JCI The Journal of Clinical Investigation

Genetic essential tremor in γ-aminobutyric acid$_A$ receptor α1 subunit knockout mice

Jason E. Kralic, Hugh E. Criswell, Jessica L. Osterman, Todd K. O'Buckley, Mary E. Wilkie, Douglas B. Matthews, Kristin Hamre, George R. Breese, Gregg E. Homanics, A. Leslie Morrow

J Clin Invest. 2005;115(3):774-779. https://doi.org/10.1172/JCI23625.

Article **Neuroscience**

Essential tremor is the most common movement disorder and has an unknown etiology. Here we report that γ-aminobutyric acid$_A$ (GABA$_A$) receptor α*1*$^{-/-}$ mice exhibit postural and kinetic tremor and motor incoordination that is characteristic of essential tremor disease. We tested mice with essential-like tremor using current drug therapies that alleviate symptoms in essential tremor patients (primidone, propranolol, and gabapentin) and several candidates hypothesized to reduce tremor, including ethanol; the noncompetitive *N*-methyl-D-aspartate receptor antagonist MK-801; the adenosine A1 receptor agonist 2-chloro-N6-cyclopentyladenosine (CCPA); the GABA$_A$ receptor modulators diazepam, allopregnanolone, and Ro15-4513; and the L-type Ca^{2+} channel antagonist nitrendipine. Primidone, propranolol, and gabapentin reduced the amplitude (power) of the pathologic tremor. Nonsedative doses of ethanol eliminated tremor in mice. Diazepam, allopregnanolone, Ro15-4513, and nitrendipine had no effect or enhanced tremor, whereas MK-801 and CCPA reduced tremor. To understand the etiology of tremor in these mice, we studied the electrophysiological properties of cerebellar Purkinje cells. Cerebellar Purkinje cells in GABA$_A$ receptor α*1*$^{-/-}$ mice exhibited a profound loss of all responses to synaptic or exogenous GABA, but no differences in abundance, gross morphology, or spontaneous synaptic activity were observed. This genetic animal model elucidates a mechanism of GABAergic dysfunction in the major motor pathway and potential targets for pharmacotherapy of essential tremor.

Find the latest version:

https://jci.me/23625/pdf

 Related Commentary, page 584

Genetic essential tremor in γ-aminobutyric acid$_A$ receptor α1 subunit knockout mice

Jason E. Kralic,[1,2] Hugh E. Criswell,[2,3] Jessica L. Osterman,[2] Todd K. O'Buckley,[2] Mary E. Wilkie,[2] Douglas B. Matthews,[4] Kristin Hamre,[5] George R. Breese,[1,2,3] Gregg E. Homanics,[6] and A. Leslie Morrow[1,2]

[1]Departments of Pharmacology and Psychiatry, [2]Bowles Center for Alcohol Studies, and [3]Department of Anesthesiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA. [4]Department of Psychology, University of Memphis, Memphis, Tennessee, USA. [5]Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, Tennessee, USA. [6]Departments of Anesthesiology and Pharmacology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

Essential tremor is the most common movement disorder and has an unknown etiology. Here we report that γ-aminobutyric acid$_A$ (GABA$_A$) receptor α*1*$^{-/-}$ mice exhibit postural and kinetic tremor and motor incoordination that is characteristic of essential tremor disease. We tested mice with essential-like tremor using current drug therapies that alleviate symptoms in essential tremor patients (primidone, propranolol, and gabapentin) and several candidates hypothesized to reduce tremor, including ethanol; the noncompetitive *N*-methyl-D-aspartate receptor antagonist MK-801; the adenosine A1 receptor agonist 2-chloro-N6-cyclopentyladenosine (CCPA); the GABA$_A$ receptor modulators diazepam, allopregnanolone, and Ro15-4513; and the L-type Ca^{2+} channel antagonist nitrendipine. Primidone, propranolol, and gabapentin reduced the amplitude (power) of the pathologic tremor. Nonsedative doses of ethanol eliminated tremor in mice. Diazepam, allopregnanolone, Ro15-4513, and nitrendipine had no effect or enhanced tremor, whereas MK-801 and CCPA reduced tremor. To understand the etiology of tremor in these mice, we studied the electrophysiological properties of cerebellar Purkinje cells. Cerebellar Purkinje cells in GABA$_A$ receptor α*1*$^{-/-}$ mice exhibited a profound loss of all responses to synaptic or exogenous GABA, but no differences in abundance, gross morphology, or spontaneous synaptic activity were observed. This genetic animal model elucidates a mechanism of GABAergic dysfunction in the major motor pathway and potential targets for pharmacotherapy of essential tremor.

Introduction

As the most common movement disorder, with higher prevalence than Parkinson disease, essential tremor can be both socially and physically debilitating, rendering patients unable to write, clothe themselves, or feed themselves (1, 2). In contrast to resting tremor found in Parkinson disease, essential tremor is characterized by postural and kinetic components (3). Postural tremor is an involuntary oscillation that occurs when a posture is maintained against gravity. Essential tremor worsens during movement, adding a kinetic component to the disorder that results in disturbances in tandem gait and ataxia in approximately 50% of patients (4). First-line treatments for essential tremor include the anticonvulsant primidone and the β-adrenergic blocker propranolol, although their mechanisms of action are unknown (5). Like primidone, gabapentin is an anticonvulsant serendipitously found to be effective in the treatment of essential tremor (6, 7). The oldest treatment for essential tremor may be ethanol, which temporarily ameliorates tremor; however, there are serious drawbacks to chronic use of ethanol for tremor management (3).

The etiology of essential tremor has not been elucidated (2). A major obstacle in the search for a mechanism is the lack of an adequate animal model for essential tremor. Current tremor models utilize drug- or lesion-induced tremors or arise from mutant strains. The only model sharing some features of essential tremor uses the γ-aminobutyric acid$_A$ (GABA$_A$) receptor inverse agonist harmaline to induce a temporary tremor in animals (8) through its action at the inferior olive. However, animals develop rapid tolerance to harmaline, enhanced physiological tremor, and locomotor deficits due to Purkinje cell degeneration and do not respond to propranolol (9–11). Although the harmaline model of tremor has made a valuable contribution to the understanding of human essential tremor, inconsistencies with essential tremor demonstrate the need for additional models.

Several lines of evidence suggest that the GABAergic system is involved in the etiology of essential tremor because this major inhibitory system has a central role in motor control pathways. GABA$_A$ receptors are the primary inhibitory receptors in brain that regulate motor function (12–14). Tremor induction by harmaline has been attributed to inhibition of GABA$_A$ receptors resulting in enhanced electrical coupling of cerebellar afferents in the inferior olive (15). Patients with essential tremor have reduced cerebrospinal fluid concentrations of GABA (16), and thalamic microinjection of the GABA agonist muscimol results in tremor arrest in patients (17). In addition, ethanol is particularly effective for treating essential tremor and has GABAergic effects that are believed to be related to its pharmacologic action (18–20).

Deletion of GABA$_A$ receptor α1 subunits results in the loss of 50% of all GABA$_A$ receptors in brain (21), including the motor pathways in brainstem, cerebellum, thalamus, and basal ganglia. Mice carrying this deletion exhibit an essential-like tremor and motor incoordination that mimics human essential tremor. To test the relevance of this model and to explore potential mechanisms underlying essential tremor, we examined the properties of the tremor and

Nonstandard abbreviations used: aCSF, artificial cerebral spinal fluid; CCPA, 2-chloro-N6-cyclopentyladenosine; GABA, γ-aminobutyric acid; Hz, hertz; mIPSC, miniature inhibitory postsynaptic current; NMDA, *N*-methyl-D-aspartate.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **115**:774–779 (2005). doi:10.1172/JCI200523625.

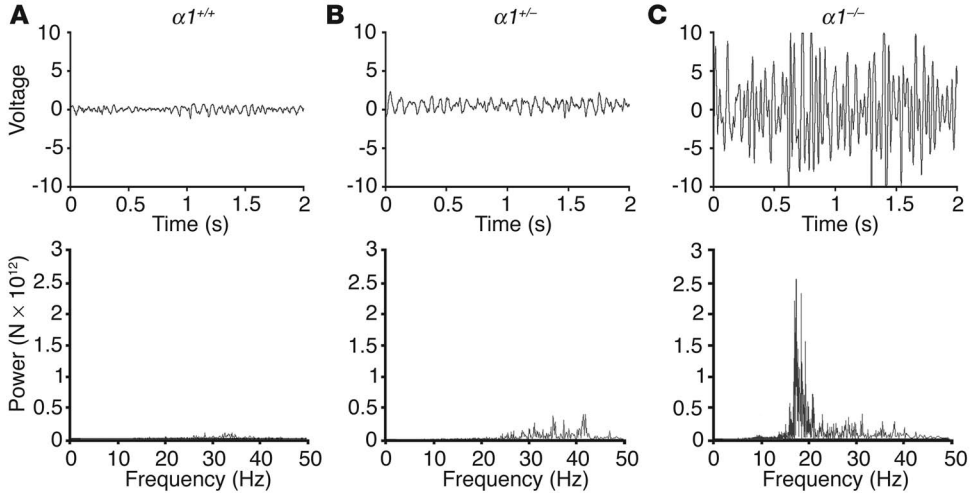

Figure 1
$GABA_A$ receptor $\alpha 1^{-/-}$ mice exhibit essential-like tremor. Normal physiologic tremor (wide range of low-power frequencies, 25–40 Hz) was observed in $\alpha 1^{+/+}$, $\alpha 1^{+/-}$, and $\alpha 1^{-/-}$ mice. Knockout mice also exhibited pathologic tremor (small range of high-power frequencies, 17–21 Hz) characteristic of essential tremor. Representative voltage tracings and Fourier transformation of tremor-induced displacement is shown for $\alpha 1^{+/+}$ (**A**), $\alpha 1^{+/-}$ (**B**), and $\alpha 1^{-/-}$ (**C**) mice. (**A**) $\alpha 1^{+/+}$ mice exhibit a tremor with a mean maximal power of $2.6 \times 10^{11} \pm 3.2 \times 10^{11}$ N and a mean frequency of 32.1 ± 0.6 Hz (n = 16); (**B**) $\alpha 1^{+/-}$ mice, mean maximal power of $6.0 \times 10^{11} \pm 1.1 \times 10^{11}$ N and mean frequency of 35.0 ± 1.9 Hz (n = 5); (**C**) $\alpha 1^{-/-}$ mice, mean maximal power of $24.3 \times 10^{11} \pm 4.2 \times 10^{11}$ N and mean frequency of 19.3 ± 0.9 Hz (n = 13). Tremor is plotted as the voltage generated upon displacement of the transducer as a function of time (seconds). The power of individual frequencies that contribute to the overall tremor was determined by Fourier transformation of the voltage trace over time.

responses to ethanol and drugs currently used to treat essential tremor. Purkinje cell morphology and function were examined in light of their involvement in the etiology of essential tremor. The results indicate that $GABA_A$ receptor $\alpha 1^{-/-}$ mice represent a useful genetic animal model of essential tremor that predicts new therapeutic targets and a potential etiology of the disease.

Results

Deletion of $GABA_A$ receptor α1 subunits produces essential-like tremor. $GABA_A$ receptor $\alpha 1^{-/-}$ mice develop normally but exhibit a tremor as they move freely about their cages. The tremor was observed in the first litters tested at the University of North Carolina at Chapel Hill (21) and has persisted through 9 generations of heterozygote breeding over more than 3 years. The tremor is most evident in the limbs, becomes significantly more pronounced upon tail suspension or movement, and is absent when the mouse is relaxed. The presence of postural and kinetic tremor is characteristic of essential tremor disorder. To determine the tremor frequency and amplitude (power) in $GABA_A$ receptor $\alpha 1^{-/-}$ mice, the animals were suspended by the tail from a force transducer and the displacement of the whole animal was measured over time. Physiologic tremor was observed in wild-type ($\alpha 1^{+/+}$) and heterozygous ($\alpha 1^{+/-}$) mice with mean frequencies between 32.2 and 35.0 hertz (Hz) and maximal power ranging between 2.6×10^{11} and 6.00×10^{11} N (Figure 1, A and B). Homozygous ($\alpha 1^{-/-}$) mice exhibited a pronounced pathologic tremor at a lower mean frequency of 19.3 ± 0.9 Hz and a greater mean maximal power of $24.3 \times 10^{11} \pm 4.2 \times 10^{11}$ N and a physiologic tremor similar to that of $\alpha 1^{+/+}$ and $\alpha 1^{+/-}$ mice (Figure 1C). In addition, motor coordination was determined by performance on the accelerating rotorod. Compared with $\alpha 1^{+/+}$ mice, $\alpha 1^{-/-}$ mice demonstrated significant impairment in their ability to remain on the rotating rod (Table 1). Deficits in motor coordination of the forearms and tandem gait are commonly observed in patients with essential tremor (4, 22, 23).

Essential tremor medications reduce tremor amplitude in $\alpha 1^{-/-}$ mice. Patients with essential tremor respond positively to a small number of drugs, including primidone, propranolol, and gabapentin (3). The efficacy of these drugs was investigated to determine whether $\alpha 1^{-/-}$ mice respond like patients with essential tremor. Primidone, gabapentin, and propranolol were effective in $\alpha 1^{-/-}$ mice, significantly reducing tremor by 45–70% (Table 2). The effect of propranolol is similar to that in essential tremor patients, where 50–60% reductions in tremor amplitude are observed (24).

Ethanol ameliorates tremor via non-GABAergic mechanisms. Although rarely prescribed to patients with essential tremor, alcohol (ethanol) markedly reduces tremor amplitude through action on a central component (20). Ethanol completely blocked the pathologic tremor in $\alpha 1^{-/-}$ mice in a dose-dependent manner (Figure 2A). Ethanol was remarkably potent with an apparent IC_{50} of 0.35 g/kg ethanol, a nonsedating dose in mice (24). Ethanol had no effect overall on the frequency or power of physiologic tremor in $\alpha 1^{+/+}$ mice, although an activating effect was observed at the lowest dose.

Ethanol acts on several neurotransmitter systems that could contribute to reduction of tremor, including inhibition of *N*-methyl-D-aspartate (NMDA) receptors (25), potentiation of $GABA_A$ receptor–mediated neurotransmission (18), and enhancement of adenosinergic transmission (26). The NMDA receptor antagonist MK-801 significantly reduced the power of the pathologic tremor in a dose-dependent manner, suggesting that ethanol may exert its effects by inhibiting the NMDA subtype of glutamate receptors (Figure 2B). MK-801 exhibited an IC_{50} of 0.024 mg/kg and a maximal effect of 65–80% inhibition. The adenosine agonist 2-chloro-N6-cyclopen-

Table 1
$GABA_A$ receptor $\alpha 1^{-/-}$ mice exhibit motor incoordination on the rotorod

Genotype	Latency to fall (seconds)	Difference (%)
$\alpha 1^{+/+}$	54.9 ± 3.1	
$\alpha 1^{-/-}$	32.0 ± 4.9[A]	41.7

Mice were tested for motor incoordination using 10 trials on an accelerating rotorod test (rod accelerates from 5 rpm to 45 rpm over 2 minutes). Data represent mean time on rod ± SEM for the final 3 trials (n = 10 per genotype). [A]$GABA_A$ receptor $\alpha 1^{-/-}$ mice are significantly impaired (P < 0.001) as measured by time to fall off the rod compared with $\alpha 1^{+/+}$ mice.

Table 2
Pharmacological modulation of tremor amplitude in $GABA_A$ receptor $\alpha 1^{-/-}$ mice

Drug	Dose (mg/kg)	Percent of vehicle response ± SEM	*n*
Primidone	50	56 ± 22[A]	10
Propranolol	10	31 ± 7[A]	12
Gabapentin	40	31 ± 11[B]	8
CCPA	1	39 ± 11[A]	8
Nitrendipine	50	159 ± 63	4
Diazepam	10	3,000 ± 737[A]	6
Allopregnanolone	16	540 ± 170[A]	6
Ro15-4513	10	112 ± 25	11

Pathologic tremor amplitude was determined in $\alpha 1^{-/-}$ mice (see Methods) following treatment with vehicle and then drug. Tremor amplitude following drug treatment is presented as percentage of vehicle response ± SEM. *n* indicates number of subjects tested per drug. The mean tremor amplitude in vehicle-treated mice was $5.5 \times 10^{12} \pm 0.8 \times 10^{12}$. [A]$P < 0.05$ and [B]$P < 0.01$ versus vehicle-treated control (paired Student's *t* test).

tyladenosine (CCPA) reduced tremor severity by approximately 60% in $\alpha 1^{-/-}$ mice (Table 2), although significant motor incoordination was observed. Therefore, the contribution of the peripheral effect of CCPA on reduction of the tremor is currently unknown. Potentiation of GABAergic neurotransmission by the benzodiazepine diazepam and the neuroactive steroid allopregnanolone exacerbated the power of the pathologic tremor in $\alpha 1^{-/-}$ mice, whereas the $GABA_A$ receptor inverse agonist Ro15-4513 had no effect (Table 2). Because primidone, gabapentin, and ethanol block voltage-gated Ca^{2+} channels, the L-type Ca^{2+} channel antagonist nitrendipine was tested at a dose that blocks ethanol withdrawal seizures; however, it did not have a measurable effect on tremor amplitude (Table 2).

Cerebellar Purkinje cells of $\alpha 1^{-/-}$ mice lack responses to endogenous or exogenous GABA. A functional disturbance of the olivocerebellar circuit is postulated to mediate human essential tremor. To understand the etiology of the tremor and motor incoordination in $\alpha 1^{-/-}$ mice, we focused on cerebellar Purkinje cells that integrate excitatory input from the inferior olive and cerebellar granule cells and inhibitory input from cerebellar interneurons to control the primary output of the cerebellar cortex, playing a central role in motor coordination. Because Purkinje cells primarily express the α1 subunit–containing subtype (type 1) of $GABA_A$ receptors (27) and Purkinje cell degeneration produces tremor, we first determined whether deletion of $GABA_A$ receptor α1 subunits caused a loss of Purkinje cells. No difference in the number (46.0 ± 2.7 cells/mm^3 in $\alpha 1^{+/+}$ mice) or morphology of calbindin-stained Purkinje cells was observed between genotypes at 4, 8, or 16 months of age, demonstrating that the tremor is not caused by Purkinje cell degeneration (Figure 3A). Furthermore, the spontaneous firing rate of Purkinje cells in $\alpha 1^{+/+}$ and $\alpha 1^{-/-}$ mice did not differ (Figure 3B).

Profound whole-cell electrophysiological changes were observed in Purkinje cells of $\alpha 1^{-/-}$ mice. Spontaneous GABAergic inhibitory postsynaptic potentials were absent in Purkinje cells recorded from dissociated neurons (Figure 3C). Similar results were found in cerebellar slices (data not shown). In addition, GABA-gated currents in dissociated Purkinje cells were undetectable at concentrations up to 100 μM GABA in $\alpha 1^{-/-}$ mice compared with a concentration-dependent response observed in $\alpha 1^{+/+}$ mice (Figure 3, D and E).

Discussion

The principal finding of this study is that deletion of the $GABA_A$ receptor α1 subunit results in a mouse with a persistent postural and kinetic tremor, characteristic of human essential tremor disease. Tremor was observed in the first generation of $GABA_A$ receptor $\alpha 1^{-/-}$ mice (21) and has persisted through more than 9 generations of mice. Our first report on these mice (28) failed to recognize the phenotype, probably owing to the focus on electrophysiological analysis rather than behavior. The tremor is easily observed in all 3 laboratories that currently breed the mice. Current drug therapy for essential tremor was efficacious in reducing tremor in $\alpha 1^{-/-}$ mice. Furthermore, the tremor in $\alpha 1^{-/-}$ mice responded to ethanol, MK-801, and CCPA, suggesting that glutamatergic and adenosinergic mechanisms are involved in the inhibitory effects of ethanol and are potential targets for novel pharmacotherapy. $GABA_A$ $\alpha 1^{-/-}$ mice exhibit a complete loss of both endogenous and exogenous GABA inhibition in cerebellar Purkinje cells. In addition, these mice exhibit the loss of 50% of all $GABA_A$ receptor binding sites throughout the brain, including brain regions in the major motor pathways from the brainstem to the thalamus and the basal ganglia (21). It is likely that loss of GABA inhibition in these motor pathways underlies tremor in $\alpha 1^{-/-}$ mice.

There are many similarities between tremor in $GABA_A$ receptor $\alpha 1^{-/-}$ mice and human essential tremor disease (Table 3). Deletion of the $GABA_A$ receptor α1 subunit produces a pathologic tremor with postural and kinetic components similar to essential tremor disorder. Furthermore, the tremor is genetic and persistent when compared with existing models that produce a chemical-induced tremor that is short-lived. Human physiologic and essential tremor occur at frequency ranges of 8–12 Hz and 4–8 Hz, respectively (29). The higher frequency of both physiologic and pathologic tremor in rodents is probably due to the smaller size of the animal. The appearance of a pathologic tremor at 19 Hz, relative to the physiologic tremor frequency of 25–40 Hz in $\alpha 1^{-/-}$ mice, is indicative of essential tremor rather than enhanced physiologic tremor.

The efficacy of drugs used in the treatment of human essential tremor was observed in $\alpha 1^{-/-}$ mice, lending further support to the model. The ability of the first-line treatments, primidone and propranolol, to alleviate essential tremor in $\alpha 1^{-/-}$ mice supports

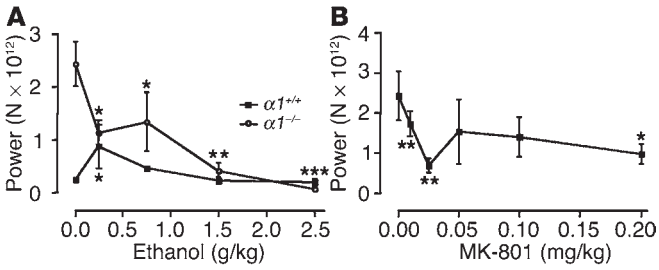

Figure 2
Inhibition of essential-like tremor in $GABA_A$ receptor $\alpha 1^{-/-}$ mice by ethanol and MK-801. (**A**) Ethanol (0.25–2.5 g/kg) inhibited the essential-like tremor (approximately 19 Hz) in $\alpha 1^{-/-}$ mice in a dose-dependent manner with an ED_{50} of 0.35 mg/kg reaching a maximal inhibition of 100% (n = 5–11 per genotype). Ethanol (0.25 g/kg) increased physiological tremor (approximately 32 Hz) in $\alpha 1^{+/+}$ mice but lacked any effect at higher doses. (**B**) MK-801 (0.01–0.2 mg/kg) reduced the approximately 19 Hz tremor power in $\alpha 1^{-/-}$ mice with an ED_{50} of 0.024 mg/kg reaching a maximal inhibition of 65–80% (n = 5–6). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

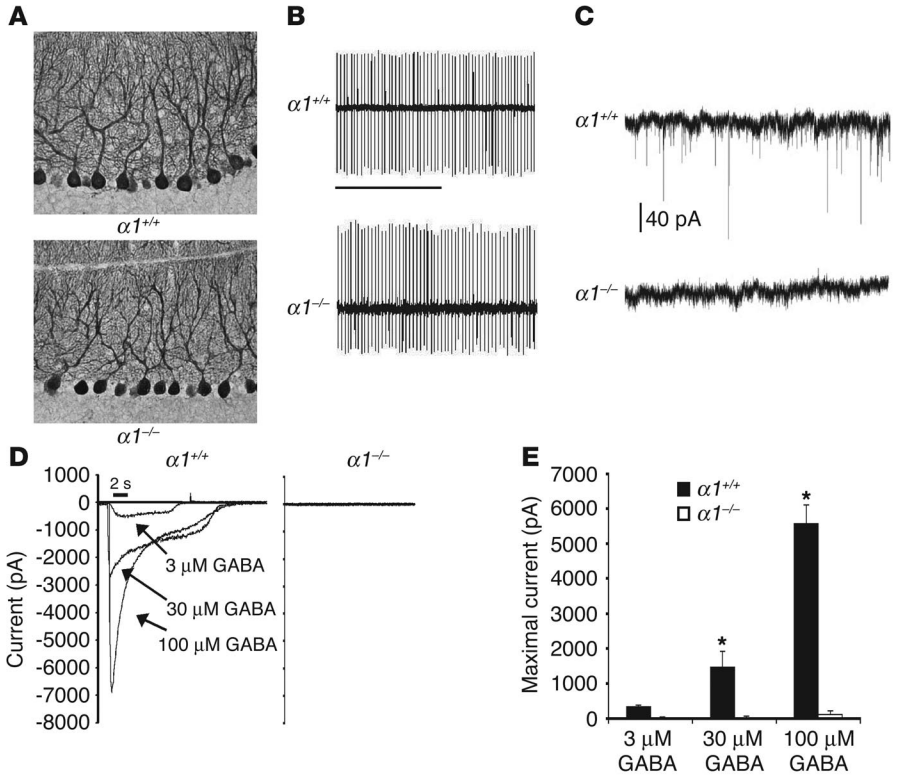

Figure 3
$GABA_A$ receptor $\alpha 1^{-/-}$ mice exhibit normal Purkinje cell number and morphology but complete loss of both spontaneous mIPSCs and exogenous GABA inhibition of whole-cell voltage-clamp electrophysiological responses. (**A**) Calbindin staining of Purkinje cell number and morphology in cerebellum of 8-month-old $\alpha 1^{+/+}$ and $\alpha 1^{-/-}$ mice. Magnification, ×400. (**B**) Similar spontaneously active Purkinje cells were found in both $\alpha 1^{+/+}$ (22 of 29 penetrations) and $\alpha 1^{-/-}$ (28 of 36 penetrations) mice. Mean rate for $\alpha 1^{-/-}$ mice (26.7 ± 3.8) did not differ from that of $\alpha 1^{+/+}$ mice (32.4 ± 5.5). (**C**) Spontaneous mIPSCs recorded over a 30-second period from a Purkinje cell mechanically dissociated from an $\alpha 1^{+/+}$ and $\alpha 1^{-/-}$ mouse. Spontaneous postsynaptic picrotoxin-sensitive currents with amplitude greater than 50 pA and fall-times greater than 4 ms were recorded from 10 of 16 $\alpha 1^{+/+}$ mice and 0 of 14 $\alpha 1^{-/-}$ mice. (**D**) Whole-cell voltage-clamp recordings were obtained from mechanically dissociated cerebellar Purkinje cells from $\alpha 1^{+/+}$ and $\alpha 1^{-/-}$ mice. GABA was applied to the neurons by a U-tube. GABA (3, 30, and 100 μM) gated a concentration-dependent inward current when applied to Purkinje cells from $\alpha 1^{+/+}$ mice, but no current was gated in $\alpha 1^{-/-}$ Purkinje cells. (**E**) Mean GABA-gated currents from cerebellar Purkinje cells mechanically isolated from $\alpha 1^{+/+}$ or $\alpha 1^{-/-}$ mice. There was a statistically reliable concentration-related increase in GABA response in the $\alpha 1^{+/+}$ mice (*$P < 0.001$) but no effect of GABA in the $\alpha 1^{-/-}$ mice ($P > 0.1$) (n = 6–11 neurons per group).

common mechanisms with human essential tremor. Furthermore, successful novel application of drugs such as the anticonvulsant gabapentin in both $\alpha 1^{-/-}$ mice and patients demonstrates the necessity for animal models in which new and existing drugs can be screened for clinical utility (7). $GABA_A$ receptor $\alpha 1^{-/-}$ mice provide a useful animal model with which to investigate the pathophysiology of essential-like tremor and the mechanisms by which current and future therapies exert their effects.

Ethanol is effective in the suppression of essential tremor symptoms in humans and $\alpha 1^{-/-}$ mice. Ethanol was the most effective treatment of tremor in $\alpha 1^{-/-}$ mice at doses that preclude the contribution of its sedative effects on tremor (30). Ethanol is believed to act centrally in the alleviation of tremor and, in human studies, has been shown to involve a central component (20). Furthermore, positron emission tomography studies have shown that ethanol consumption increases cerebral blood flow in the inferior olivary nucleus, a central oscillator, which coincides with alleviation of tremor (31).

The mechanism responsible for ethanol's effects on tremor appears to involve inhibition of excitatory glutamatergic transmission but not activation of inhibitory GABAergic receptors. The efficacy of the glutamatergic antagonist MK-801 but not of GABAergic agonists diazepam and allopregnanolone suggest that the tremor may result from inactivation of inhibitory neurons. Although the lack of tremor inhibition by diazepam in $\alpha 1^{-/-}$ mice is consistent with low responsiveness in patients with essential tremor (20), the augmentation of tremor by diazepam was unexpected. Ethanol has several other targets in the brain that could account for its effects. Ethanol-sensitive adenosine receptors may be involved; however, studies with the adenosine agonist CCPA did not eliminate the possibility for peripheral effects of the drug, because significant motor incoordination was also observed in the mice.

Disruption of Purkinje cell function following deletion of $GABA_A$ receptor α1 subunits likely contributes, in part, to the etiology of the tremor in $\alpha 1^{-/-}$ mice. The loss of GABAergic inhibition on Purkinje cells suggests that α1 subunit expression is critical for normal function of motor circuits and likely contributes to tremor and motor incoordination observed in $\alpha 1^{-/-}$ mice. Tremor induced by alcohol withdrawal is clinically similar to essential tremor and also results in decreased α1 subunit expression in rat cerebellum (32, 33). The lack of change in spontaneous activity of Purkinje cells is consistent with the lack of resting tremor and implies some adaptation to the loss of α1 subunits in these mice. Although compensatory changes in other $GABA_A$ receptor subunits have been observed in some brain regions of $\alpha 1^{-/-}$ mice (21), no GABAergic compensation in cerebellar Purkinje cells was found (data not shown). Because α1 subunits are normally expressed in other cells of this motor pathway, including the deep cerebellar nuclei and thalamus (but not the inferior olive), it is likely that communication among these regions is also disturbed by α1 subunit deletion and subsequent adaptations in receptor subunit expression. Although we have described altered GABA responses in the cerebellum, impairment of GABAergic inhibition is likely throughout the motor pathways that could contribute to the observed tremor. Additional studies will be required to determine if loss of α1 subunits in any single brain area is sufficient to mimic the clinical manifestations of human essential tremor disease. Furthermore, a common target of the major drugs used to treat essential tremor (primidone and propranolol) has not been elucidated. Therefore, treatment of essential tremor may target several pathways in the brain.

Table 3
$GABA_A$ receptor $\alpha 1^{-/-}$ mouse model reflects aspects of human essential tremor

Characteristic	Human essential tremor	$GABA_A$ receptor $\alpha 1^{-/-}$ mice
Tremor type	Persistent, visible, bilateral, postural, kinetic	Persistent, visible, bilateral, postural, kinetic
Tremor location[A]	Upper limbs and, less frequently, lower limbs, affecting ability to clothe and feed oneself	Fore and hind limbs equally effected, causing impairment of balance on rotating rod
Tremor frequency[B]	4–8 Hz compared with 8–12 Hz physiological tremor	15–19 Hz compared with 25–40 Hz physiological tremor
Response to drug treatment	+: primidone, propranolol, ethanol, gabapentin	+: primidone, propranolol, ethanol, gabapentin, MK-801, CCPA; –: diazepam, allopregnanolone, Ro15-4513, nitrendipine
Etiology	Unknown	Disruption of inhibitory GABAergic signaling throughout brain, complete loss of GABA inhibition in cerebellar Purkinje cells
Response to thalamotomy and deep brain stimulation	+	?

[A]Upper limb motor incoordination in humans is comparable to incoordination of mice on rotating rod. [B]Difference in tremor frequency between rodent and human is species-dependent, but both exhibit pathologic tremor of lower frequency than physiologic tremor.

The similarities between human essential tremor and pathologic tremor in $\alpha 1^{-/-}$ mice suggest that expression of α1 subunits in the brains of patients with essential tremor may be abnormal. Further studies are needed to test this possibility. The $GABA_A$ receptor $\alpha 1^{-/-}$ mouse model of essential tremor provides a behavioral paradigm with predictive validity for testing novel potential treatments for this prevalent disorder.

Methods

Mutant mice. Production of $GABA_A$ receptor $\alpha 1^{+/+}$, $\alpha 1^{+/-}$, and $\alpha 1^{-/-}$ mice was previously described (28). Wild-type controls were homozygous for a floxed α1 allele that was functionally equivalent to the wild-type allele. $GABA_A$ receptor $\alpha 1^{-/-}$ mice were homozygous for a cre-mediated global deletion of exon 8 of the α1 gene. All mice were of a mixed C57BL/6J, 129Sv/SvJ, and FVB/N genetic background of F_{8+} generations and genotyped by Southern blot analysis. Mice were group-housed, provided ad libitum access to food and water, and maintained under a 12-hour light/12-hour dark schedule with lights on at 0700. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals by the NIH and were approved by the Institutional Animal Care and Use Committees at the University of North Carolina and the University of Memphis.

Drugs. Vehicles were saline for ethanol (AAEPER Alcohol), MK-801 (Sigma-Aldrich), CCPA (Sigma-Aldrich), primidone (Sigma-Aldrich), propranolol (MP Biochemicals), nitrendipine (Axxora), and gabapentin (Sigma-Aldrich); 20% 2-hydroxypropyl-β-cylcodextrin (Sigma-Aldrich)/saline for allopregnanolone (provided by R. Purdy, The Scripps Research Institute, La Jolla, California, USA); and 1% Tween-80 (MP Biochemicals)/saline for diazepam (Biomol) and Ro-154513 (Sigma-Aldrich).

Tremor measurement. Tremor was quantified by determination of amplitude and frequency using a tremor monitor built in the Bowles Center for Alcohol Studies at the University of North Carolina. Tremor was measured in mice suspended by the tail for 22 seconds so that the animals held a posture against gravity and the tremor was easily observed and measured. The mice exhibited no signs of stress (defecation, vocalization, biting, or jumping) during the measurement as the mice were accustomed to handling by tail suspension in the laboratory. Each mouse was suspended by its tail from a cord attached to the center of a stereo speaker (Archer 3" 8Ωmax2W). Tremor resulted in vibration of the speaker surface and the resulting signal was amplified before passing through an analog-to-digital converter that assigned voltage equivalents to the vibrations transmitted to the speaker. These voltage measurements were analyzed via a Pascal program developed in house. Voltage recordings were made over a 22-second period at a rate of 454 recordings per second. The data obtained from the program was analyzed using MatLab (Mathworks) by Fourier transformation to determine the peak amplitude and frequency of the tremor for each animal.

For all drugs tested (except ethanol), each animal served as its own control in which a baseline measurement of tremor was obtained following administration of vehicle. Dose-response curves for MK-801 were generated by administration of each dose to a group of animals in a χ^2 design. Animals were tested at 3-day intervals to allow clearance of drug. Each animal was used to test 1 drug. For ethanol, every dose was tested in a separate group of mice. Drugs and vehicles were administered intraperitoneally in a 10 ml/kg injection volume 1 hour (45 minutes for CCPA) before measurement of tremor.

Motor coordination. Mice were tested on an accelerating rotorod (Columbus Instruments) with a rod diameter of 3 inches that accelerated from 5 to 45 rpm over 2 minutes. Mice were placed on the rotating rod (5 rpm) and each trial lasted until the mouse fell off the rod. Each mouse was given 10 trials on the rotorod. For each trial, the length of time the mouse stayed on the rotorod was recorded. The mean was calculated from the last 3 trials for each mouse.

Immunohistochemical methods. Mice were sacrificed at 4, 8, and 16 months of age, perfused, and processed as previously described (34), with cerebella serially sectioned (10 μm) in the sagittal plane. Purkinje cells were labeled using an anti-calbindin rabbit polyclonal antibody (Chemicon). Calbindin-positive cells were profile-counted throughout the entire cerebellum. The area of each cerebellum tissue section was determined using the Bioquant system and data expressed as the number of Purkinje cells per mm^2.

Electrophysiological recording. Action potentials from cerebellar Purkinje cells in a slice preparation were recorded extracellularly using an Axon Instruments Axopatch-200A in track mode. Cerebellar slices (250 μm) from 20- to 40-day-old mice were cut in cold artificial cerebrospinal fluid (aCSF) (124 mM NaCl, 3.25 mM KCl, 1.25 mM KH_2PO_4, 1 mM $CaCl_2$, 20 mM $NaHCO_3$, 2 mM $MgSO_4$, and 10 mM glucose). Slices were maintained with continuous 95% O_2 and 5% CO_2 for at least 1 hour before use. Slices were transferred to a recording chamber under an upright microscope where they were continuously bathed in aCSF. Purkinje cells were identified using infrared illumination and differential interference contrast optics. Recording pipettes were fabricated from N51A capillary glass (Drummond Scientific)

and filled with HEPES-buffered aCSF (described below). The electrode was apposed to a Purkinje cell, and if no spontaneous activity was observed, it was lowered through the Purkinje layer for 100 μm or until an active neuron was found. Probability of finding a neuron during the 100-μm penetration and firing rate of each neuron were recorded.

Spontaneous GABA-gated miniature inhibitory postsynaptic currents (mIPSCs) and currents generated by exogenously applied GABA were measured in the slice preparation and in mechanically dissociated cerebellar Purkinje cells using standard whole-cell voltage-clamp recording. For dissociation, slices (as described above) were transferred into standard HEPES-buffered recording medium (145 mM NaCl, 5 mM KCl, 10 mM HEPES, 2 mM $CaCl_2$, and 10 mM glucose, pH 7.4; 340 milliosmoles per liter) with room air, and a vibrating probe (60 Hz; 0.5–1 mm amplitude) was touched to the surface of the slice for 30–60 seconds. The slice was removed and the dissociated neurons allowed to settle onto the floor of the recording chamber. This procedure dissociates Purkinje cells with intact presynaptic terminals, allowing recording of mIPSCs.

Voltage-clamp (–60 mV) recording in the whole-cell configuration was accomplished using an Axopatch-1D amplifier. Recording pipettes were fabricated from N51A capillary glass (Drummond Scientific). The internal solution used for measuring mIPSCs and ion currents induced by GABA included 150 mM KCl, 3.1 mM $MgCl_2$, 15 mM HEPES, 2 mM K-ATP, 5 mM EGTA, 15 mM phosphocreatine, 500 nM tetrodotoxin, and 50 U/ml creatine phosphokinase. This solution was adjusted to pH 7.4 with KOH and osmolality of 310 milliosmoles per liter with sucrose. A U-tube was used to apply varying concentrations of GABA to neurons. Counts of action potentials and mIPSCs were detected off-line using Mini Analysis software (Synaptosoft).

Statistical analysis. Data were analyzed using Prism (GraphPad). All data were presented as mean (or mean percent control) ± SEM and subjected to Student's *t* test or ANOVA with an appropriate post hoc test. Dose responses were analyzed by nonlinear regression. The IC_{50} was defined as the drug dose that inhibited 50% of the pathologic tremor and maximal effect as the best-fit bottom value estimated by nonlinear regression.

Acknowledgments

This research was supported by NIH grants AA11605 (to A.L. Morrow and G.R. Breese), AA09013 (to A.L. Morrow), MH61971 (to D.B. Matthews and K. Hamre), AA10422, and GM52035 (to G.E. Homanics). We thank Jean-Marc Fritschy and Kirk Wilhelmson for discussion of the manuscript.

Received for publication October 14, 2004, and accepted in revised form December 21, 2004.

Address correspondence to: A. Leslie Morrow, University of North Carolina School of Medicine, CB No. 7178, 3027 Thurston-Bowles Building, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7178, USA. Phone: (919) 966-7682; Fax: (919) 966-9099; E-mail: morrow@med.unc.edu.

Jason E. Kralic's present address is: Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland.

Jason E. Kralic, Hugh E. Criswell, Jessica L. Osterman, and A. Leslie Morrow contributed equally to this work.

1. Louis, E.D., Ottman, R., and Hauser, W.A. 1998. How common is the most common adult movement disorder? Estimates of the prevalence of essential tremor throughout the world. *Mov. Disord.* **13**:5–10.
2. Chen, J.J., and Swope, D.M. 2003. Essential tremor: diagnosis and treatment *Pharmacotherapy*. **23**:1105–1122.
3. Pahwa, R., and Lyons, K.E. 2003. Essential tremor: Differential diagnosis and current therapy. *Am. J. Med.* **115**:134–142.
4. Hubble, J.P., Busenbark, K.L., Pahwa, R., Lyons, K., and Koller, W.C. 1997. Clinical expression of essential tremor: effects of gender and age. *Mov. Disord.* **12**:969–972.
5. Gorman, W.P., Cooper, R., Pocock, P., and Campbell, M.J. 1986. A comparison of primidone, propranolol, and placebo in essential tremor, using quantitative analysis. *J. Neurol. Neurosurg. Psychiatr.* **49**:64–68.
6. O'Brien, M.D., Upton, A.R., and Toseland, P.A. 1981. Benign familial tremor treated with primidone. *Br. Med. J. (Clin. Res. Ed.).* **282**:178–180.
7. Gironell, A., et al. 1999. A randomized placebo-controlled comparative trial of gabapentin and propranolol in essential tremor. *Arch. Neurol.* **56**:475–480.
8. Wilms, H., Sievers, J., and Deuschl, G. 1999. Animal models of tremor. *Mov. Disord.* **14**:557–571.
9. Iwata, S., Nomoto, M., and Fukuda, T. 1993. Effects of β-adrenergic blockers on drug-induced tremors. *Pharmacol. Biochem. Behav.* **44**:611–613.
10. O'Hearn, E., and Molliver, M.E. 1993. Degeneration of Purkinje cells in parasagittal zones of the cerebellar vermis after treatment with ibogaine or harmaline. *Neuroscience.* **55**:303–310.
11. Wang, G., and Fowler, S.C. 2001. Concurrent quantification of tremor and depression of locomotor activity induced in rats by harmaline and physostigmine. *Psychopharmacology.* **158**:273–280.
12. Barnard, E.A., et al. 1998. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric $acid_A$ receptors: classification on the basis of subunit structure and receptor function. *Pharmacol. Rev.* **50**:291–313.
13. Rudolph, U., et al. 1999. Benzodiazepine actions mediated by specific gamma-aminobutyric $acid_A$ receptor subtypes. *Nature.* **401**:796–800.
14. Sieghart, W. 2000. Unraveling the function of $GABA_A$ receptor subtypes. *Trends Pharmacol. Sci.* **21**:411–413.
15. Stratton, S.E., and Lorden, J.F. 1991. Effect of harmaline on cells of the inferior olive in the absence of tremor: differential response of genetically dystonic and harmaline-tolerant rats. *Neuroscience.* **41**:543–549.
16. Mally, J., Baranyi, M., and Vizi, E.S. 1996. Change in the concentrations of amino acids in CSF and serum of patients with essential tremor. *J. Neural. Transm. Gen. Sect.* **103**:555–560.
17. Pahapill, P.A., et al. 1999. Tremor arrest with thalamic microinjections of muscimol in patients with essential tremor. *Ann. Neurol.* **46**:249–252.
18. Suzdak, P.D., Schwartz, R.D., Skolnick, P., and Paul, S.M. 1986. Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. *Proc. Natl. Acad. Sci. U. S. A.* **83**:4071–4075.
19. Mihic, S.J., et al. 1997. Sites of alcohol and volatile anaesthetic action on $GABA_A$ and glycine receptors. *Nature.* **389**:385–389.
20. Zeuner, K.E., et al. 2003. Effect of ethanol on the central oscillator in essential tremor. *Mov. Disord.* **18**:1280–1285.
21. Kralic, J.E., Korpi, E.R., O'Buckley, T.K., Homanics, G.E., and Morrow, A.L. 2002. Molecular and pharmacological characterization of $GABA_A$ receptor α1 subunit knockout mice. *J. Pharmacol. Exp. Ther.* **302**:1037–1045.
22. Lou, J.S., and Jankovic, J. 1991. Essential tremor: clinical correlates in 350 patients. *Neurology.* **41**:234–238.
23. Elble, R.J. 2002. Essential tremor is a monosymptomatic disorder. *Mov. Disord.* **17**:633–637.
24. Koller, W.C., and Vetere-Overfield, B. 1989. Acute and chronic effects of propranolol and primidone in essential tremor. *Neurology.* **39**:1587–1588.
25. Lovinger, D.M., White, G., and Weight, F.F. 1989. Ethanol inhibits NMDA-activated ion currents in hippocampal neurons. *Science.* **243**:1721–1724.
26. Gordon, A.S., Collier, K., and Diamond, I. 1986. Ethanol regulation of adenosine receptor-stimulated cAMP levels in a clonal neural cell line: an in vitro model of cellular tolerance to ethanol. *Proc. Natl. Acad. Sci. U. S. A.* **83**:2105–2108.
27. Laurie, D.J., Seeburg, P.H., and Wisden, W. 1992. The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *J. Neurosci.* **12**:1063–1076.
28. Vicini, S., et al. 2001. $GABA_A$ receptor α1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. *J. Neurosci.* **21**:3009–3016.
29. Elble, R.J. 1986. Physiologic and essential tremor. *Neurology.* **36**:225–231.
30. Kralic, J.E., et al. 2003. Deletion of $GABA_A$ receptor alpha1 subunit-containing receptors alters responses to ethanol and other anesthetics. *J. Pharmacol. Exp. Ther.* **305**:600–607.
31. Boecker, H., et al. 1996. The effect of ethanol on alcohol-responsive essential tremor: a positron emission tomography study. *Ann. Neurol.* **39**:650–658.
32. Morrow, A.L., Herbert, J.S., and Montpied, P. 1992. Differential effects of chronic ethanol administration on $GABA_A$ receptor α1 and α6 subunit mRNA levels in rat cerebellum. *Mol. Cell. Neurosci.* **3**:251–258.
33. Mhatre, M.C., Pena, G., Sieghart, W., and Ticku, M.K. 1993. Antibodies specific for $GABA_A$ receptor α subunits reveal that chronic ethanol treatment down-regulates α-subunit expression in rat brain regions. *J. Neurochem.* **61**:1620–1625.
34. Crews, F.T., Nixon, K., and Wilkie, M.E. 2004. Exercise reverses ethanol inhibition of neural stem cell proliferation. *Alcohol.* **33**:63–71.