Characterization of the Properties of Seven Promoters in the Motor Cortex of Rats and Monkeys After Lentiviral Vector-Mediated Gene Transfer

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Abstract

Lentiviral vectors deliver transgenes efficiently to a wide range of neuronal cell types in the mammalian central nervous system. To drive gene expression, internal promoters are essential; however, the *in vivo* properties of promoters, such as their cell type specificity and gene expression activity, are not well known, especially in the nonhuman primate brain. Here, the properties of five ubiquitous promoters (murine stem cell virus [MSCV], cytomegalovirus [CMV], CMV early enhancer/chicken β -actin [CAG], human elongation factor-1 α [EF-1 α], and Rous sarcoma virus [RSV]) and two cell type-specific promoters (rat synapsin I and mouse α -calcium/calmodulin-dependent protein kinase II [CaMKII α]) in rat and monkey motor cortices *in vivo* were characterized. Vesicular stomatitis virus G (VSV-G)-pseudotyped lentiviral vectors expressing enhanced green fluorescent protein (EGFP) under the control of the various promoters were prepared and injected into rat and monkey motor cortices. Immunohistochemical analysis revealed that all of the VSV-G-pseudotyped lentiviral vectors had strong endogenous neuronal tropisms in rat and monkey brains. Among the seven promoters, the CMV promoter showed modest expression in glial cells (9.4%) of the rat brain, whereas the five ubiquitous promoters (MSCV, CMV, CAG, EF-1 α , and RSV) showed expression in glial cells (7.0–14.7%) in the monkey brain. Cell type-specific synapsin I and CaMKII α promoters showed excitatory neuron-specific expression in the monkey brain (synapsin I, 99.7%; CaMKII α , 100.0%), but their specificities for excitatory neurons were significantly lower in the rat brain (synapsin I, 94.6%; CaMKII α , 93.7%). These findings could be useful in basic and clinical neuroscience research for the design of vectors that efficiently deliver and express transgenes into rat and monkey brains.

Introduction

VIRAL VECTORS are promising tools for altering the expression of specific genes in the CNS to elucidate fundamental physiological mechanisms of the brain and to achieve therapeutic success (Davidson and Breakefield, 2003; Thomas *et al.*, 2003). Lentiviral and adeno-associated viral (AAV) vectors are particularly suitable for *in vivo* gene transfer into the CNS because they transduce nondividing cells, sustain the expression of transgenes, and exhibit low toxicity (Naldini *et al.*, 1996b; McCown, 2005). Combined with genetic methods such as optogenetics, these viral vectors

allow targeted gene expression, in particular CNS cell types, and the manipulation of the particular CNS cell functions in mammalian species, including primates as well as rodents, in a region- and time-dependent manner (Luo *et al.*, 2008; Han *et al.*, 2009, 2011; Diester *et al.*, 2011; Tsubota *et al.*, 2011, 2012; Inoue *et al.*, 2012; Kinoshita *et al.*, 2012; Tamura *et al.*, 2012).

Initial efforts to drive transgene expression in the CNS, using lentiviral vectors, relied on strong and ubiquitous promoters, such as the cytomegalovirus (CMV) promoter (Thomsen *et al.*, 1984; Naldini *et al.*, 1996a; Blomer *et al.*, 1997). These strong and ubiquitous promoters are useful for the labeling of cells with LacZ or fluorescent proteins.

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However, when optimal transgene expression levels and patterns are required (e.g., when overproduced transgene products are toxic in specific cells), weaker or cell type-specific promoters need to be considered. To fulfill this requirement, gene-regulatory elements have been developed, including cell type-specific promoters that can be inserted into the lentiviral genome (Dittgen *et al.*, 2004; Hioki *et al.*, 2007). Therefore, it is important to select the optimal promoter for every experimental design. Several previous studies have compared the properties of various promoters in lentiviral vectors (Jakobsson *et al.*, 2003; Dittgen *et al.*, 2004; Hioki *et al.*, 2007; Kuroda *et al.*, 2008; Takayama *et al.*, 2008; Li *et al.*, 2010); however, there have been no previous reports of side-by-side comparisons of the properties of various promoters in primate brains or of interspecies differences in promoter properties.

Among the many structures of the primate brain, the cerebral cortex is considered to be the center of extraordinary cognitive abilities, which were acquired during the course of evolution (Miyashita, 2004; Rakic, 2009). Therefore, by targeting the nonhuman primate cerebral cortex *in vivo*, using viral vectors, the molecular and cellular bases of higher mammalian cognitive abilities will be elucidated. Anatomically, the cerebral cortex is an intricate laminar structure composed of six layers of neurons surrounded by astrocytes. These neurons are classified into two main groups: glutamatergic excitatory neurons and GABAergic inhibitory neurons. Therefore, to accomplish successful *in vivo* gene transfer into nonhuman primate brains, detailed knowledge of the extent, efficiency, and cellular pattern of transgene expression in nonhuman primate brains is needed.

Here, we prepared vesicular stomatitis virus G (VSV-G)-pseudotyped lentiviral vectors expressing enhanced green fluorescent protein (EGFP) under the control of five ubiquitous promoters (murine stem cell virus [MSCV; Hawley *et al.*, 1994], cytomegalovirus [CMV; Thomsen *et al.*, 1984], CMV early enhancer/chicken β -actin [CAG; Niwa *et al.*, 1991], human elongation factor-1 α [EF-1 α ; Kim *et al.*, 1990], and Rous sarcoma virus [RSV; Yamamoto *et al.*, 1980]) and two cell type-specific promoters (rat synapsin I [Hoesche *et al.*, 1993; Dittgen *et al.*, 2004] and mouse α -calcium/calmodulin-dependent protein kinase II (CaMKII α) [Dittgen *et al.*, 2004]). After *in vivo* viral injection into rat and monkey motor cortices, the EGFP expression patterns from the seven promoters were compared via histological analysis. Gene expression levels, neuronal/glial cell specificities, excitatory/inhibitory neuron specificities, and interspecies differences between rats and monkeys for these promoters are reported here.

Materials and Methods

All the experiments were conducted in accordance with the National Institutes of Health (Bethesda, MD) *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Review Committee of the University of Tokyo School of Medicine (Tokyo, Japan).

Cell culture

Human embryonic kidney (HEK) 293T cells used for transient transfection were obtained from the RIKEN Bio-Resource Center (Tsukuba, Ibaraki, Japan; cell no. RCB2202).

HEK293T cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) containing 10% (v/v) fetal bovine serum (FBS), penicillin G (100 U/ml), and streptomycin (100 μ g/ml) (Invitrogen) at 37°C in a 5% CO₂ atmosphere.

Plasmid construction

The following is a general outline of the construction of the individual plasmids used in this study. Further details are available on request.

pCL20c-MSCV-GFP-WPRE. The *Cla*I-digested wood-chuck hepatitis posttranscriptional regulatory element (WPRE) fragment from CS-CA-MCS (kindly provided by H. Miyoshi, RIKEN BioResource Center) was subcloned into the *Cla*I site of pCL20c-MSCV-GFP (kindly provided by A.W. Nienhuis, St. Jude Children's Research Hospital, Memphis, TN).

pCL20c-CMV-GFP-WPRE. The *Eco*RI/*Age*I-digested CMV promoter (0.6 kb) from CS-CDF-CG-PRE (kindly provided by H. Miyoshi) was inserted (by blunt-end ligation) into the *Mlu*I/*Eco*RI-digested 7.0-kb fragment of the pCL20c-MSCV-GFP-WPRE vector, from which the MSCV promoter was removed.

pCL20c-CAG-GFP-WPRE. The *Pst*I-digested CAG promoter (1.7 kb) from CS-CA-MCS was inserted (by blunt-end ligation) into the *Mlu*I/*Eco*RI-digested 7.0-kb fragment of the pCL20c-MSCV-GFP-WPRE vector, from which the MSCV promoter was removed.

pCL20c-EF1 α -GFP-WPRE. The primers 5'-GGA CGC GTC AAG CTT CGT GAG GCT CCG-3' and 5'-CGG AAT TCG ACC GGT AGC GTG TTC ACG-3' were used to amplify the EF-1 α promoter (1.2 kb) from CS-CDF-EG-PRE (kindly provided by H. Miyoshi). The *Mlu*I/*Eco*RI-digested EF-1 α promoter (1.2 kb) was then inserted (by blunt-end ligation) into the *Mlu*I/*Eco*RI-digested 7.0-kb fragment of the pCL20c-MSCV-GFP-WPRE vector, from which the MSCV promoter was removed.

pCL20c-RSV-GFP-WPRE. The primers 5'-CAT GAT TTC GAA TTC GAT GTA CGG GCC AGA TAT ACG CGT-3' and 5'-GAA CCG GTA GGT GCA CAC CAA TGT GGT GAA TGG TC-3' were used to amplify the RSV promoter (0.4 kb) from pCMV-VSV-G-RSV-Rev (kindly provided by H. Miyoshi). The *Bst*BI/*Age*I-digested RSV promoter (0.4 kb) was then inserted (by blunt-end ligation) into the *Bst*BI/*Age*I-digested 7.0-kb fragment of the pCL20c-MSCV-GFP-WPRE vector, from which the MSCV promoter was removed.

pCL20c-Synapsin I-GFP-WPRE. The primers 5'-GGG TTT TGG CTA CGT CCA GAG-3' and 5'-GGG ATC CAA GGG GCA GTG-3' were used to amplify the synapsin I promoter (1.1 kb) from rat genomic DNA. The PCR product was then electrophoresed on an agarose gel (2%, w/v), extracted with a QIAquick gel extraction kit (Qiagen, Hilden, Germany), subcloned into the pGEM-T Easy vector (Promega, Madison, WI), and sequenced (ABI PRISM 310 genetic analyzer; Applied Biosystems/Life Technologies, Foster

City, CA). The *EcoRI*-digested synapsin I promoter (1.1 kb) was inserted (by blunt-end ligation) into the *MluI/EcoRI*-digested 7.0-kb fragment of the pCL20c-MSCV-GFP-WPRE vector, from which the MScV promoter was removed.

pCL20c-CaMKII α -GFP-WPRE. The *PacI/BamHI*-digested mouse CaMKII α promoter (1.3 kb) from pLenti-CaMKII α -hChR2-mCherry-WPRE (kindly provided by K. Deisseroth, Stanford University, Stanford, CA) was inserted (by blunt-end ligation) into the *MluI/EcoRI*-digested 7.0-kb fragment of the pCL20c-MSCV-GFP-WPRE vector, from which the MScV promoter was removed.

All the inserts in the above-constructed plasmids were sequenced (Ohashi *et al.*, 2004).

Lentiviral preparation

Lentiviral preparations and titrations were performed as described previously (Ohashi *et al.*, 2011; Tsubota *et al.*, 2011; Tamura *et al.*, 2012). Briefly, for the functional titration using a fluorescent protein (GFP titer), cultured HEK293T cells were transduced, transduction efficiency was estimated by counting the EGFP-positive cells with an EPICS XL flow cytometer with EXPO 32 software (Beckman Coulter, Brea, CA) and FlowJo software (Tree Star, San Carlos, CA), and the GFP titer was calculated from a linear range (Tamura *et al.*, 2012). For the DNA titration assay (DNA titer), cultured HEK293T cells were transduced, their genomic DNA was extracted, and the copy number of integrated lentiviral genomes was analyzed by TaqMan real-time PCR for the WPRE sequence (Tsubota *et al.*, 2011). Lentiviral vectors derived from transfer vector plasmids, namely pCL20c-MSCV-GFP-WPRE, pCL20c-CMV-GFP-WPRE, pCL20c-CAG-GFP-WPRE, pCL20c-EF1 α -GFP-WPRE, pCL20c-RSV-GFP-WPRE, pCL20c-Synapsin I-GFP-WPRE, and pCL20c-CaMKII α -GFP-WPRE, were subsequently termed Lenti-MSCV-GFP, Lenti-CMV-GFP, Lenti-CAG-GFP, Lenti-EF1 α -GFP, Lenti-RSV-GFP, Lenti-Synapsin I-GFP, and Lenti-CaMKII α -GFP, respectively.

Stereotactic injection

For rat experiments, lentiviral vectors were stereotactically injected into the right motor cortex of 10-week-old male Wistar rats (230–250 g; Nihon SLC, Hamamatsu, Japan). Each rat was anesthetized with ketamine–xylazine (90 and 10 mg/kg, respectively) by intraperitoneal injection and positioned in a stereotactic apparatus (SR-6R; Narishige, Tokyo, Japan). A midline sagittal incision was made, and the skull was exposed. A small hole was drilled 1.0 mm anterior to the bregma and 1.0 mm lateral to the midline. The tip of a 32-gauge needle attached to a 10- μ l gas-tight Hamilton syringe (Hamilton Company, Reno, NV) was placed in the motor cortex, and 3 μ l of the virus solution, at a titer of 5×10^9 transducing units (TU)/ml (GFP titer), was injected at a rate of 100 nl/min, using a micropump (UltraMicroPump III; World Precision Instruments [WPI], Sarasota, FL) and a microprocessor-based controller (Micro4; WPI) (Fig. 1B). In the case of Lenti-CaMKII α -GFP, the functional titer could not be measured precisely because the activity of the CaMKII α promoter was weak in the HEK293T cells used for titration. The needle was left in place for an additional 5 min before being retracted from the brain. The scalp incision was su-

tured, and the rat was returned to a standard cage after recovering from the anesthesia.

For monkey experiments, lentiviral vectors were stereotactically injected into the motor cortex of two macaque monkeys (monkey M, *Macaca fuscata*, body weight 6.0 kg; monkey H, *Macaca fuscata*, body weight 11.1 kg) under aseptic conditions and general anesthesia with sodium pentobarbital (4 mg/kg/hr, intravenous injection) and xylazine (2 mg/kg, intramuscular injection) (Matsui *et al.*, 2007). Before the surgery, monkeys were scanned by magnetic resonance imaging (MRI; 4.7 T) (BioSpec 47/40; Bruker BioSpin, Rheinstetten, Germany) to visualize the anatomical landmarks of the proposed injection target sites. A craniotomy was made in the skull over the motor cortex, the tip of a 32-gauge needle attached to a 10- μ l gas-tight Hamilton syringe was placed within the motor cortex, and the lentiviral injections were performed. In monkey M, four sets of stereotaxic injections into the motor cortex were performed bilaterally (Fig. 1C). Each set consisted of seven (MSCV, CMV, CAG, EF-1 α , RSV, synapsin I, and CaMKII α) lentiviral injections at 4-mm intervals. In monkey H, two sets of stereotaxic injections into the motor cortex were performed bilaterally. Each set consisted of five (MSCV, CMV, CAG, EF-1 α , and RSV) lentiviral injections at 4-mm intervals. During surgery, blood pressure, electrocardiogram (ECG), heart rate, body temperature, and oxygen saturation were continuously monitored. Body temperature was kept constant with a heating blanket, and glucose–lactated Ringer's solution was given intravenously at a rate of 5–10 ml/kg/hr. After surgery, the monkeys were given postsurgical analgesics (ketoprofen at 2 mg/kg/day, intramuscular injection) and postsurgical prophylactic antibiotics (benzylpenicillin at 15,000 units/kg/day, intramuscular injection) for 1 week.

Immunohistochemistry

Two weeks after the last injection, the rats and monkeys were deeply anesthetized with an overdose (rats: 200 mg/kg, intraperitoneal injection; monkeys: 60 mg/kg, intravenous injection) of sodium pentobarbital and then transcardially perfused with saline, followed by 4% paraformaldehyde in phosphate buffer. The brains were postfixed in 4% paraformaldehyde for 2 hr (rats) or 24 hr (monkeys) and sunk in 20% (rats) or 30% (monkeys) sucrose in phosphate-buffered saline (PBS). For fluorescence staining, 24- μ m-thick (rats) or 40- μ m-thick (monkeys) coronal sections were cut with a cryostat and each section was stored in PBS at 4°C. Three sections per injection site (every fifth section) were stained. Sections were permeabilized with 0.3% Triton X-100 in PBS, blocked with 5% normal goat serum (Vector Laboratories, Burlingame, CA) for 30 min at room temperature, and incubated for 24 hr at 4°C with primary antibodies: mouse anti-neuron-specific nuclear protein (NeuN) monoclonal antibody (diluted 1:1000; Millipore, Temecula, CA) and rabbit anti-gial fibrillary acidic protein (GFAP) polyclonal antibody (diluted 1:1000; Dako, Glostrup, Denmark) or rabbit anti- γ -aminobutyric acid (GABA) polyclonal antibody (diluted 1:1000; Sigma-Aldrich, St. Louis, MO). The sections were then incubated for 2 hr at room temperature with secondary antibodies: biotin-conjugated goat anti-mouse antibody (diluted 1:500; Vector Laboratories) and Alexa Fluor 546-conjugated goat anti-rabbit antibody (diluted 1:1000; Invitrogen),

FIG. 1. Schematic diagrams of the lentiviral vectors and injections. **(A)** Schema of the lentiviral constructs. Each of the seven promoters (MSCV, CMV, CAG, EF-1 α , RSV, synapsin I, and CaMKII α) was inserted into the transfer vector. LTR, long terminal repeat; ψ , packaging region; cPPT, central polypurine tract; CTS, central termination sequence; EGFP, enhanced green fluorescent protein; RRE, Rev-responsive element; WPRE, woodchuck hepatitis posttranscriptional regulatory element. **(B)** Lentiviral injection site in the rat brain. Coronal, sagittal, and dorsal views are shown. Lentiviral vectors were stereotactically injected into the motor cortex of the right hemisphere. **(C)** Lentiviral injection sites in the monkey brain (monkey M). Coronal, sagittal, and dorsal views are shown. Four sets of stereotaxic injection into the motor cortex were performed bilaterally. Red squares represent a set of injections. Each set consisted of seven lentiviral injections at 4-mm intervals. Each number corresponds to each lentiviral vector: 1, Lenti-MSCV-GFP; 2, Lenti-CMV-GFP; 3, Lenti-CAG-GFP; 4, Lenti-EF1 α -GFP; 5, Lenti-RSV-GFP; 6, Lenti-Synapsin I-GFP; and 7, Lenti-CaMKII α -GFP. Color images available online at www.liebertpub.com/hgtb

followed by incubation for 30 min at room temperature with Alexa Fluor 405-conjugated streptavidin (diluted 1:500; Invitrogen). The sections were mounted onto Matsunami adhesive silane (MAS)-coated glass slides, coverslipped with Fluoromount (Diagnostic BioSystems, Pleasanton, CA), and stored at 4°C. The fluorescence images were obtained with a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan) and a confocal laser-scanning microscope (TCS-SPE; Leica Microsystems, Wetzlar, Germany).

Cell counting

Double-positive cells (GFP⁺/NeuN⁺, GFP⁺/GFAP⁺) and triple-positive cells (GFP⁺/NeuN⁺/GABA⁺) were counted manually in images from all fields of GFP-positive areas within the motor cortex. The neuron-to-astrocyte ratio was calculated from NeuN/GFAP double-stained sections and the excitatory-to-inhibitory neuron ratio was calculated from NeuN/GABA double-stained sections. Laser-scanning confocal microscopic analysis was used to confirm coexpression or triple expression of GFP and the antigenic marker(s) in double-positive and triple-positive cells, respectively. The

number of excitatory neurons was calculated by subtracting the number of triple-positive cells (GFP⁺/NeuN⁺/GABA⁺, inhibitory neurons) from that of double-positive cells (GFP⁺/NeuN⁺).

Statistics

For statistical analysis, the data were evaluated for significance by performing one-way analysis of variance (ANOVA) followed by multiple comparison (post hoc test) analysis, using Statcel software (OMS, Saitama, Japan) after modification of percentage values through arcsine transformation. Probability values less than 0.05 were considered to be statistically significant. Data are expressed as means $\pm$ standard error of the mean (SEM).

Results

To systemically test the properties of commonly used promoters in rat and monkey motor cortices *in vivo*, we constructed seven lentiviral vectors that expressed EGFP from different internal promoters: MSCV, CMV, CAG, EF-1 α ,

RSV, synapsin I, and CaMKII α (Fig. 1A and Table 1). All lentiviral vectors were pseudotyped with a VSV-G envelope. Titer-matched lentiviral vectors were stereotactically injected into rat motor cortex ($n=3$ rats for each lentiviral vector) (Fig. 1B) and monkey motor cortex ($n=2$ monkeys) (Fig. 1C). The animals survived for more than 2 weeks in good health without apparent behavioral abnormalities. After sacrifice, brain tissues were processed for immunohistochemistry and sections containing successful injection sites were used for further analysis.

Distribution patterns of transduced cells and strength of promoter activities in motor cortices

To compare the cellular distribution patterns of GFP expressed from the various promoters and across different species, exposure times for each section were adjusted so that each image captured a similar level of fluorescence (Fig. 2). The distribution patterns of the transduced cortical cells were similar for ubiquitous and cell type-specific promoters both in rats and monkeys (Fig. 2). Fluorescence images in which exposure times were equalized between sections are also shown (Supplementary Fig. S1; supplementary data are available online at <http://www.liebertpub.com/hgtb>). Because the GFP fluorescence range was different between rats and monkeys, we obtained images at different exposure times (rat, 1/30 sec; monkey, 1/100 sec). In the rat sections, GFP fluorescence levels were similar between the seven promoters (Supplementary Fig. S1A). By contrast, large differences in GFP fluorescence were observed between the promoters in the monkey sections. Sections infected with the lentiviral vectors containing the CMV or RSV promoter exhibited stronger GFP fluorescence than did the other promoters (Supplementary Fig. S1B). These results suggest that the species differences affect promoter activity and that the CMV and RSV promoters are suitable for robust transgene expression in the monkey motor cortex.

Neuronal and glial cell specificities

To investigate neuronal/glial cell specificities between the promoters and species, we next measured the total number of GFP-positive neurons and astrocytes in the sections double-stained with anti-NeuN and anti-GFAP antibodies (Fig. 3). The average cell number per section and the percentages of neurons (GFP-positive/NeuN-positive cells) and

astrocytes (GFP-positive/GFAP-positive cells) to total transduced cells (GFP-positive cells) are shown in Table 2. All of the promoters, with the exception of the CMV promoter, drove highly specific expression in neurons (MSCV, $100\pm0.0\%$; CAG, $99.5\pm0.3\%$; EF-1 α , $99.9\pm0.1\%$; RSV, $99.5\pm0.3\%$; synapsin I, $100\pm0.0\%$; CaMKII α , $100\pm0.0\%$) (Table 2A). A modest number of GFP-expressing astrocytes, which were located mainly at the edge of transduced areas, was observed with administration of the CMV promoter in the rat motor cortex ($9.4\pm3.4\%$). The number of GFP-expressing astrocytes observed with administration of the CMV promoter was significantly greater than with other promoters in the rat motor cortex ($p<0.01$, one-way ANOVA followed by Tukey–Kramer multiple comparison test) (Fig. 3A and Table 2A). The numbers of transduced cells in different cortical layers were then analyzed (Table 2A). This analysis showed that the number of GFP-expressing astrocytes with administration of the CMV promoter was greater in deep layers. In the monkey sections, on the other hand, modest numbers of GFP-expressing astrocytes, located mainly at the edges of transduced areas, were observed with all of the ubiquitous promoters (MSCV, $13.4\pm5.3\%$; CMV, $7.4\pm2.2\%$; CAG, $14.7\pm2.2\%$; EF-1 α , $7.8\pm2.7\%$; RSV, $7.0\pm2.8\%$) (Fig. 3B and Table 2B). GFP-expressing astrocytes were evenly distributed across cortical layers (Table 2B). As in rats, the synapsin I and CaMKII α promoters drove highly specific expression in the neurons of the monkey motor cortex (synapsin I, $99.9\pm0.2\%$; CaMKII α , $99.9\pm0.2\%$). These results indicate that (1) there are species differences between rat and monkey in terms of the neuronal/glial cell specificities of the ubiquitous promoters, with the exception of the CMV promoter, suggesting that these species differences may be due to differences in the transduction specificity of VSV-G pseudotype and/or in the transcriptional mechanisms in neuronal/glial cells between rats and monkeys; (2) the CMV promoter functions in both neuronal and glial cells in both rats and monkeys; and (3) the synapsin I and CaMKII α promoters maintain their neuronal specificities in both rats and monkeys.

Excitatory and inhibitory neuron specificities

To investigate promoter specificities for excitatory and inhibitory neurons in rat and monkey, we measured the number of GFP-expressing excitatory/inhibitory neurons in sections double-stained with anti-GABA and anti-NeuN

TABLE 1. PROMOTERS USED IN THE PRESENT STUDY

Promoter	Length (kb)	Species	Specificity	Reference	Titer (TU/ml)
MSCV	1.5	Murine stem cell virus	Ubiquitous	Hawley <i>et al.</i> , 1994	5×10^9
CMV	0.6	Cytomegalovirus	Ubiquitous	Thomsen <i>et al.</i> , 1984	5×10^9
CAG	1.7	CMV early enhancer/ chicken β -actin	Ubiquitous	Niwa <i>et al.</i> , 1991	5×10^9
EF-1 α	1.2	Human	Ubiquitous	Kim <i>et al.</i> , 1990	5×10^9
RSV	0.4	Rous sarcoma virus	Ubiquitous	Yamamoto <i>et al.</i> , 1980	5×10^9
SynapsinI	1.1	Rat	Panneuronal	Hoesche <i>et al.</i> , 1993, Dittgen <i>et al.</i> , 2004	5×10^9
CaMKII α	1.3	Mouse	Excitatory neuron	Dittgen <i>et al.</i> , 2004	—

CAG, cytomegalovirus early enhancer element and chicken β -actin promoter; CaMKII α , α -calcium/calmodulin-dependent protein kinase II, CMV, cytomegalovirus; EF-1 α , elongation factor-1 α ; GFP, green fluorescent protein; MSCV, murine stem cell virus; RSV, Rous sarcoma virus; TU, transducing units.

FIG. 2. GFP expression in transduced cortical cells. Representative images of lentiviral vector-injected rat (**A**) and monkey (**B**) coronal sections through the motor cortex are shown. The promoter types of the lentiviral vectors are indicated at the top of each image. Images were obtained at various exposure times. Scale bars: 250 μ m. Color images available online at www.liebertpub.com/hgfb

antibodies (Fig. 4). Sections adjacent to those used for the neuron/glia analysis were used in this experiment. The average cell number per section and the percentages of excitatory/inhibitory neurons are shown in Table 3. We defined NeuN⁺/GABA⁻ cells as excitatory neurons and NeuN⁺/GABA⁺ cells as inhibitory neurons. The percentages of excitatory/inhibitory neurons were calculated by dividing the number of excitatory neurons (GFP-positive/NeuN-positive/GABA-negative) or the number of inhibitory neurons (GFP-positive/NeuN-positive/GABA-positive) by that of transduced neurons (GFP-positive/NeuN-positive). GFP-expressing inhibitory neurons were observed on administration of all promoters in the rat motor cortex (MSCV, $8.7 \pm 1.5\%$; CMV, $9.2 \pm 4.3\%$; CAG, $5.8 \pm 0.6\%$; EF-1 α , $5.4 \pm 1.0\%$; RSV, $4.6 \pm 1.6\%$; synapsin I, $5.4 \pm 1.4\%$; CaMKII α , $6.3 \pm 1.1\%$) (Table 3A). There were no significant differences in the percentages of GFP-expressing inhibitory neurons between the promoters ($p = 0.604$, one-way ANOVA). The percentages of GFP-expressing inhibitory neurons on administration of all promoters were lower compared with the percentages ($17.3 \pm 1.6\%$, $n = 6$ rats) of inhibitory neurons in noninjected areas of the rat motor cortex ($p < 0.05$, one-way ANOVA followed by Dunnett's test). The percentages of inhibitory neurons in noninjected areas of the rat motor cortex were calculated by dividing the number of NeuN-positive/GABA-positive cells by that of NeuN-positive cells. We also confirmed that the low percentages of GFP-expressing inhibitory neurons were not due to specific cell loss of inhibitory neurons in injected areas: the percentages of inhibitory neurons (GABA-positive cells) in injected areas were as follows: MSCV, $15.3 \pm 1.2\%$; CMV, $18.3 \pm 0.9\%$; CAG, $15.8 \pm 1.2\%$; EF-1 α , $17.5 \pm 1.6\%$; RSV, $16.1 \pm 0.6\%$; synapsin I, $17.5 \pm 2.8\%$; CaMKII α , $17.1 \pm 0.9\%$; and these values were not significantly different from that in noninjected areas ($p > 0.9$, one-way ANOVA followed by the Tukey-Kramer test) (Supplementary Fig. S2).

In the monkey motor cortex sections, by contrast, almost all GFP-expressing neurons were excitatory with administration of the synapsin I and CaMKII α promoters (synapsin I, $0.3 \pm 0.1\%$; CaMKII α , $0.0 \pm 0.0\%$), although GFP-expressing inhibitory neurons were present at a low percentage with administration of the ubiquitous promoters (MSCV, $5.8 \pm 2.6\%$; CMV, $13.4 \pm 2.0\%$; CAG, $3.7 \pm 1.3\%$; EF-1 α , $7.8 \pm 2.0\%$; RSV, $4.9 \pm 0.7\%$). The percentages of GFP-expressing inhibitory neurons with administration of all the promoters, except the CMV promoter, were lower compared with the percentages ($17.9 \pm 1.4\%$, $n = 2$ monkeys) of inhibitory neurons in noninjected areas of the monkey motor cortex ($p < 0.01$, one-way ANOVA followed by Dunnett's test). We also confirmed that the low percentages of GFP-expressing inhibitory neurons were not due to specific cell loss of inhibitory neurons in injected areas: the percentages of inhibitory neurons (GABA-positive cells) in injected areas were as follows: MSCV, $17.5 \pm 1.2\%$; CMV, $18.3 \pm 2.8\%$; CAG, $19.6 \pm 3.6\%$; EF-1 α , $19.7 \pm 0.5\%$; RSV, $19.3 \pm 2.3\%$; synapsin I, $19.0 \pm 3.4\%$; CaMKII α , $18.0 \pm 0.2\%$; and these values were not significantly different from that in noninjected areas ($p > 0.9$, one-way ANOVA followed by the Tukey-Kramer test) (Supplementary Fig. S2). Furthermore, the difference in excitatory neuronal specificity of the synapsin I and CaMKII α promoters between rats and monkeys was statistically significant (rat synapsin I vs. monkey synapsin I, $p < 0.05$; rat CaMKII α vs. monkey CaMKII α , $p < 0.0005$, t test). With the MSCV and CMV promoters in rats, the percentages of inhibitory neurons were higher in layer 5 than in the other layers (Table 3A). GFP-expressing inhibitory neurons in connection with ubiquitous promoters in monkeys were evenly distributed across all cortical layers (Table 3B and Fig. 4). These results suggest that (1) transgene expression from ubiquitous promoters is significantly biased toward excitatory neurons, which probably reflects the preferential tropisms of VSV-G-pseudotyped lentiviral vectors for excitatory

FIG. 3. Confocal microscopy of lentivirus-infected neurons and astrocytes in rat brain (**A**) and monkey brains (**B**). (**A**) Confocal images of rat brain sections infected with Lenti-MSCV-GFP (a–h) and Lenti-CMV-GFP (i–p). Magnified views of rectangular area in (d) and (l) are shown in (e–h) and (m–p), respectively. Arrows indicate GFP-expressing neurons. Arrowheads indicate GFP-expressing astrocytes. (**B**) Confocal images of monkey brain sections infected with Lenti-MSCV-GFP (a–h) and Lenti-CMV-GFP (i–p). Magnified views of rectangular area in (d) and (l) are shown in (e–h) and (m–p), respectively. Arrowheads indicate GFP-expressing astrocytes. Scale bars: (A and B, panels a–d and i–l) 50 μ m; (A and B, panels e–h and m–p) 10 μ m.

TABLE 2. NUMBER OF GREEN FLUORESCENT PROTEIN-POSITIVE NEURONS AND ASTROCYTES IN RAT AND MONKEY^a

A. Rat: Cell Counts								
Virus	Animal	Injection	Section	Total				
				NeuN ⁺	GFAP ⁺			
Lenti-MSCV-GFP	3	3	9	326.2±74.1 (100.0)	0.0±0.0 (0.0)			
Lenti-CMV-GFP	3	3	9	84.9±3.6 (90.6)	8.8±3.1 (9.4)			
Lenti-CAG-GFP	3	3	9	241.1±32.8 (99.5)	1.1±0.8 (0.5)			
Lenti-EF1 α -GFP	3	3	9	307.7±16.7 (99.9)	0.2±0.2 (0.1)			
Lenti-RSV-GFP	3	3	9	145.8±15.8 (99.5)	0.8±0.5 (0.5)			
Lenti-SynapsinI-GFP	3	3	9	272.2±46.5 (100.0)	0.0±0.0 (0.0)			
Lenti-CaMKII α -GFP	3	3	9	682.0±26.6 (100.0)	0.0±0.0 (0.0)			
	L2/3		L5		L6			
Virus	NeuN ⁺ GFAP ⁺		NeuN ⁺ GFAP ⁺		NeuN ⁺ GFAP ⁺			
Lenti-MSCV-GFP	17.1±5.3 (100.0)	0.0±0.0 (0.0)	137.1±10.1 (100.0)	0.0±0.0 (0.0)	172.0±47.8 (100.0)	0.0±0.0 (0.0)		
Lenti-CMV-GFP	44.2±9.9 (94.3)	2.7±0.8 (5.7)	36.3±8.1 (87.9)	5.0±2.5 (12.1)	4.3±2.9 (79.6)	1.1±1.1 (20.4)		
Lenti-CAG-GFP	49.4±9.1 (99.1)	0.4±0.3 (0.9)	147.3±24.8 (99.5)	0.7±0.7 (0.5)	44.3±13.5 (100.0)	0.0±0.0 (0.0)		
Lenti-EF1 α -GFP	67.6±17.5 (99.7)	0.2±0.2 (0.3)	173.2±19.1 (100.0)	0.0±0.0 (0.0)	66.9±19.2 (100.0)	0.0±0.0 (0.0)		
Lenti-RSV-GFP	23.9±4.6 (98.6)	0.3±0.2 (1.4)	114.4±16.7 (99.6)	0.4±0.4 (0.4)	7.4±3.9 (100.0)	0.0±0.0 (0.0)		
Lenti-SynapsinI-GFP	43.3±12.6 (100.0)	0.0±0.0 (0.0)	196.7±26.9 (100.0)	0.0±0.0 (0.0)	32.2±12.3 (100.0)	0.0±0.0 (0.0)		
Lenti-CaMKII α -GFP	158.9±21.9 (100.0)	0.0±0.0 (0.0)	327.7±34.1 (100.0)	0.0±0.0 (0.0)	195.4±33.4 (100.0)	0.0±0.0 (0.0)		
B. Monkey: Cell Counts								
Virus	Animal	Injection	Section	Total				
				NeuN ⁺	GFAP ⁺			
Lenti-MSCV-GFP	2	3	12	104.0±21.9 (86.6)	16.0±11.4 (13.4)			
Lenti-CMV-GFP	2	3	9	190.0±53.3 (92.6)	15.0±3.3 (7.4)			
Lenti-CAG-GFP	2	4	12	103.0±16.3 (85.3)	15.0±5.2 (14.7)			
Lenti-EF1 α -GFP	2	4	12	105.0±27.1 (92.2)	9.0±6.0 (7.8)			
Lenti-RSV-GFP	2	4	12	185.0±33.2 (93.0)	13.0±5.5 (7.0)			
Lenti-SynapsinI-GFP	1	3	9	212.0±43.6 (99.9)	0.0±0.2 (0.1)			
Lenti-CaMKII α -GFP	1	3	9	128.0±15.7 (99.9)	0.0±0.2 (0.1)			
	L2/3		L4		L5		L6	
Virus	NeuN ⁺ GFAP ⁺		NeuN ⁺ GFAP ⁺		NeuN ⁺ GFAP ⁺		NeuN ⁺ GFAP ⁺	
Lenti-MSCV-GFP	18.8±5.2 (80.4)	4.4±1.8 (19.6)	10.0±2.2 (87.5)	2.4±1.5 (12.5)	26.2±6.3 (91.2)	2.9±1.5 (8.8)	48.8±9.2 (91.2)	6.2±2.7 (8.8)
Lenti-CMV-GFP	49.1±11.3 (88.1)	5.3±1.2 (11.9)	22.6±4.6 (79.6)	2.6±1.2 (20.4)	83.6±11.5 (92.6)	5.8±1.3 (7.4)	34.3±16.9 (97.3)	1.6±0.8 (2.7)
Lenti-CAG-GFP	38.8±8.6 (74.8)	6.9±1.5 (25.2)	17.8±5.5 (89.4)	3.1±1.7 (10.6)	26.1±5.6 (91.2)	2.3±0.7 (8.8)	19.8±9.2 (85.9)	2.8±1.3 (14.1)
Lenti-EF1 α -GFP	41.3±9.3 (96.1)	2.0±0.8 (3.9)	16.6±4.6 (92.6)	1.9±0.8 (7.4)	40.5±8.4 (84.8)	3.5±1.9 (15.2)	6.4±2.9 (88.1)	1.3±0.9 (11.9)
Lenti-RSV-GFP	56.8±8.3 (92.8)	4.5±1.3 (7.2)	21.8±4.0 (93.2)	1.9±1.1 (6.8)	66.7±16.4 (91.8)	4.2±1.4 (8.2)	39.2±13.5 (96.4)	2.3±1.5 (3.6)
Lenti-SynapsinI-GFP	19.1±5.1 (100.0)	0.0±0.0 (0.0)	11.9±3.3 (100.0)	0.0±0.0 (0.0)	64.9±10.3 (100.0)	0.0±0.0 (0.0)	116.4±21.3 (99.8)	0.2±0.2 (0.2)
Lenti-CaMKII α -GFP	28.6±5.6 (99.5)	0.1±0.1 (0.5)	21.5±6.3 (100.0)	0.0±0.0 (0.0)	54.8±12.5 (100.0)	0.0±0.0 (0.0)	23.4±15.8 (100.0)	0.0±0.0 (0.0)

^aPercentages are given in parentheses. Data represent means±standard error.

neurons; and (2) there are species differences between rats and monkeys in terms of excitatory/inhibitory neuron specificities of the synapsin I and CaMKII α promoters. In particular, the CaMKII α promoter in rat motor cortex does not restrict expression only to excitatory neurons.

Discussion

In the present study, we have shown the first side-by-side comparative analysis of the properties of seven promoters (MSCV, CMV, CAG, EF-1 α , RSV, synapsin I, and CaMKII α) in the monkey motor cortex after their lentiviral vector-mediated gene transfer. This study is also the first to demonstrate that promoters can show different properties in rat and monkey motor cortices when they are introduced as part of lentiviral vectors. To compare promoter differences, factors that can potentially affect cell type specificity and gene expression (e.g., components in the viral genome, viral pseu-

dotyping, and viral titer) require careful adjustment. In this study, we used the same lentiviral backbone and pseudotyped all of the lentiviral vectors with a VSV-G envelope (Hanawa *et al.*, 2002). VSV-G-pseudotyped viral vectors bind nonselectively to membrane phospholipids on all mammalian cells (Burns *et al.*, 1993). However, several studies have reported that VSV-G-pseudotyped lentiviral vectors predominantly transduce neurons (Naldini *et al.*, 1996a; Blomer *et al.*, 1997; Kordower *et al.*, 1999; Deglon *et al.*, 2000). Our findings also suggest that VSV-G-pseudotyped lentiviral vectors have strong neuronal tropisms (Fig. 3 and Table 2). Likewise, viral titer may affect viral tropisms, and more importantly, gene expression levels after infection. Therefore, in this study, we adjusted the titers (5×10^9 TU/ml; Table 1) of the lentiviral vectors with the exception of the CaMKII α vector, which could not be measured precisely by functional titration assays because the activity of the CaMKII α promoter we employed was weak in HEK293T cells. The DNA/GFP

ratios were confined to the narrow range (DNA/GFP ratios, 1.4–2.0) for the six promoters (MSCV, CMV, CAG, EF-1 α , RSV, and synapsin I), but the DNA/GFP ratio for the CaMKII α promoter (DNA/GFP ratio, 17.3) was much higher than those for the six promoters (Supplementary Table S1), suggesting that a functional titration assay using HEK293T cells is not adequate for the titration of a lentiviral vector containing the CaMKII α promoter as an internal promoter. Therefore, for more precise adjustments with cell type-specific promoters such as the CaMKII α promoter, a DNA titration method that is not affected by promoter activity (Sastry *et al.*, 2002) will be used in future studies.

In terms of GFP fluorescence levels, there were no significant differences between the properties of the seven promoters in the rat motor cortex. On the other hand, the CMV and RSV promoters showed more robust GFP fluorescence than did the other promoters in the monkey motor cortex. This difference is possibly due to species differences between rat and monkey. Our findings suggest that the CMV and RSV promoters are suited for robust gene expression in the monkey motor cortex, but not always in the rat motor cortex. Moreover, the sizes of the CMV (0.6 kb) and RSV (0.4 kb) promoters are relatively short, which is advantageous because viral titers decrease significantly as the size of transfer vector increases (al Yacoub *et al.*, 2007). In addition, although the CMV promoter is inactivated by CpG methylation in several tissue and cell types (Prosch *et al.*, 1996; Brooks *et al.*, 2004; Krishnan *et al.*, 2006), the CMV promoter used in the present study was active for at least 2 weeks, which is consistent with another report (Blomer *et al.*, 1997). However, when gene expression in nontargeted cells is a concern, cell type-specific promoters such as the synapsin I, CaMKII α , and GFAP promoters (Kuroda *et al.*, 2008) are more appropriate.

The specificities of two cell type-specific promoters, synapsin I and CaMKII α , in rat and monkey motor cortices were also examined in this study. A previous report by Dittgen and colleagues (2004) showed that the CaMKII α (1.3 kb) promoter in a VSV-G-pseudotyped lentiviral vector drove specific expression in excitatory neurons in rodents, whereas the synapsin I (1.1 kb) promoter also caused strong expression in excitatory neurons with weaker expression in cortical interneurons. Consistent with previous reports (Dittgen *et al.*, 2004; Nathanson *et al.*, 2009), the synapsin I (1.1 kb) promoter shows preferential expression in excitatory

FIG. 4. Confocal microscopy of lentivirus-infected excitatory and inhibitory neurons in rat brain (**A**) and monkey brain (**B**). (**A**) Confocal images of rat brain sections infected with Lenti-Synapsin I-GFP (a–h) or Lenti-CaMKII α -GFP (i–p). Magnified views of rectangular area in (d) and (l) are shown in (e–h) and (m–p), respectively. Arrows indicate GFP-expressing excitatory neurons. Arrowheads indicate GFP-expressing inhibitory neurons. (**B**) Confocal images of monkey brain sections infected with Lenti-CMV-GFP (a–h), Lenti-CAG-GFP (i–p), Lenti-Synapsin I-GFP (q–x), or Lenti-CaMKII α -GFP (y–ff). Magnified views of the rectangular area in (d), (l), (t), and (bb) are shown in (e–h), (m–p), (u–x), and (cc–ff), respectively. Arrows indicate GFP-expressing excitatory neurons. Arrowheads indicate GFP-expressing inhibitory neurons. Scale bars: 50 μ m (**A**, panels a–d and i–l; **B**, panels a–d, i–l, q–t, y–bb) and 10 μ m (**A**, panels e–h, m–p; **B**, panels e–h, m–p, u–x, cc–ff).

TABLE 3. NUMBER OF GREEN FLUORESCENT PROTEIN-POSITIVE EXCITATORY NEURONS AND INHIBITORY NEURONS IN RAT AND MONKEY^a

A. Rat: Cell Counts								
Virus	Animal	Injection	Section	Total				
				Excitatory neuron	Inhibitory neuron			
Lenti-MSCV-GFP	3	3	9	233.3±68.5 (91.3)	22.0±6.7 (8.7)			
Lenti-CMV-GFP	3	3	9	73.4±3.7 (90.8)	8.0±4.4 (9.2)			
Lenti-CAG-GFP	3	3	9	181.8±45.1 (94.2)	11.8±3.6 (5.8)			
Lenti-EF1 α -GFP	3	3	9	273.8±8.4 (94.6)	15.8±3.6 (5.4)			
Lenti-RSV-GFP	3	3	9	99.1±9.6 (95.4)	4.7±1.6 (4.6)			
Lenti-SynapsinI-GFP	3	3	9	215.8±24.5 (94.6)	12.8±4.3 (5.4)			
Lenti-CaMKII α -GFP	3	3	9	505.3±66.8 (93.7)	33.1±4.6 (6.3)			
	L2/3		L5		L6			
Virus	Excitatory neuron	Inhibitory neuron	Excitatory neuron	Inhibitory neuron	Excitatory neuron	Inhibitory neuron		
Lenti-MSCV-GFP	29.9±15.1 (97.1)	0.9±0.5 (2.9)	63.4±17.1 (85.1)	11.1±3.2 (14.9)	140.0±39.9 (93.3)	10.0±2.7 (6.7)		
Lenti-CMV-GFP	34.4±7.9 (95.1)	1.8±0.4 (4.9)	33.2±6.1 (84.9)	5.9±3.2 (15.1)	5.8±3.7 (94.5)	0.3±0.2 (5.5)		
Lenti-CAG-GFP	45.3±12.4 (96.5)	1.7±0.7 (3.5)	106.7±22.6 (93.8)	7.1±1.1 (6.3)	29.8±10.2 (90.8)	3.0±1.3 (9.2)		
Lenti-EF1 α -GFP	59.6±9.0 (95.2)	3.0±0.9 (4.8)	161.9±8.5 (94.1)	10.2±1.4 (5.9)	52.3±10.2 (95.3)	2.6±0.7 (4.7)		
Lenti-RSV-GFP	26.3±4.5 (98.3)	0.4±0.2 (1.7)	70.4±6.0 (94.3)	4.2±1.0 (5.7)	2.3±1.6 (95.5)	0.1±0.1 (4.5)		
Lenti-SynapsinI-GFP	50.0±18.9 (94.5)	2.9±1.2 (5.5)	138.8±22.2 (94.0)	8.9±2.2 (6.0)	27.0±10.4 (96.4)	1.0±0.6 (3.6)		
Lenti-CaMKII α -GFP	125.1±20.4 (95.3)	6.2±2.3 (4.7)	202.8±18.9 (93.0)	15.3±2.1 (7.0)	177.4±34.5 (93.9)	11.6±3.5 (6.1)		
B. Monkey: Cell Counts								
Virus	Animal	Injection	Section	Total				
				Excitatory neuron	Inhibitory neuron			
Lenti-MSCV-GFP	2	4	12	128.8±36.2 (94.2)	8.0±3.3 (5.8)			
Lenti-CMV-GFP	2	3	9	162.3±39.2 (86.6)	31.3±11.7 (13.4)			
Lenti-CAG-GFP	2	4	12	149.0±27.6 (96.3)	5.3±1.5 (3.7)			
Lenti-EF1 α -GFP	2	4	12	126.7±32.6 (92.2)	8.8±5.5 (7.8)			
Lenti-RSV-GFP	2	4	12	313.4±41.4 (95.1)	19.3±3.0 (4.9)			
Lenti-SynapsinI-GFP	1	3	9	292.1±74.7 (99.7)	0.8±0.3 (0.3)			
Lenti-CaMKII α -GFP	1	3	9	135.0±29.7 (100.0)	0.0±0.0 (0.0)			
	L2/3		L4		L5		L6	
Virus	Excitatory neuron	Inhibitory neuron	Excitatory neuron	Inhibitory neuron	Excitatory neuron	Inhibitory neuron	Excitatory neuron	Inhibitory neuron
Lenti-MSCV-GFP	36.3±9.6 (91.7)	2.1±0.8 (8.3)	16.0±3.0 (91.9)	1.1±0.4 (8.1)	30.3±6.3 (91.5)	2.2±0.4 (8.5)	46.3±13.3 (96.0)	2.6±1.5 (4.0)
Lenti-CMV-GFP	44.3±18.2 (90.5)	7.8±3.1 (9.5)	25.9±5.7 (85.9)	4.8±1.3 (12.8)	73.8±16.2 (86.9)	11.1±3.1 (13.1)	18.3±12.9 (87.0)	1.6±1.0 (13.0)
Lenti-CAG-GFP	55.8±11.4 (92.3)	2.4±1.0 (7.7)	11.8±3.3 (97.5)	0.4±0.3 (2.5)	41.6±10.9 (97.3)	1.4±0.5 (2.1)	38.1±12.6 (97.4)	1.1±0.5 (2.6)
Lenti-EF1 α -GFP	55.8±14.1 (95.4)	4.4±2.1 (4.6)	17.3±3.3 (94.4)	1.3±0.5 (5.6)	46.2±12.3 (92.6)	2.9±0.9 (0.9)	7.5±4.1 (96.9)	0.2±0.2 (3.1)
Lenti-RSV-GFP	97.4±16.5 (93.6)	6.6±1.0 (6.4)	41.0±7.5 (93.1)	2.8±0.8 (6.9)	102.6±20.1 (93.9)	6.3±1.5 (1.5)	72.4±18.3 (97.0)	3.6±1.4 (3.0)
Lenti-SynapsinI-GFP	36.3±16.7 (98.6)	0.2±0.1 (1.4)	17.9±6.4 (100.0)	0.0±0.0 (0.0)	104.0±12.1 (99.5)	0.4±0.3 (0.3)	133.9±30.0 (99.9)	0.1±0.1 (0.1)
Lenti-CaMKII α -GFP	41.3±12.8 (100.0)	0.0±0.0 (0.0)	12.9±4.1 (100.0)	0.0±0.0 (0.0)	51.0±15.9 (100.0)	0.0±0.0 (0.0)	29.8±11.1 (100.0)	0.0±0.0 (0.0)

^aPercentages are given in parentheses. Data represent means±standard error.

neurons (94.4%), with weaker expression in inhibitory neurons (5.6%), in the rat motor cortex. The CaMKII α (1.3 kb) promoter also resulted in a expression pattern (excitatory, 93.9%; inhibitory, 6.1%) similar to that of the synapsin I (1.1 kb) promoter. Although the CaMKII α promoter result is inconsistent with the findings of Dittgen and colleagues (2004), mentioned previously, it is consistent with a study by Nathanson and colleagues (2009) that used the CaMKII α promoter (1.3 kb) in rAAV2/1. Similar to Nathanson and colleagues (2009), our results suggest that the 1.3-kb mouse CaMKII α promoter cannot drive fully restricted expression in excitatory neurons in rodents. However, the synapsin I and CaMKII α promoters we employed resulted in preferential expression in the excitatory neurons of monkey motor cortex (synapsin I, 99.7%; CaMKII α , 100.0%). This result is consistent with findings by Han and colleagues (2009), who used the CaMKII α (1.3 kb) promoter in a VSV-G-pseudotyped

lentiviral vector in the monkey cerebral cortex (Han *et al.*, 2009). These species differences may be due to differences in the amount of the VSV-G receptor (Burns *et al.*, 1993) or in the transcriptional mechanisms in excitatory and inhibitory neurons between rats and monkeys.

In summary, we have reported side-by-side comparative analysis of the properties of seven promoters (MSCV, CMV, CAG, EF-1 α , RSV, synapsin I, and CaMKII α) in rat and monkey motor cortices after their lentiviral vector-mediated gene transfer. These findings will be useful in basic and clinical neurosciences for the design of vectors for delivering transgenes into rat and monkey brains.

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Author Disclosure Statement

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