Spontaneous epileptic seizures in transgenic rats harboring a human ADNFLE missense mutation in the β2-subunit of the nicotinic acetylcholine receptor

（ヒト常染色体優性夜間前頭葉てんかん由来ニコチン性アセチルコリン受容体β2 サブユニット変異ラットの自発性発作）

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Abstract

We generated a transgenic rat strain with a missense mutation in V286L (V286L-TG), in the gene encoding the neuronal nicotinic acetylcholine receptor β2 subunit *(CHRNB2)* found in patients with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). To confirm that V286L-TG rats exhibit seizures similar to those observed in humans, gene expression patterns and behavioral phenotypes were analyzed. In situ hybridization using a V286L *Chrnb2*-selective probe indicated that the transgene was expressed at higher levels in the cortex, hippocampus, and cerebellum of V286L-TG than wild-type littermates (non-TG). Spontaneous epileptic seizures with ictal discharges in electroencephalograms were detected in 45% of V286L-TG rats and the frequency of seizures was 0.73 times a week. This seizure type is similar to "paroxysmal arousals" that are observed in human ADNFLE. V286L-TG rats displayed nicotine-induced abnormal motor activity including seizures in comparison to non-TGs. Response time following nicotine administration occurred faster in V286L-TG than in non-TG rats.

V286L-TG rats demonstrated spontaneous epileptic seizures, which are similar to human ADNFLE, and also showed a higher sensitivity to nicotine administration. Thus, the V286L-TG rat model could be a valuable tool for developing novel

mechanism-driven treatment strategies for epilepsy and provide a better understanding of ADNFLE.

Highlights

- Rats with a missense mutation in the nicotinic receptor β2 subunit were generated.
- V286L-TG showed spontaneous epileptic seizures, similar to human ADNFLE.
- V286L-TG rats demonstrated hypersensitivity to nicotine administration.
- V286L-TG may cause an imbalance of excitation vs. inhibition leading to seizures.

Keywords

autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), nicotinic acetylcholine receptor (nAChR) mutation, β2-subunit, epilepsy, seizures, transgenic rat

Abbreviations

ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; EEG, electroencephalogram; nAChR, nicotinic acetylcholine receptor; non-TG; wild-type littermates of V286L-TG; PDGF, platelet derived growth factor; V286L-TG, rats harboring a valine to leucine substitution at position 286 of the nicotinic acetylcholine receptor β2 subunit

1. Introduction

In the last decade, abnormalities of genes encoding ion channels expressed in the brain have been identified as the underlying cause of some 20 idiopathic epilepsy syndromes. Dysfunction of ion channels using *in vitro* mutations has been demonstrated in certain types of idiopathic epilepsy syndromes. However, the mechanisms linking channel abnormalities and epilepsy phenotypes are poorly understood. Animal models bearing mutations in genes encoding ion channels that have been identified in human epilepsy and exhibiting similar phenotypic characteristics could be used to develop new treatment measures for seizures caused by channel abnormalities.

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein et al., 1995; Steinlein et al., 1997; Hirose et al., 1999; De Fusco et al., 2000; Steinlein et al., 2000; Phillips et al., 2001; Bertrand et al., 2005) is an idiopathic epilepsy syndrome with mutations of genes encoding ion channels. Mutations have been identified in the genes encoding α4-, β2-, and α2-subunits of nicotinic acetylcholine receptors (nAChRs), *CHRNA4*, *CHRNB2*, and *CHRNA2*, respectively. The nAChR is a ligand-gated ion channel known to function as a pentamer consisting of mainly α4- and β2-subunits in the brain. The heteromeric nAChR are thought to be present in two main stoichiometries, the high-affinity $(\alpha4)_2(\beta2)_3$ and the low-affinity $(\alpha4)_3(\beta2)_2$ forms (Nelson et al., 2003).

In neocortex, high-affinity form of nACh receptors mainly functioned (Aracri et al., 2013). However, ADNFLE mutations caused an increase of low-affinity forms and nicotine exposure to α4S248F and β2V287L mutations lead to return the WT stoichiometry (Son et al., 2009).

In ADNFLE, three missense, S280F (Steinlein et al., 1995), S284L (Hirose et al., 1999), T293I (Leniger et al., 2003), and one insertional (insL) (Steinlein et al., 1997) mutation of *CHRNA4* have been identified. The numbering of the mutations given here are based on the deduced amino acid sequence of the human α4-subunit, which differs from that used in the original articles based on the *Torpedo* sequence and nomenclature system. Additionally three missense mutations of V287L (De Fusco et al., 2000), V287M (Phillips et al., 2001), and I312M (Bertrand et al., 2005) of *CHRNB2* have been identified in ADNFLE. Moreover, a heterozygous missense I1287N of *CHRNA2* was described in a pedigree where the phenotype of affected individuals was comparable to ADNFLE (Aridon et al., 2006). All of the identified ADNFLE mutations were heterozygous and within the nAChR gating regulator or pore region (Wonnacott, 1997; Combi et al., 2004; Bertrand et al., 2005) and dysfunction of the corresponding channels have been demonstrated *in vitro*.

Recently, we generated a transgenic rat that contains transgenes with a rat *Chrna4*

missense S284L mutation, corresponding to a mutation identified in both ADNFLE and NFLE that demonstrated seizure phenotypes resembling those of human ADNFLE/NFLE (Zhu et al., 2008). We also produced a transgenic rat with a rat *Chrnb2* missense V286L mutation, corresponding to the human mutation of V287L (De Fusco et al., 2000). Here, we report the underlying neurobiological abnormalities in these rats and compare the changes to those that occur with a mutation in *Chrna4*.

2. Materials and methods

2.1. Animals

Animal experiments conformed to the guidelines issued by the Hirosaki University on the ethical use of animals in experimentation, and all efforts were made to minimize the number of animals used and their suffering.

Male rats that were heterozygous for V286L-TG and wild-type littermates (non-TG) were used for all experiments. All animals were housed in ventilated racks (two to three rats per cage) at the Hirosaki University experimental animal vivarium. The colony room was maintained on a 12-h light-dark cycle (lights were on: 8 am to 8 pm) and rats received standard laboratory food and water *ad libitum*.

2.2. Generation of V286L transgenic rats

The rat cDNA of *Chrnb2* was cloned from a rat fetal brain cDNA panel (Clontech, Palo Alto, CA, USA) by PCR. The cDNA was subcloned with a pCRII-TOPO vector (Invitrogen, Carlsbad, CA, USA). Mutation V286L corresponding to human V287L and an exchange of 20 nucleotides (1359–1418), was introduced in the rat cDNA in the same vector using the QuickChange site-direct mutagenesis kit (Stratagene Inc., La Jolla, CA, USA). The nucleotide exchange was c.856 G>C, which replaced a valine at 286 amino acid with a leucine according to the sequence of the rat *Chrnb2* (GenBank accession No. L31622).

For generation of V286L-TG, the rat cDNA was excised from the vector with EcoRI and transferred into pCI-neo vector (Promega, Madison, WI, USA). The cDNA was excised from the pCI-neo vector with XhoI and NotI and transferred to a vector equipped with the PDGF-β promoter constructed based on pCI-neo vector (a kind gift from Dr. Takashima, RIKEN, Wako, Japan). The platelet derived growth factor-β (PDGF-β) promoter upstream of the multiple cloning site is a mammalian expression promoter that drives ectopic expression of transgenes mainly in the cortex and hippocampus, whereas the expression and distribution in transgenic animals are subject to change depending on the genetic location of transgenes (Kuteeva et al., 2004). The 4.0-kb fragment containing the PDGF-β promoter and V286L rat *Chrnb2* cDNA, and a

3′ untranslated sequence, were prepared with SnaBI and NaeI and used as the transgene to generate V286L-TG on a Sprague-Dawley rat background. Microinjection of the transgene and generation of V286L-TG rats was conducted at Japan SLC (Shizuoka, Japan) using standard procedures. Genomic DNA was prepared from tails of the animals by a standard method and direct sequencing using the primer pair: CCAAGGACTCGCCGGC and CGGTCCCAAAGACACAGACA.

2.3. Morphological studies

Eight-week-old (n = 3) and 2-year-old (n = 3) rats were sacrificed by decapitation. The brains were harvested and then immersed in 4% paraformaldehyde with 0.1 M phosphate-buffer (pH 7.4) for 24 h. Paraffin-embedded sagittal three sections (4 μm thick, lateral, 3.0 mm relative to bregma) were stained by the Klüver-Barrera method, and immunostained by the avidin-biotin-peroxidase complex (ABC) method using the Vectastain kit (Vector Laboratories, Burlingame, CA, USA), with anti-ssDNA (A4506; Dako Cytomation, Japan; 1:100) as the primary antibody. The reaction was visualized with 0.02% 3, 3′-diaminobenzidine tetrahydrochloride (DAB) and 0.005% H_2O_2 in 0.05 M Tris-HCl buffer (pH 7.6) for 10 min. Subsequently, the sections were counterstained with hematoxylin.

For immunohistochemistry of α4- and β2-subunits of nAChRs, V286L-TG (n = 3) and non-TG (n = 3) rats at 8 weeks of age were anesthetized with sodium pentobarbital (20 mg/kg, intraperitoneally) and perfused transcardially with 0.1 M phosphate-buffer followed by a mixture of 2% paraformaldehyde and 0.3% glutaraldehyde in 0.1 M phosphate-buffer. The brains were quickly removed, immersed in the same fixative for 3 h at 4°C, and stored overnight in 7.5% sucrose containing 0.1 M phosphate-buffer. Vibratome sections (50 μm thick, 3 sections of each region) were cut from the frontal cortex/striatum (caudal, 1.0 mm relative to bregma), hippocampus/thalamus (caudal, 4.0 mm relative to bregma), and occipital cortex/mesencephalon (caudal, 7.0 mm relative to bregma), and immersed in 1% H_2O_2 for 15 min, then blocked with 5% normal serum for 30 min, incubated with an anti-nAChR α4-subunit antibody (mAb299, Sigma-Aldrich, St. Louis, MO, USA; anti-rat, 1:500) or with anti-nAChR β2-subunit antibody (mAb290, Sigma-Aldrich; anti-rat, 1:500) for 24 h at 4°C, followed by incubation with a biotinylated secondary antibody (Vector Laboratories; 1:200) for 1 h and ABC (Vector Laboratories; 1:200) for 1 h. The reaction was developed with DAB (0.1 mg/mL) containing 0.0015 % H_2O_2.

2.4. *In situ* hybridization

V286L-TG (n = 3) and non-TG (n = 3) rats were killed at 2, 4, 6, 8, and 12 weeks of age

by decapitation, brains removed quickly, frozen on powdered dry ice, and stored at −80°C. Three sagittal brain sections (20 μm thick, lateral, 3.0 mm relative to bregma) were cut with a microtome cryostat at −15°C, thaw-mounted on glass slides coated with 3-aminopropyltriethoxysilane, and kept at −80°C.

The expression of mutant and wild-type mRNA was investigated by using ^{33}P-labeled oligonucleotide probes. The data presented here were obtained with antisense oligonucleotide probes for a wild-type gene (5′-CCA GCC AAG CCC TGC ACT GAT GCA GGG TT and 5′-GGG CCG GCT GCA GTG GGC TCA GCT CGG AAA GCC) from Sprague-Dawley rat *Chrnb2* cDNA (GenBank accession No. L31622) and for the mutant gene (5′-CCG GCG AGT CCT TGG ACG GAG GCG GGG TT and 5′-GGG CCA GCG GCG GTA GGT TCG GCC CTA AAG GCG). These probes were synthesized to correspond to the nucleotide residues: (AACCCCGCCTCCGTCCAAGGACTCGCCGGCGCCTTTAGGGCCGAACCTACCG CCGCTGGCCC), of which the amino acid sequence is the same as that of the wild-type nAChRβ2. The procedures for probe labeling were similar to those reported previously (Mori et al., 2010). X-ray film autoradiograms were digitized with a flatbed scanner (Epson GT-X970, Tokyo, Japan) and evaluated semi-quantitatively in terms of gray levels with Adobe Photoshop CS3. The data were normalized by subtracting the film

background, and the mean value was calculated from three sections and the values of each sample were analyzed by one-way ANOVA with post-hoc Scheffe's test.

2.5. Electroencephalography

For free-moving electroencephalography (EEG), recording and reference electrodes were screwed onto the skull over the frontal (anterior, 2.7 mm; lateral, 1.8 mm relative to bregma) and occipital (midline 1.0 mm caudal to lambda) regions. Simultaneous video/EEG recordings were obtained continuously for 1 week from 8-, 10-, or 12-week-old rats using a digital telemetry system (Unimec, Tokyo, Japan). The obtained EEG data were analyzed using NOTOCORD-hem Evolution® software (Notocord, Croissy, France). The implantation procedure was performed under anesthesia (intraperitoneal administration of a mixture containing: 0.3 mg/kg medetomidine, 4.0 mg/kg midazolam, and 5.0 mg/kg butorphanol).

2.6. Nicotine-induced seizures

Thirteen-week-old rats received a single intraperitoneal injection of 0.5 mg/kg or 1 mg/kg nicotine, were observed for 10 min and scored for seizure activity using a modified behavioral scale (Kedmi et al., 2004): score 1, immobility; score 2, mild head tremors or straub tail; score 3, more severe tremors and repetitive rapid movements of the forelimbs; score 4, wild running and/or complete loss of righting response; score 5,

clonic seizures; score 6, tonic seizures and death. Simultaneous video/EEG recordings were obtained from the day before injection of nicotine and throughout the experiment.

2.7. Statistical analysis

Data analyses were performed using the Student's *t*-test for comparisons between two groups, or one-way ANOVA with post-hoc Scheffe's test. Results are expressed as mean and standard error of the mean (S.E.M.). The level of significance was set at $P < 0.05$.

3. Results

3.1. Generation and characterization of V286L-TG

The proportion of transgenic rats among littermates followed Mendelian law. The non-TG and V286L-TG rat littermates were equally fertile and survived for 2 years.

Histological examination revealed no abnormality in the overall structure of the brains of V286L-TG rats (Figure 1). There was no clear increase in the number of apoptotic ssDNA-positive cells in V286L-TG compared with non-TGs (from 8 to 100 weeks of age).

3.2. Regional distribution of transgene and mutant nAChR expression in V286L-TG

In situ hybridization using a non-selective probe (sensitive to both wild-type and V286L

mutant *Chrnb2* mRNA) revealed no differences in the expression of *Chrnb2* mRNA in brains of non-TG and V286L-TG (Figure 2A–D). Furthermore, *in situ* hybridization using a V286L *Chrnb2*-selective probe indicated that the transgene was expressed at significantly higher levels in brains of V286L-TG compared with non-TGs ($p<0.01$), with higher expression occurring predominantly in the cortex, hippocampus, and cerebellum (Figure 2E–H). In the sensorimotor cortex (caudal, 2.7–3.0 mm; lateral, 1.0–5.0 mm relative to bregma), immunohistochemical analyses showed no differences in the protein levels of the nAChR β2-subunit between non-TG and V286L-TG (Figure 3).

3.3. V286L-TG demonstrated spontaneous epileptic seizures

V286L-TG rats developed spontaneous abnormal EEG with high amplitude spike at 8–12 weeks of age, whereas there was no difference between non-TG (n = 4) and V286L-TG (n = 11) rats in the baseline of EEG for wakefulness and sleep (Figure.4). Simultaneous video recordings of behavior revealed that V286L-TG rats suddenly jumped and ran with EEG abnormalities. This type of seizure is similar to “paroxysmal arousals”, which are brief episodes characterized by sudden frightened expression observed in human ADNFLE (Provini et al., 1999; Combi et al., 2004). Spontaneous epileptic seizures with ictal discharges in the EEG were detected in 45% of V286L-TG rats (n = 11) and the frequency of seizures was 0.73 times a week. All seizures occurred

in the light phase of the day (8 am to 8 pm).

3.4. Enhanced sensitivity to nicotine-induced seizures in V286L-TG rats

Nicotine administration induced seizure behaviors with EEG abnormalities in both non-TG and V286L-TG rats (Figure 5). However, V286L-TG rats demonstrated a higher sensitivity to nicotine. Following administration of 0.5 mg/kg of nicotine, more than 90% of V286L-TG rats exhibited convulsions or convulsion-like behavior (score 3-5), while all of the non-TG rats only exhibited mild head tremors (score 2), (Figure 6A). The mean maximal behavioral score in V286L-TG rats was higher than that of the non-TGs (Figure 6C). When 1 mg/kg of nicotine was administered, there was no significant difference in the mean score between V286L-TG and non-TG rats (Figure 6B and C); however, 78.6% of V286L-TG displayed a straub tail where the tail was bent 180° from its natural position and slight changes in the EEG traces, while non-TG displayed no changes in these parameters (Figure 6E).

The latent period from nicotine administration to onset of response in V286L-TG rats was significantly shorter than that of non-TGs (Figure 6D). Following administration of 0.5 mg/kg nicotine, the latency to response was 116 ± 23 s (mean ± SEM; non-TG) and 28 ± 4 s (V286L-TG). After 1 mg/kg of nicotine, the latency to response was 73 ± 9 s (non-TG) and 31 ± 9 s (V286L-TG).

4. Discussion

In the present study, we established a transgenic rat strain harboring a human ADNFLE mutation of the β2-subunit of nAChR (V286L-TG). V286L-TG rats developed spontaneous epileptic seizures and a hypersensitivity to nicotine administration. The seizures in these transgenic rats are similar to “paroxysmal arousals” of ADNFLE patients. V286L-TG also caused a pathological phenotype related to those observed in ADNFLE.

4.1. Spontaneous epileptic seizures

V286L-TG rats demonstrated spontaneous epileptic seizures where ictal discharges in the EEG were characterized by sudden jumps and/or bursts of behavior associated with high-amplitude EEG. These bursts of seizure activity were detected in 45% of the transgenic rats with a frequency of 0.73 episodes in 1 week. All seizures occurred in the light phase of the day (8 am to 8 pm). The ictal hypermoter events “jump and run” we observed were similar to “paroxysmal arousals”, which are one of the characteristic seizure types occurring in ADNFLE patients. Paroxysmal arousals are abrupt frequently recurring arousals lasting from 2–20 sec. The most common pattern consists of a sudden arousal during which patients raise their heads, sit on the bed with a frightened

expression. It is reported that the mean seizure frequency is 16 ± 12 per month (Provini et al., 1999).

There was no difference in the expression levels of wild-type nAChRβ2 mRNA between V286L-TG and non-TG rats, although the transgene was expressed in cortical, hippocampal, and cerebellar brain regions of V286L-TG. In the sensorimotor cortex, there was no difference in the expression of β2-nAChR protein between non-TG and V286L-TG rats. Co-expression of normal and mutated gene products in V286L-TG satisfied the construct validity of our transgenic rat as an animal model of ADNFLE.

Previously, two mouse models carrying V287L mutations of the β2-subunit of the nAChR have been engineered, both of which are comparable to the V286L-TG rat developed in the current study. One is a conditional transgenic mouse strain, which displayed a spontaneous epileptic phenotype with frequent interictal spikes and seizures (Manfredi et al., 2009). However, the seizure phenotype in the conditional transgenic mouse may have been dependent on the overexpression of mutated β2 receptor. The other model was a knock-in mouse strain, which did not display spontaneous seizures but did show enhanced sensitivity to seizures induced by nicotine (Xu et al., 2011; O'Neill et al., 2013).

Thus, the rat model we developed here can accurately reproduce many of the

properties of ADNFLE in human patients. We chose to use rats in our studies because they exhibit many physiological characteristics similar to humans and are one of the best characterized mammalian model systems for studying seizures (Steinlein, 2010). In our models, a transgenic method rather than a knock-in strategy was used, because knock-in engineering is not, as yet available to perform in rats. An additional advantage is that various experimental procedures, such as microsurgical techniques or intrathecal administration of drugs, can be performed with ease in rats; whereas they are much more difficult to use in mice.

4.2. Nicotine sensitivity

V286L-TG rats demonstrated a much higher sensitivity to nicotine by responding to relatively low concentrations of nicotine with marked behavioral changes such as straub tail, and an abnormal EEG pattern. The non-TGs did not display such distinct behavioral responses to nicotine. Previous studies in ADNFLE knock-in strains with a mutated α4-subunit (Fonck et al., 2003; Fonck et al., 2005; Klaassen et al., 2006; Teper et al., 2007) or β2-subunit (Xu et al., 2011; O'Neill et al., 2013) have reported a higher sensitivity to nicotine-induced seizures, suggesting that increased response to nicotine might relate to seizure phenotypes in ADNFLE. Furthermore, using knock-in mice, high doses of nicotine (10 mg/kg) result in early-onset seizures accompanied by a behavioral

response called the dystonic arousal complex (DAC) and a late-onset response with classic tonic-clonic seizures (Fonck et al., 2005; Teper et al., 2007). The DAC response includes straub tail, tonic forelimb extension, tonic digit splaying, and occasionally forelimb clonus (Teper et al., 2007). The nicotine-induced behavioral responses we observed in the V286L-TG rats used in the present study were remarkably similar to those observed in other mutant mice expressing ADNFLE mutations, except for the EEG pattern. V286L-TG rats demonstrated a slightly higher amplitude and rhythmical EEG pattern in the early stages of seizures.

4.3. Gain of function

Spontaneous epileptic seizures and enhanced nicotine sensitivity have been suggested to be gain of function effects of mutating the β2-subunit. When HEK293 cells express a mutant β2-subunit, a prolonged inward current and slower desensitization to cholinergic stimulation was observed and is likely to cause seizures through a gain of function effect (De Fusco et al., 2000).

Connections between the thalamus and cortex with a negative feedback from the cortical-thalamic projections are believed to contribute to ADNFLE (Steinlein and Bertrand, 2010). At present, it is hypothesized that the gain of function of β2 nAChR variants may cause an imbalance of excitation versus inhibition in this brain circuit,

which in turn promotes the triggering of seizures (Steinlein and Bertrand, 2010).

4.4. Comparison with α4 transgenic model

The α4 S284L-TG rat previously generated by our group also experienced seizures during slow-wave sleep that are similar to those in ADNFLE exhibiting three characteristic phenotypes: “paroxysmal arousals”, “paroxysmal dystonia”, and “episodic wandering”. More than 90% of S284L-TG rats exhibited spontaneous epileptic seizures (Zhu et al., 2008). In contrast, V286L-TG rats exhibited only one characteristic seizure type “paroxysmal arousals”, which were detected in only 45% of V286L-TG. The reasons for these distinct seizure activities with the different mutations are not yet known. Further studies are required to determine the mechanism of epileptogenesis by ion channel mutation. Patients with ADNFLE demonstrate various types of seizure behavior (Provini et al., 1999; Combi et al., 2004; Steinlein et al., 2012), thus the study of V286L may lead to an improved understanding of the phenotype-genotype relationship in this patient population. Furthermore, our previous study of S284L-TG rats revealed a perturbed GABAergic inhibitory system in brains of the TG, which has led to a novel prophylactic treatment for ADNFLE (Yamada et al., 2013). Understanding the differences in pathogenesis between S284L-TG and V286L-TG rats may contribute to the development of a novel mechanism-driven class of antiepileptic

drugs.

In conclusion, V286L-TG rats demonstrated spontaneous epileptic seizures that are similar to human ADNFLE, and also showed a hypersensitive response to nicotine administration. The molecular and pathophysiological study using this genetically modified rat model of epilepsy may lead to a better understanding of ADNFLE, and contribute to the development of new type of mechanism-driven antiepileptic drug. Our studies may also result in the design of a prophylatic and/or radical cure treatment strategy for epilepsy.

Disclosure of conflicts of interest

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure legends

Figure 1.

Sagittal sections from brains of 2-year-old non-TG (A, B) and V286L-TG rats (C, D). Right: Higher magnification views of cortical areas marked by the rectangles in (A) and (C). Klüver–Barrera method. Scale bar = 2 mm.

Figure 2.

In situ hybridization analysis of non-TG (A, B, E, F) and V286L-TG rats (C, D, G, H) at 8 weeks of age using ^{33}P-labeled oligonucleotide probes specific for wild Chrnb2 mRNA (A–D) and V286L-transgenic Chrnb2 mRNA (E-H). The regional expression of wild Chrnb2 mRNA was higher in the thalamus, hippocampus, and cerebellum both in non-TG (A, B) and V286L-TG rats (C, D). No signal of V286L-transgenic Chrnb2 mRNA was seen in non-TG (E, F), although the expression of V286L-transgenic Chrnb2 mRNA was significantly higher in each brain region of V286L-TG rats, especially in the cortex, hippocampus, and cerebellum (G, H). The data were normalized by subtracting the film background. non-TG, n = 3; V286L, n = 3, 3 sections of each region. The mean values of each case were analyzed using one-way ANOVA with post-hoc Scheffe's test ($p<0.01$).

Scale bar = 2 mm. Cx: Cortex, Th: Thalamus, Cpu: Corpus striatum, Hip: Hippocampus, Hy: Hypothalamus, Cb: Cerebellum, Bst: Brain stem

Figure 3.

Light micrographs of β2-nAChR immunoreactivity in the sensorimotor cortex of 8-week-old non-TG and V286L-TG rats. Note the immunostained neuronal cell bodies and apical dendrites in layers II–VI. Scale bar = 100 μm.

Figure 4.

Electroencephalogram (EEG) during ictal discharge in a V286L-TG rat. (A) Baseline for wakefulness and sleep. There was no difference between non-TG (n = 4) and V286L-TG (n = 11) rats. (B) A typical spontaneous epileptic seizure EEG for paroxysmal arousal. (C) Higher magnification of the boxed wave in (B). Paroxysmal arousal behaviors such as sudden jumping and running were observed using the simultaneous video recording.

Figure 5.

Representative EEG traces after a single injection of 1 mg/kg intraperitoneal nicotine (non-TG, n = 8; V286L, n = 14). (A) Non-TG rats did not show scores of 3 or 4. (B)

V286L-TG rats displayed straub tail, rapid movement of the forelimbs, and complete loss of righting response (score 2, score 3, and score 4, respectively) with abnormal EEG, which was rhythmical wave with high amplitude and frequency. (C) Extended EEG trace of framed part in (B), which appeared as a rhythmical sharp wave and/or sharp-and-slow-wave complex.

Figure 6.

(A) Maximal scores rats displayed for 0.5 mg/kg nicotine administration (non-TG, n = 9; V286L, n = 11). All non-TG rats displayed only mild tremors (score 2), in contrast more than 90% V286L-TG rats exhibited convulsions or convulsion-like behaviors (score 3–5). (B) Maximal scores for 1 mg/kg nicotine (non-TG, n = 8; V286L, n = 14). Almost all rats displayed scores of 4–6. (C) The mean of maximal scores rats displayed. Each column represents the mean ± SEM. *** $P < 0.001$, unpaired Student's *t*-test. (D) The latent periods from nicotine administration to occurrence of the first response for 0.5 mg/kg and 1 mg/kg. Each column represents mean ± SEM, ** $P < 0.01$, *** $P < 0.001$, unpaired Student's *t*-test.

Scores: 1, immobility; 2, mild head tremors or straub tail; 3, more severe tremors and repetitive rapid movements of the forelimbs; 4, wild running and/or complete loss of righting response; 5, clonic seizures; 6, tonic seizures and death.

0.5 mg/kg intraperitoneal (non-TG, n = 9; V286L, n = 11) and 1 mg/kg intraperitoneal. (E) Enhanced sensitivity to nicotine-induced straub tail in V286L-TG rats. Data show the positive ratio of nicotine-induced straub tail behavior. Each point represents mean ± SEM, * $P < 0.05$, ** $P < 0.01$, unpaired Student's t-test.

Figure 1.

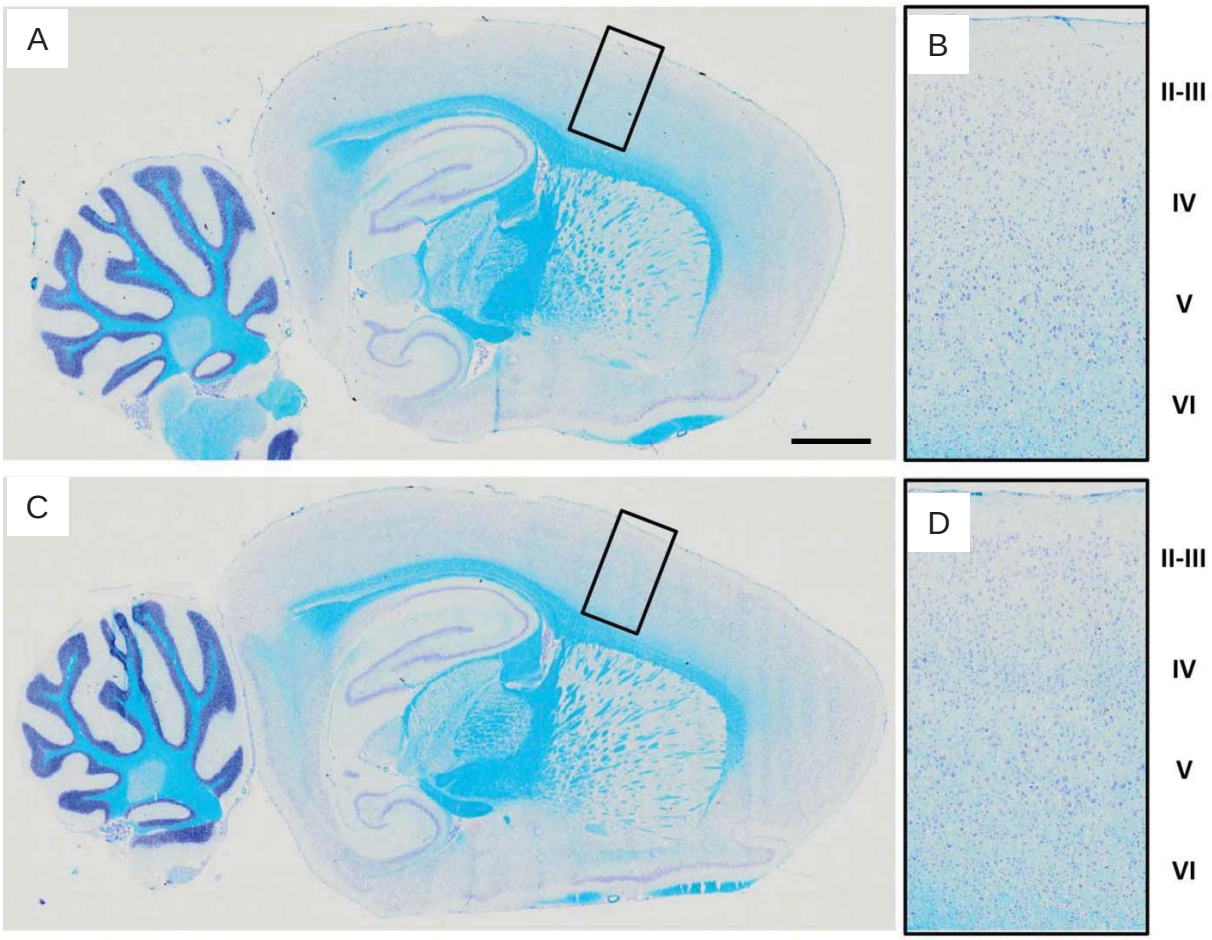
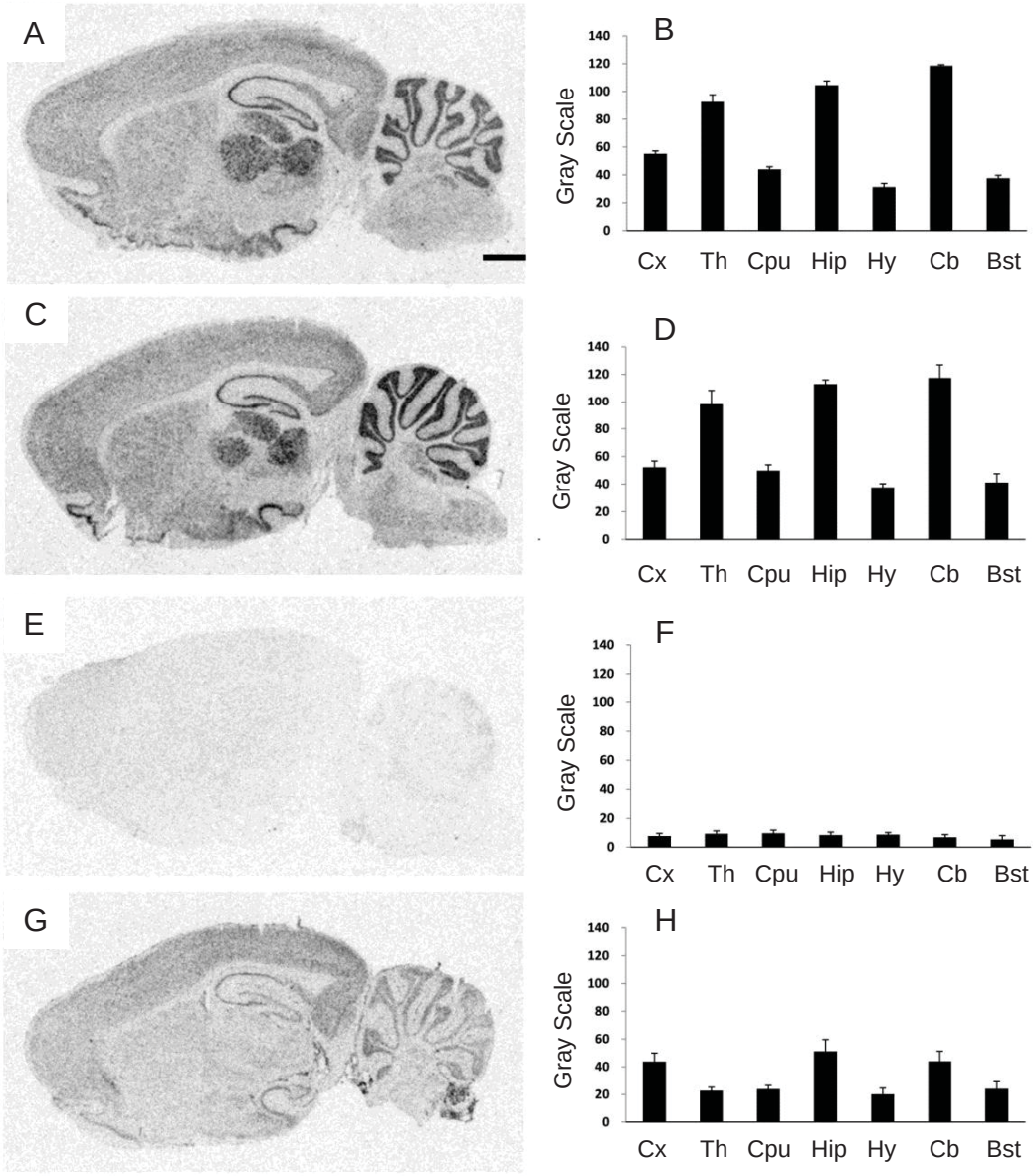


Figure 2.


A
B
Gray Scale
140
120
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0
Cx Th Cpu Hip Hy Cb Bst
C
D
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140
120
100
80
60
40
20
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Cx Th Cpu Hip Hy Cb Bst
E
F
Gray Scale
140
120
100
80
60
40
20
0
Cx Th Cpu Hip Hy Cb Bst
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140
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Figure 3.

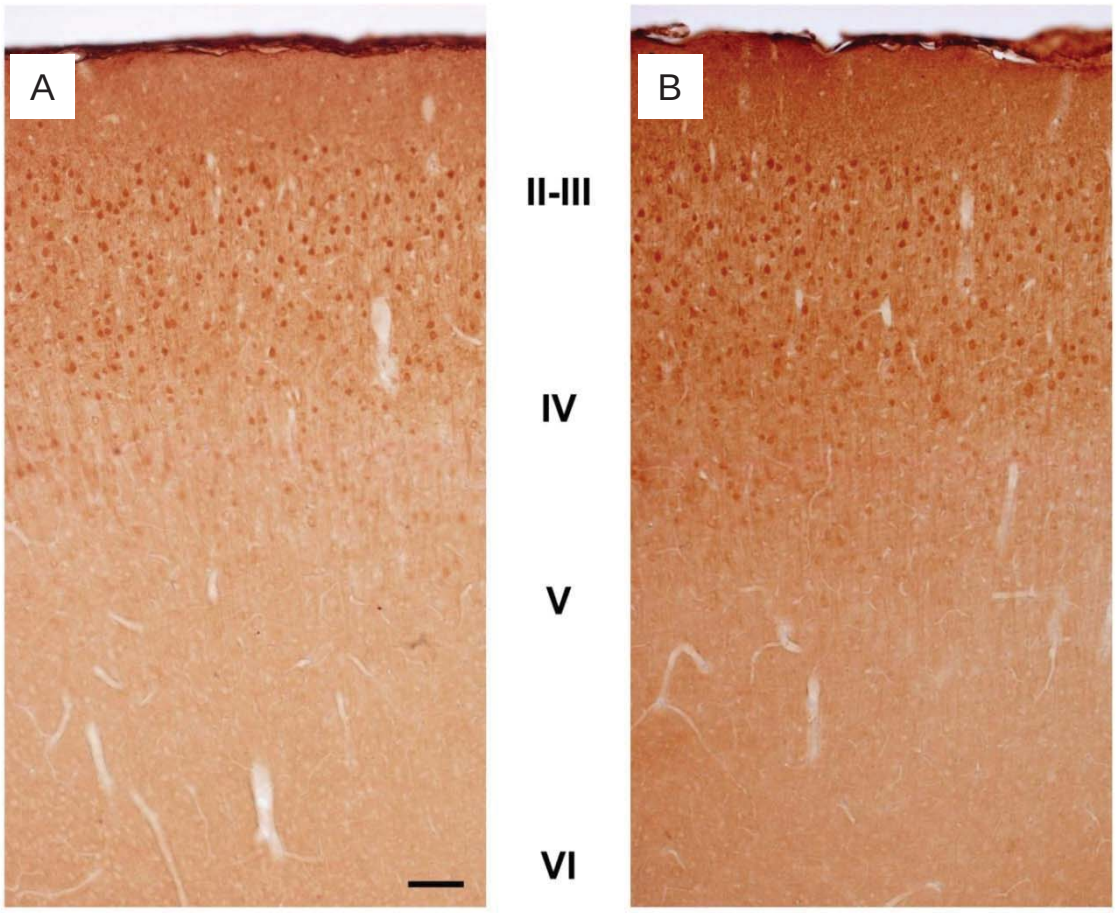


Figure 4.

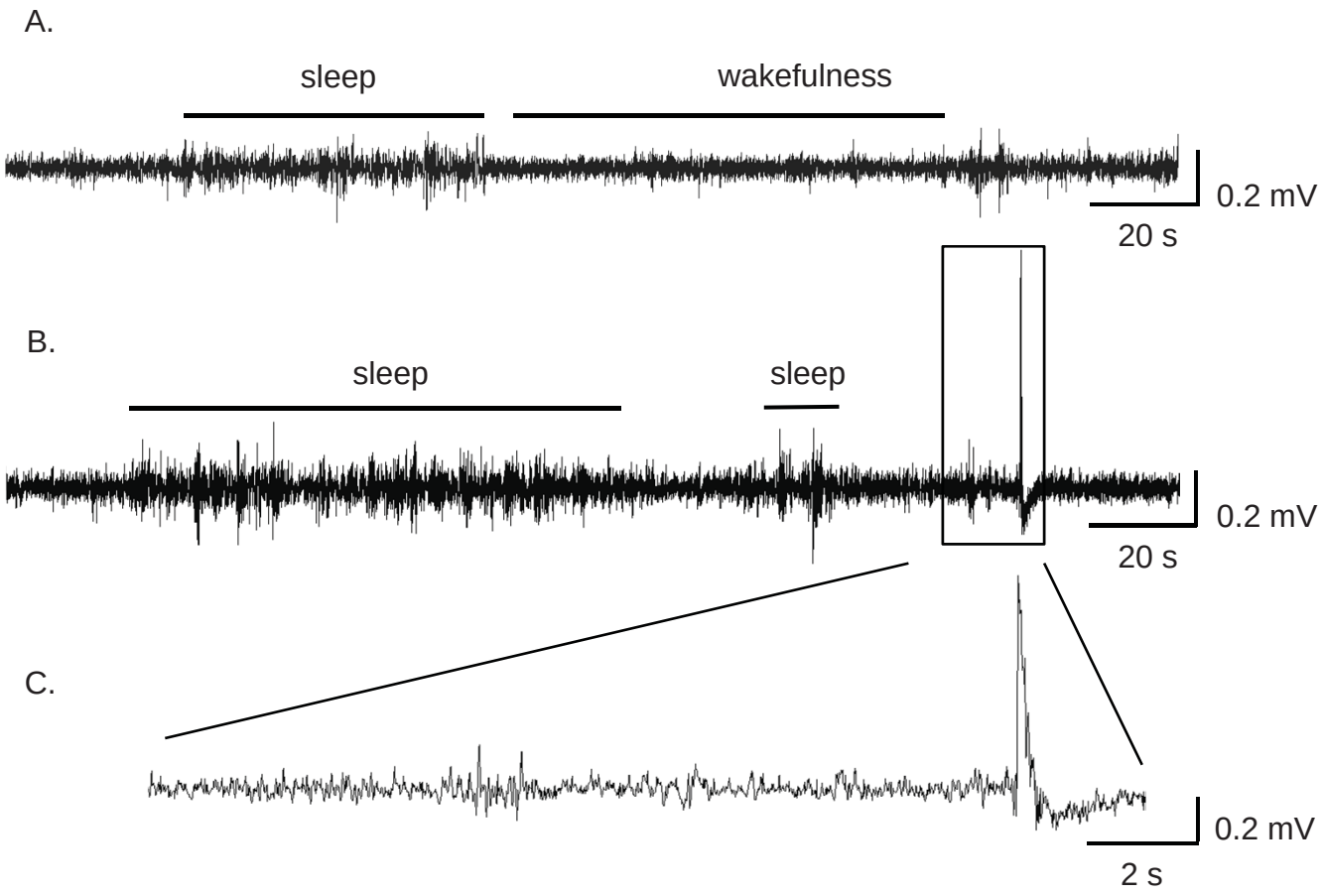


Figure 5.

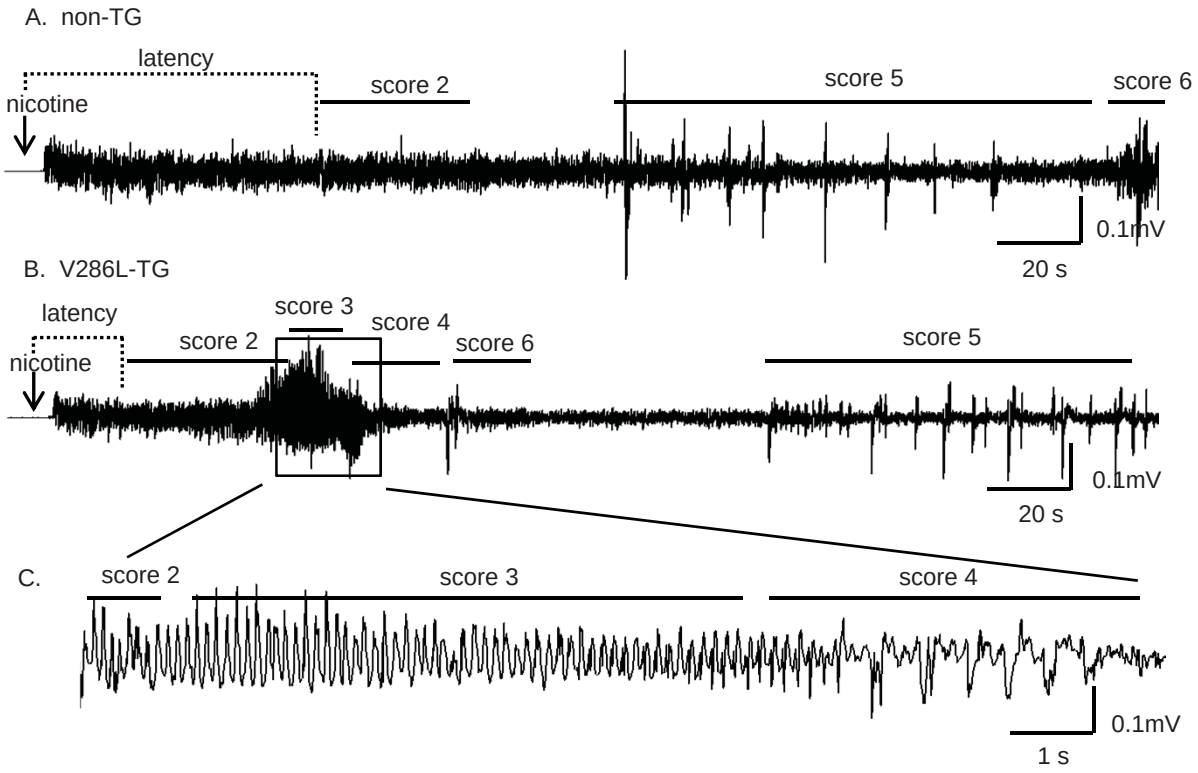
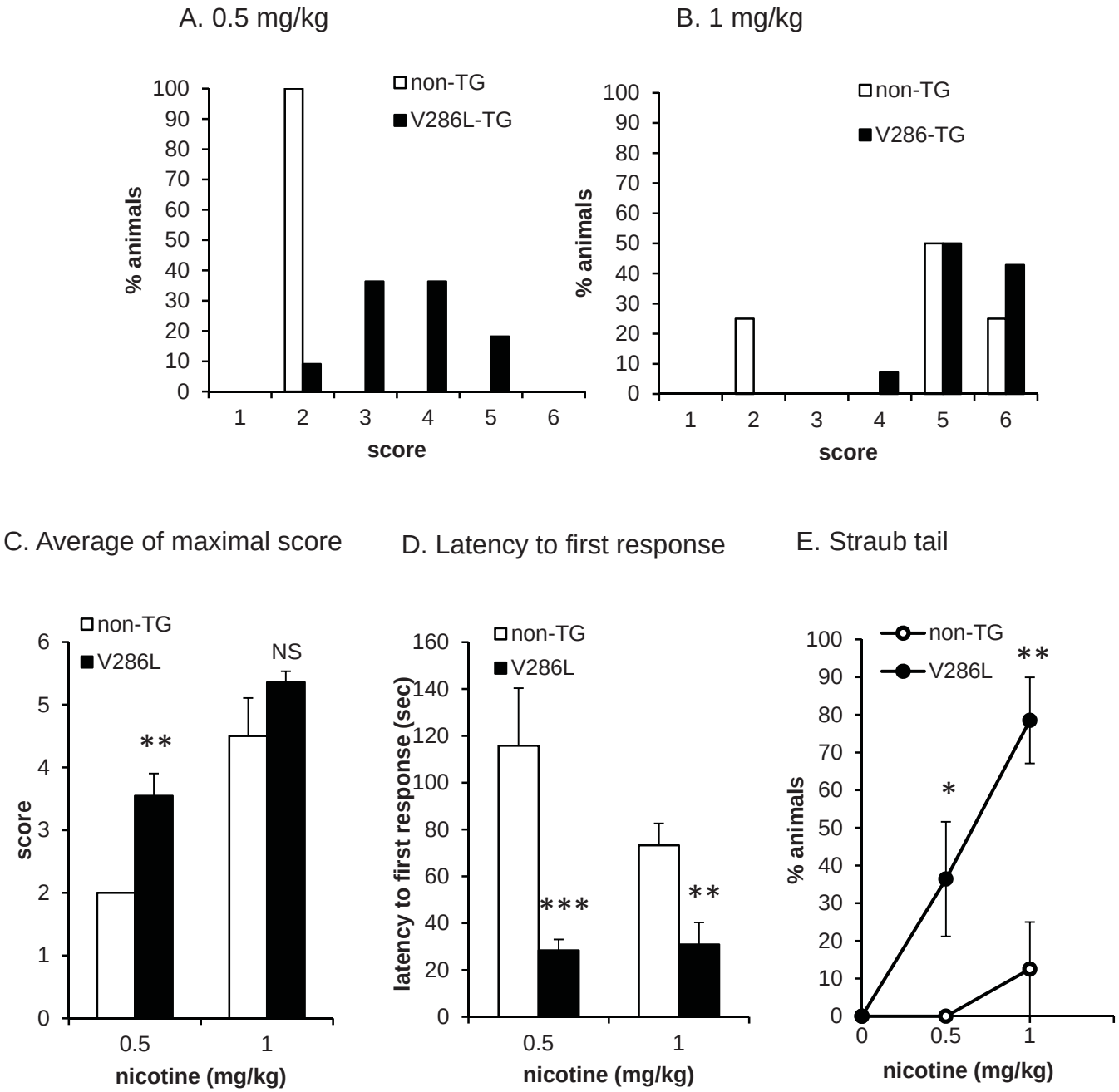

A. non-TG
latency
nicotine
score 2
score 5
score 6
0.1mV
20 s
B. V286L-TG
latency
nicotine
score 2
score 3
score 4
score 6
score 5
0.1mV
20 s
C.
score 2
score 3
score 4
0.1mV
1 s

Figure 6.


A. 0.5 mg/kg
B. 1 mg/kg
non-TG
V286L-TG
V286-TG
% animals
score
C. Average of maximal score
D. Latency to first response
E. Straub tail
V286L
NS
**
***
*
latency to first response (sec)
nicotine (mg/kg)