- [24] I. Dusart, J.L. Guenet, C. Sotelo, Purkinje cell death: Differences between developmental cell death and neurodegenerative death in mutant mice, *Cerebellum* 5 (2006) 163–173.
- [25] H. Deng, W.D. Le, J. Jankovic, Genetics of essential tremor, *Brain* 130 (2007) 1456–1464.
- [26] J. Jankovic, Essential tremor: A heterogeneous disorder, *Movement Disorders* 17 (2002) 638–644.
- [27] R. Llinas, Y. Yarom, Electrophysiology of mammalian inferior olivary neurons invitro - different types of voltage-dependent ionic conductances, *Journal of Physiology-London* 315 (1981) 549.
- [28] R. Llinas, Y. Yarom, Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurons invitro, *Journal of Physiology-London* 315 (1981) 569–584.
- [29] A. Devor, J.M. Fritschy, Y. Yarom, Spatial distribution and subunit composition of GABA(A) receptors in the inferior olivary nucleus, *Journal of Neurophysiology* 85 (2001) 1686–1696.
- [30] E.M. Talley, L.L. Cribbs, J.H. Lee, A. Daud, E. Perez-Reyes, D.A. Bayliss, Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels, *Journal of Neuroscience* 19 (1999) 1895–1911.
- [31] A.M.R. Yunker, A.H. Sharp, S. Sundarraj, V. Ranganathan, T.D. Copeland, M.W. McEnery, Immunological characterization of T-type voltage-dependent calcium channel Ca(V)3.1 (alpha1G) Ca(V)3.3 (alpha1I) isoforms reveal differences in their localization, expression, and neural development, *Neuroscience* 117 (2003) 321–335.
- [32] S. Vicini, C. Ferguson, K. Prybylowski, J. Kralic, A.L. Morrow, G.E. Homanics, GABA(A) receptor alpha 1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons, *Journal of Neuroscience* 21 (2001) 3009–3016.
- [33] G. Deuschl, C.H. Lucking, E. Schenck, Essential tremor - electrophysiological and pharmacological evidence for a subdivision, *Journal of Neurology Neurosurgery and Psychiatry* 50 (1987) 1435–1441.
- [34] A. Handforth, G.E. Homanics, D.F. Covey, K. Krishnan, J.Y. Lee, K. Sakimura, F.C. Martin, A. Quesada, T-type calcium channel antagonists suppress tremor in two mouse models of essential tremor, *Neuropharmacology* 59 (2010) 380–387.