Original Research Paper

Running Title: **Somatostatin Gene Therapy in Epilepsy**

Adeno-associated viral vector-mediated preprosomatostatin expression suppresses
induced seizures in kindled rats

Gowri Natarajan^{a-f}, Jeffrey A. Leibowitz^b, Junli Zhou, Ph.D.^{a, c, d}, Yang Zhao^g, Jessica A.
McElroy^{a, c}, Michael A. King, Ph.D.^{f-h}, Brandi K. Ormerod, Ph.D.^{b, e, f}, Paul R. Carney,
M.D.^{a-f, **}

^aWilder Center of Excellence for Epilepsy Research, ^bJ. Crayton Pruitt Family
Department of Biomedical Engineering, Departments of ^cPediatrics and ^dNeurology,
^eDepartment of Neuroscience, ^fMcKnight Brain Institute, ^gDepartment of Pharmacology
and Therapeutics and ^hNF/SG VA Medical Center, University of Florida, Gainesville, FL
32611, U.S.A

****Corresponding Author:**

Paul R. Carney
Department of Neurology
University of North Carolina at Chapel Hill
170 Manning Drive, Campus Box 7025
Chapel Hill, North Carolina 27599
Phone: +1-919-966-4401
E-mail: paulcarney@unc.edu

ABSTRACT

Somatostatin is expressed widely in the hippocampus and notably in hilar GABAergic neurons that are vulnerable to seizure neuropathology in chronic temporal lobe epilepsy. We previously demonstrated that sustained bilateral preprosomatostatin (preproSST) expression in the hippocampus prevents the development of generalized seizures in the amygdala kindling model of temporal lobe epilepsy. Here we tested whether sustained preproSST expression is anticonvulsant in rats already kindled to high-grade seizures. Rats were kindled until they exhibited 3 consecutive Racine Grade 5 seizures before adeno-associated virus serotype 5 (AAV5) vector driving either eGFP (AAV5-CBa-eGFP) or preproSST and eGFP (AAV5-CBa-preproSST-eGFP) expression was injected bilaterally into the hippocampal dentate gyrus and CA1 region. Retested 3 weeks later, rats that received control vector (AAV5-CBa-eGFP) continued to exhibit high-grade seizures whereas 6/13 rats that received preproSST vector (AAV5-CBa-preproSST-eGFP) were seizure-free. Of these rats, 5/6 remained seizure-free after repeated stimulation sessions and when the stimulation current was increased. These results suggest that vector-mediated expression of preproSST may be a viable therapeutic strategy for temporal lobe epilepsy.

Key Words hippocampus, gene therapy, neuropeptides, somatostatin, kindling, adeno-associated viral vectors

1. INTRODUCTION

Over 20 million people continue to have seizures despite pharmacotherapy, leaving seizure-free rates unchanged for the last 15 years (Annegers, Hauser, & Elveback, 1979; Brodie, 2005; Cascino, 2008; Kwan et al., 2010; Kwan & Brodie, 2000; Kwan, Schachter, & Brodie, 2011). Developing novel strategies for these individuals is critical because drug resistant epilepsy is a potentially life-threatening condition accompanied by progressive cognitive impairment that compromises quality of life (Cramer, 1994; Kwan & Brodie, 2001, 2002). New surgical techniques, laser thermal ablation and responsive devices that detect seizures and automatically stimulate the brain to pre-empt them are exciting new options for these individuals, but they are in their infancy and have not yet substantially changed seizure-free rates (Cascino, 2008; Fisher et al., 2010; Jobst & Cascino, 2015; Morrell & Group, 2011).

Viral vector-mediated neuropeptide gene delivery may open a promising treatment avenue for temporal lobe epilepsy [TLE; (Riban, Fitzsimons, & During, 2009; Vezzani, 2004)] for individuals that are resistant to antiepileptic drug treatment or deemed not good candidates for resective epilepsy surgery. The temporal lobe structures involved in seizure genesis and propagation are permissive to neurotropic vector-mediated gene transfer, which has already demonstrated safety in clinical trials for a variety of human disorders [www.clinicaltrials.gov and (Freese et al., 1997; McCown, 2004, 2010; O'Connor et al., 1997; Riban et al., 2009; Vezzani, 2004, 2007; Weinberg & McCown, 2013; Weinberg, Samulski, & McCown, 2013)]. Some neuropeptides exhibit properties that could contribute to the effective treatment of seizures. For example, they may be neuroprotective and reduce excitability when

released during high-frequency neuronal activity(Baraban & Tallent, 2004; Hökfelt, 1991). The endogenous expression and synaptic release of some neuropeptides (Csaba et al., 2004; Schwarzer et al., 1996; Simonato et al., 1998; Sperk et al., 1992) and the expression of their receptor subtypes(Csaba et al., 2005; Csaba et al., 2004) is altered by seizure activity, suggesting a role for dysregulated neuropeptide signaling in seizure development and maintenance. Neuropeptides have been shown to suppress seizures in experimental epilepsy(Mazarati & Wasterlain, 2002; Zafar, King, & Carney, 2012). Sustained adeno-associated viral (AAV) vector-mediated expression of galanin, neuropeptide Y or preprosomatostatin (preproSST) has been shown to delay epileptogenesis or suppress seizures in several animal models of TLE (Haberman, Samulski, & McCown, 2003; Kanter-Schlifke et al., 2007; Lin et al., 2003; McCown, 2006; Noè et al., 2008; Richichi et al., 2004; Sørensen et al., 2009; Woldbye et al., 2010; Zafar et al., 2012).

Somatostatin (SST) is a particularly attractive treatment candidate for TLE(Brazeau et al., 1973; Epelbaum, 1986; Tallent & Qiu, 2008; Vezzani & Hoyer, 1999). The 116 amino acid prohormone preproSST is cleaved by proteases into biologically active SST-14, SST-28 and neuronostatin neuropeptides(Billova, Galanopoulou, Seidah, Qiu, & Kumar, 2007; Galanopoulou, Seidah, & Patel, 1995; Goodman, Aron, & Roos, 1983; Samson et al., 2008; Tavianini, Hayes, Magazin, Minth, & Dixon, 1984; Winsky-Sommerer et al., 2000). In the naïve hippocampus, SST is predominantly expressed in CA1 region, CA3 region and hilar GABAergic interneurons (Freund & Buzsáki, 1996) although one report describes SST immunoreactivity in hippocampal granule neurons and pyramidal neurons (Billova et al., 2007). SST levels

are responsive to neuronal activity and are altered across neurological diseases that include experimental and human TLE (Riekkinen & Pitkänen, 1990; Robbins et al., 1991). Specifically, SST expression and release is modulated by seizures (Csaba et al., 2004; Simonato et al., 1998; Tallent & Qiu, 2008; Vezzani & Hoyer, 1999). Moreover, a highly selective loss of SST containing hilar GABAergic neurons occurs in both animal models and humans with TLE (Buckmaster & Dudek, 1997; Robbins et al., 1991; Sloviter, 1987; Sun, Mtchedlishvili, Bertram, Erisir, & Kapur, 2007). This neuronal loss along with changes in the morphology and connectivity of surviving SST containing neurons has been postulated to mediate the chronic hyper-excitability associated with epileptogenesis (Peng et al., 2013; Zhang et al., 2009). SST knockout mice demonstrate increased severity of induced seizures (Buckmaster, Otero-Corchón, Rubinstein, & Low, 2002) and specific SST receptor agonists have been shown to effectively treat status epilepticus in experimental epilepsy (Aourz et al., 2011; Kozhemyakin et al., 2013). Together these data support the hypothesis that sustained hippocampal SST expression could ameliorate TLE. To this end, we previously demonstrated that sustained AAV5 vector-mediated hippocampal preproSST expression prevented the development of generalized or high-grade seizures in the majority of adult rats (Zafar et al., 2012).

The primary objective of the current study was to test whether sustained AAV5 vector-mediated hippocampal preproSST expression suppressed seizures when initiated *after* a stable seizure state was established in the rat amygdala kindling model (Goddard, McIntyre, & Leech, 1969; McNamara, Byrne, Dasheiff, & Fitz, 1980; Sato, Racine, & McIntyre, 1990). We hypothesized that sustained preproSST transgene

expression in the hippocampi of *kindled* rats may be anticonvulsant and therefore a promising TLE treatment strategy. To test this hypothesis, an AAV serotype 5 vector was employed to bilaterally express the preproSST gene in the dentate gyrus and CA1 region of amygdala kindled adult rats. Our results showed that sustained hippocampal preproSST expression significantly reduced seizures in this experimental TLE model and that the preproSST anticonvulsant effect persisted over time.

2. METHODS

2.1 Subjects

This study was conducted in accordance with Federal and University of Florida Institutional Animal Care and Use Committee policies regarding the ethical use of animals in research. Adult male Sprague Dawley rats (n=23; 250-275g upon arrival from Harlan) were housed in pairs in corncob-lined ventilated shoebox cages located in a standard colony room maintained at $24\pm 1^{\circ}\text{C}$ on a 12:12h light:dark cycle (lights on at 0600h). The rats were given Harlan Teklad Rodent Food Diet #7912 and reverse osmosis-filtered water *ad libitum* for the duration of the experiment.

Two weeks after arrival, rats were implanted with local electrical field potential recording and stimulating electrodes and allowed 10 days to recover from surgery. A baseline kindling session was employed to determine the after-discharge (AD) threshold current used for daily kindling sessions until rats reached the criterion of exhibiting 3 consecutive Grade 5 Racine seizures (Racine, 1972a, 1972b). The following week, rats were randomly assigned to groups that were transfected with either AAV5 vector driving preproSST and eGFP expression or control AAV5 vector driving eGFP expression.

Three weeks later rats were retested to determine the stability and endurance of vector effects.

2.2 Bipolar electrode and connector strip preparation

Connector strips were 3D printed at the University of Florida Infinity Fabrication laboratory (<http://fablab.arts.ufl.edu/>). Bipolar twisted stimulating and recording electrodes were custom made in-house by cutting quadruple Teflon-coated 316 stainless steel wires (Sigmund Cohn Corp.; Mount Vernon, NY) into 6cm lengths, removing the insulation at both ends and then soldering the male Amphenol gold pins (A-M systems; Sequim, WA) to both ends. Following this, the electrode wires with the Amphenol pins at either end were twisted. The loop generated at the end of the twisted wires was cut to produce an uninsulated tip that would make contact with the target tissue and deliver the administered current. For ground and reference screw electrodes, quadruple Teflon-coated 316 stainless steel wires (2.0cm and 2.5cm respectively), uninsulated at both ends were soldered to male Amphenol gold pins at one end. Stainless steel bone screws (FHC Inc.; Bowdoin, ME) were connected with a Unitek spot welder to the other end. Electrode wires were checked for continuity and impedance tested with an LCR/ESR meter (B&K Precision, Yorba Linda, CA). Only electrodes with impedance $<1.8\Omega$ were implanted.

2.3 Surgical implantation of electrodes

Surgical procedures were conducted as described previously (Zafar et al., 2012). Rats were sedated with xylazine (10mg/kg, subcutaneous) before anesthesia was induced with 4% isoflurane in 1L/min oxygen and maintained at 1.5% isoflurane in

0.5L/min oxygen. Anesthetized rats were placed in a Kopf stereotaxic frame and their shaven heads sterilized with alternating scrubs of 1% povidone-iodine solution and 70% ethanol. A midline incision exposed bregma and lambda. Two bipolar electrodes (330 μ m d) were implanted bilaterally in the amygdala [-2.2mm AP, $\pm$ 4.8mm ML, -8.3mm DV; (Paxinos & Watson, 2007)] to stimulate and record activity in the left and right hemispheres counterbalanced randomly across groups. Two small diameter plastic hex nuts containing removable screws were affixed to the skull over dentate gyrus (-3.8mm AP, $\pm$ 1.8mm ML) and CA1 region (-3.8mm AP, $\pm$ 1.8mm ML) target coordinates (Paxinos & Watson, 2007) to keep the skull free of dental cement for later vector delivery. Ground and reference metal screw electrodes were implanted rostral to bregma and caudal to lambda respectively. Electrodes were attached to male Amphenol pins for insertion into connector strips before the entire electrode assembly was secured with dental cement anchored by skull bone screws.

2.4 Electrical kindling

Kindling sessions were initiated 10 days after surgery. Synchronized behavioral and electroencephalographic (EEG) data for all rats were recorded across kindling sessions and after vector treatment. ADs were defined as spikes >1Hz with amplitudes at least twice the pre-stimulation baseline amplitude detected in the EEG (Zafar et al., 2012). Baseline EEG AD threshold currents were determined for each rat by identifying the minimum current intensity (starting at 50 μ A and then increased in 50 μ A steps delivered 1 minute apart) at which a standard 2s, 1ms pulse duration, 50Hz biphasic square wave pulse generated an AD (Racine, 1972a; Zafar et al., 2012).

Kindling sessions were initiated 24h later and conducted twice daily (>6h apart) using each rat's AD threshold current.

Seizures were graded according to the Racine scale, a method by which seizure severity is quantified in animal models of epilepsy (Racine, 1972b). Briefly, Grade 0 included no evidence of behavioral seizure; Grade 1 included staring, immobility, and facial movements; Grade 2 included head nodding and chewing; Grade 3 included unilateral forelimb clonus; Grade 4 included bilateral forelimb clonus with rearing and Grade 5 included bilateral forelimb clonus with rearing, loss of balance, and falling. Kindling sessions were terminated once the rats exhibited 3 consecutive Grade 5 seizures. Upon reaching this criterion, rats were assigned randomly to the AAV5-CBa-preproSST-eGFP group (n=13) or the AAV5-CBa-eGFP group (n=10). The synchronized behavioral and EEG data for all rats across kindling sessions and after vector treatment on test and post-test sessions were analyzed blinded to score: 1) latency (s) to Grade 4 seizure after the onset of electrical stimulation 2) AD duration (s), 3) time (s) spent in high-grade seizure and 4) seizure grade (Grade 0-5).

2.5 Plasmids and vector construct

CBa-preproSST-eGFP and CBa-eGFP plasmids were packaged into serotype 5 AAV capsids by the University of Florida vector core facility (gtc.ufl.edu/core/vector-core-lab.htm). Final dot blot titer values were 4.19×10^{13} vector genomes (vg)/ml for AAV5-CBa-preproSST-eGFP and 8.9×10^{12} vg/ml for AAV5-CBa-eGFP. The plasmid sequence consisted of the hybrid cytomegalovirus enhancer/chicken β -actin (CBa) promoter (Klein et al., 2002; Niwa, Yamamura, & Miyazaki, 1991) driving either preproSST transgene tagged with a downstream eGFP reporter (pAAV5-CBa-

preproSST-eGFP) expression or eGFP (pAAV5-CBa-eGFP) expression. The CBa-preproSST-eGFP plasmid contained an internal ribosomal entry site (IRES) sequence between the preproSST and the eGFP coding sequences that made eGFP expression an inert reporter of preproSST expression in this vector (Wong, Ngoi, & Lee, 2002). Fig. 1 shows the preproSST sequence and the AAV5-CBa-preproSST-eGFP vector construct.

2.6 AAV vector injections

The week after reaching fully kindled criterion, rats were infused bilaterally with AAV5-CBa-preproSST-eGFP vector (n=13) or AAV5-CBa-eGFP control vector (n=10) in the dentate gyrus (-3.8mm AP, $\pm$ 1.8mm ML, -3.4mm DV) and CA1 region [-3.8mm AP, $\pm$ 1.8mm ML, -2.4mm DV; (Paxinos & Watson, 2007)]. Anesthetized rats were mounted in the stereotaxic frame and the placeholder screws were removed from the hex nuts placed over dentate gyrus and CA1 region targets. Holes were drilled through the hex nuts to expose the surface of the brain. Vectors were injected using a 10 μ L Hamilton syringe fitted with a 27G steel needle (Hamilton, Reno, NV) attached to an infusion system (Cole-Parmer, Vernon Hills, IL). A 2 μ L volume of the vector was delivered in each of 4 injection sites (i.e. 2 μ L of vector was injected into the left and right CA1 region and dentate gyrus) through polyaryletherketone tubing (ID 0.381mm, OD 0.794mm, length 0.5m; Restek; Bellafonte, PA) by convection-enhanced delivery (Bankiewicz et al., 2000; Bobo et al., 1994) at a flow rate of 0.3 μ L/min. The injection needle was held in place for 3-5 minutes to allow for uniform vector distribution and to minimize backflow.

2.7 Post vector injection testing

After a 3-week interval to ensure that vector expression plateaued (Klein et al., 2002; Zafar et al., 2012), each rat was given a single test kindling session at the original

AD threshold current. Rats were subsequently kindled 2-3 times per week for up to 3 additional weeks during post-test sessions to determine the stability and persistence of vector effects. Rats that exhibited Grade 0 seizures on the test session were also tested at higher currents (+50 μ A, +50 μ A, +100 μ A above the AD threshold current) on the final 3 post-test sessions. As with kindling sessions, the synchronized behavioral and EEG data for all rats in test and post-test sessions administered after vector treatment were scored blind to compare latencies to Grade 4 seizure, AD durations, time spent in high-grade seizures, and seizure grade.

2.8 Perfusion and histology

Forty-eight hours after the final test stimulation, rats were deeply anesthetized with an intraperitoneal xylazine (10mg/kg)/ketamine (80mg/kg) cocktail injection and then perfused with ice cold isotonic saline followed by ice cold freshly prepared 4% paraformaldehyde in phosphate buffered saline (PBS). Brains were extracted and submerged overnight in perfusate and then cryoprotected in 30% sucrose PBS solution for ~3 days. The brains were sectioned coronally at 40 μ m through the rostral-caudal extent of the hippocampus on a freezing stage microtome (American Optical Corporation, Buffalo, N.Y., USA) and stored in cryoprotectant solution (25% glycerol, 30% ethylene glycol and 45% 0.1M PBS) at -20°C.

2.9 Electrode tract and gene expression analysis

Electrode and vector injection placements were confirmed by brightfield and epifluorescence microscopy by an experimenter who was blind to the treatment conditions and treatment outcomes. Placements were localized through the anterior-posterior extent of the dorsal hippocampus [-2.04mm to -5.4mm AP to bregma,

DV<5mm; (Paxinos & Watson, 2007)] and in underlying subcortical regions and overlying cortical regions using the GFP filter on an Olympus IX71 epifluorescence microscope.

2.10 SST immunohistochemistry

Sections from 3 representative rats in the AAV5-CBa-preproSST-eGFP group and the AAV5-CBa-eGFP group were immunolabeled with an SST specific antibody to confirm SST expression. The sections were rinsed repeatedly in tris-buffered saline (TBS) prior to immunolabeling and between each step. The sections were blocked in a solution of 3% normal donkey serum and 0.1% Triton-X in TBS and then incubated overnight at 4°C in rabbit polyclonal SST primary antibody [1:5000, Peninsula laboratories; (Peng et al., 2013)] dissolved in antibody diluent (1% normal donkey serum and 0.1% Triton-X in TBS). The following day, the sections were incubated in anti-rabbit secondary antibody conjugated to cy3 (1:500, Jackson laboratories) in antibody diluent for 4h at room temperature, incubated with DAPI (1:10,000; Chemicon) for 10 minutes and then mounted under PVA-DABCO (2.5% diazobicyclooctane in TBS with 10% polyvinyl alcohol and 20% glycerol). At least 50 immunolabeled neurons per hippocampal region were examined through their z-plane to confirm eGFP expression and/or SST immunoreactivity using a Zeiss meta LSM 710 fully spectral laser scanning confocal microscope with 405, 488, 543, and 633nm laser lines (Thornwood, N.Y., USA) under a 20x objective and 2.3x digital zoom. The percentages of neurons producing transgenic SST (eGFP⁺/SST⁺ neurons), endogenous SST (eGFP⁻/SST⁺ neurons) and transgenic and/or endogenous SST (eGFP⁺/SST⁺ neurons and eGFP⁻/SST⁺ neurons) were compared between AAV5-CBa-preproSST-eGFP-treated and AAV5-CBa-eGFP-

treated rats (n=3 per group) across hippocampal regions to confirm that the AAV5-CBa-preproSST-eGFP vector drove SST expression.

2.11 Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 24 software (www.onthehub.com/spss). T-tests (preproSST vs. eGFP treatment) and one-way ANOVAs (eGFP controls versus preproSST-treated responders versus preproSST-treated non-responders) were used to compare group differences for dependent variables (kindling sessions to criterion, latency to Grade 4 seizure, AD duration, number and time in high-grade (Grade 4/5) seizures and percentages of eGFP⁺ and/or SST⁺ neurons). Group differences in dependent variables yielding non-parametric data [seizure grade after vector treatment, AD threshold currents (μ A)] were compared using Mann-Whitney U tests (preproSST versus eGFP treatment) or Kruskal Wallance ANOVA tests (eGFP controls versus preproSST-treated responders versus preproSST-treated non-responders) followed by Mann-Whitney U tests. Significant effects revealed by the Kruskal Wallance ANOVA were explored with a Mann-Whitney U test. Effect sizes were calculated using Cliff's *d* (Cliff, 1993). Alpha levels were set at $p < 0.05$ and reported as two-tailed unless stated otherwise.

3. RESULTS

3.1 Kindling acquisition proceeded similarly in rats assigned to the preproSST or eGFP control group

Behavioral changes in all 23 rats progressed through the classic Racine scale (Racine, 1972b) from Grades 0-5 across kindling sessions. The average AD threshold current across rats was $243.5 \pm 16.4 \mu$ A. Rats exhibited their first Grade 5 seizures after

20.1±1.3 sessions (~10 days) and reached the fully kindled criteria of 3 consecutive Grade 5 seizures after 24.7±1.1 sessions (~12 days).

Rats assigned to the preproSST and eGFP vector groups required similar numbers of kindling sessions to reach criterion (eGFP=24.8±1.8 and preproSST=24.5±1.7; $t_{(21)}=0.11$; $p=0.92$) and exhibited similar AD threshold currents (eGFP=220±21.3µA and preproSST=261.5±23.4µA; $U=46.5$, $n_1=10$, $n_2=13$, $p=0.20$). They also exhibited similar numbers of high-grade (Grade 4/5) seizures during kindling acquisition (eGFP=6.8±1.1 and preproSST=6.5±0.9; $t_{(21)}=0.19$; $p=0.85$), latencies to Grade 4 seizure (eGFP=23.9±3.9s and preproSST=24.9±3.0s; $t_{(21)}=-0.20$; $p=0.22$), time spent in high-grade seizures (eGFP=47.4±3.5s and preproSST=50.7 ±6.0s; $t_{(21)}=0.45$; $p=0.66$) and AD durations (eGFP=69.0±3.9s and preproSST=74.4±5.7s; $t_{(21)}=-0.73$; $p=0.66$).

3.2 Sustained preproSST expression produced seizure resistance in a subset of rats

Fig.2a shows the individual and vector group seizure grade exhibited during the test session. The average seizure grade exhibited by preproSST-treated rats (2.5±0.7) was significantly lower than the average seizure grade exhibited by eGFP-treated rats (4.0±0.5; $U=39$, $n_1=13$, $n_2=10$, $p=0.048$, one-tailed). The effect size for this analysis ($d=-0.40$) approached Cliff's (Cliff, 1993) convention for an intermediate effect. Fig.2a also shows that of the eGFP-treated rats 10% exhibited Grade 1 seizures, 20% exhibited Grade 2 seizures and 70% exhibited Grade 4/5 seizures. For preproSST-treated rats, 46% remained seizure-free while 54% exhibited seizures higher than Grade 3 on the test session. On the basis of this response separation, preproSST-treated rats were divided into preproSST-treated responder and preproSST-treated non-responder

groups (Fig.2b). Seizure grade varied between the eGFP-treated, preproSST-treated responder and preproSST-treated non-responder groups ($H_{(2,20)}=14.2$; $p<0.001$), such that seizure grade was significantly lower in the preproSST-treated responder ($p<0.01$), but not in the preproSST-treated non-responder ($p=0.73$) group relative to the eGFP-treated control group (Fig.2b). Importantly, the effect size of the difference between eGFP-treated and preproSST-treated responder groups ($d\geq-0.99$) far exceeded Cliff's (Cliff, 1993) convention for a large effect.

EEG traces from representative rats (Fig.2c) illustrate kindling and vector effects. The top 2 traces were obtained from an eGFP-treated and preproSST-treated responder rat respectively, on the final kindling session prior to vector injection and show EEG ADs (the arrow shows AD onset) coincident with a Grade 5 behavioral seizure. The third and fourth traces were obtained from the same rats after vector injection. The AAV5-CBa-eGFP vector-treated control rat exhibited EEG ADs coincident with a Grade 5 behavioral seizure, whereas the AAV5-CBa-preproSST-eGFP vector-treated rat exhibited neither EEG ADs nor a behavioral seizure.

3.3 The effects of vector on kindled seizure susceptibility were stable and persistent

Vector-treated rats were given 2-3 post-test sessions per week for up to 3 weeks. Responders that exhibited Grade 0 seizures on the initial test session ($n=6/13$ AAV5-CBa-preproSST-eGFP-treated rats) underwent 5 subsequent post-test sessions using their AD threshold currents and then 3 more sessions during which the stimulus current was delivered at $50\mu\text{A}$, $50\mu\text{A}$ and then $100\mu\text{A}$ above their AD threshold current. On the first 5 post-test sessions, 3/6 rats remained completely seizure-free, 2 rats exhibited 1

or 2 high-grade seizures among multiple Grade 0 seizures and 1 rat repeatedly exhibited high-grade seizures. On the last 3 sessions when higher current intensities were employed, 2/3 rats that were seizure-free across post-test sessions remained seizure-free while 1 rat exhibited high-grade seizures at the highest stimulus currents. The 2 rats that exhibited high-grade seizures during 1 or 2 post-test sessions produced variable grade seizures at higher stimulus currents. The rat that produced high-grade seizures on all the post-test sessions was seizure-free (Table1).

eGFP-treated and preproSST-treated non-responders underwent 3-5 post-test sessions over a 2-3 week period using the AD threshold current for each rat. Since all preproSST-treated non-responder rats exhibited Grade 4 to 5 seizures during each of these sessions, current intensities were not increased during final sessions. Of the eGFP-treated rats, 9/10 exhibited seizures equal to or greater in severity across post-test sessions to those exhibited during the test session. The remaining eGFP-treated rat exhibited Grade 5 seizures on all but 2 post-test sessions during which he exhibited Grade 0 seizures.

3.4 eGFP expression was similar in preproSST-treated responders and non-responders

eGFP expression was found within the dentate gyrus (hilus, sub-granular zone and granule cell layer), the CA1 region (pyramidal cell layer, stratum oriens and stratum radiatum) and the CA3 region (predominantly in the pyramidal cell layer) and in several cases, the subiculum of the dorsal hippocampus of vector-treated rats (Fig.3). Table2 shows eGFP expression in each region of the hippocampus of preproSST-treated responder and non-responder rats. Note that 1 preproSST-treated non-responder rat

was excluded from this analysis because its brain was frozen prior to cryopreservation making the resolution of fine detail difficult to resolve. However, the rat was retained in other analyses because eGFP⁺ cells could be resolved grossly throughout hippocampal tissue.

In all rats, bilateral eGFP expression was detected in at least 1 major hippocampal region. In most rats, eGFP expression was detected through the anterior-posterior extent of 1 region on at least 1 side of the hippocampus. Moderate to strong eGFP expression was detected through the CA1 region of all rats and through the CA3 region of all rats except for 1 of the preproSST-treated responder rats. In these regions, eGFP expression could be localized or diffuse. eGFP expression was undetectable in the dentate gyrus of 1 preproSST-treated responder and 1 preproSST-treated non-responder rat but was detected in the dentate gyrus of all other rats. Intense localized eGFP expression was detected in the hilus and sub-granular zone of all rats except 1 preproSST-treated non-responder and 2 preproSST-treated responder rats and the granule cell layer was intensely transduced in a few rats. Cells in the subiculum were similarly transduced in responder and non-responder rats. Overall, eGFP expression patterns did not differ overtly between preproSST-treated responder and non-responder rats with respect to the laterality of expression or the hippocampal regions transduced. There were also no overt differences between groups with respect to the localization or morphologies of the cells transduced in each region.

Extrahippocampal eGFP expression was also compared across responder and non-responder rats. Only 3 preproSST-treated rats (1 responder and 2 non-responders) had eGFP expression in the thalamic region ventral to the hippocampus. Cortical eGFP

expression dorsal to the hippocampus was found in all but 2 rats and was consistent between preproSST-treated responders and non-responders.

3.5 Co-labeling for eGFP and SST was consistent with vector transduction in hippocampal principal neurons

Our IRES vector employs downstream eGFP expression to report preproSST transcription and presumably the generation of SST peptide in transduced neurons (Wong et al., 2002). Figs.4a and 4b show examples of eGFP⁺, eGFP⁺/SST⁺ and SST⁺ neurons found in the CA3 region of rats treated with the control (Fig.4a) or preproSST (Fig.4b) vector. Fig.4 also shows the percentages of eGFP⁺/SST⁺ neurons (Fig.4c), eGFP⁻/SST⁺ neurons (Fig.4d) and eGFP⁺/SST⁺ plus eGFP⁻/SST⁺ neurons (Fig.4e). Our initial assumption was that the population of eGFP⁺/SST⁺ neurons expressed transgenic SST and that the population of eGFP⁻/SST⁺ neurons naturally expressed SST. Few eGFP⁺ neurons were immunoreactive for SST in the granule or pyramidal cell layers of control vector-treated rats whereas most eGFP⁺ neurons were immunoreactive for SST in the CA3 region pyramidal cell layer and ~30% of eGFP⁺ neurons were immunoreactive for SST in the CA1 region pyramidal cell layer and dentate gyrus granule cell layers of preproSST vector-treated rats (Fig.4c). In both groups, a few eGFP⁻/SST⁺ neurons were found typically outside of the CA1 and CA3 region pyramidal cell layers and in the hilus of the dentate gyrus. The majority of hilar neurons were eGFP⁺ making the distinction between neurons expressing transgenic and natural SST more difficult and reinforcing that at least among hilar eGFP⁺/SST⁺ neurons, eGFP⁺ neurons were immunoreactive for transgenic and/or endogenous SST. In control rats, ~30% of hilar neurons were eGFP⁺/SST⁺ and this percentage surprisingly doubled in

the preproSST vector-treated rats (Fig.4c).

SST was co-labeled in a greater percentage of eGFP⁺ CA3 region ($t_{(4)}=59.78$; $p<0.0001$) and CA1 region ($t_{(4)}=5.60$; $p<0.01$) pyramidal cell layer neurons, granule cell layer neurons ($t_{(4)}=4.75$; $p<0.01$) and hilar neurons ($t_{(4)}=4.50$; $p<0.05$) in preproSST vector-treated rats versus control vector-treated rats (Fig.4c). The percentage of eGFP⁺/SST⁺ neurons increased in the granule cell layer ($t_{(4)}=8.92$; $p<0.001$) and hilus ($t_{(4)}=3.33$; $p<0.05$) but not the CA3 region ($t_{(4)}=1.23$; $p=n.s.$) or CA1 region ($t_{(4)}=2.37$; $p=n.s.$) pyramidal cell layers of preproSST vector-treated rats versus control vector-treated rats (Fig.4d). The percentage of neurons immunoreactive for transduced and/or endogenous SST increased in the CA3 region pyramidal cell layer ($t_{(4)}=19.63$; $p<0.0001$), CA1 region pyramidal cell layer ($t_{(4)}=5.55$; $p<0.01$), granule cell layer ($t_{(4)}=8.63$; $p<0.001$) and hilus ($t_{(4)}=16.84$; $p<0.0001$) of preproSST vector-treated versus control vector-treated rats (Fig.4e).

3.6 PreproSST-treated responders and non-responders exhibited similar behavioral responses and EEG characteristics during kindling acquisition

Retrospective analysis of kindling properties revealed that the eGFP, preproSST-treated responder and preproSST-treated non-responder groups required equivalent numbers of kindling sessions to reach criterion ($F_{(2,20)}=0.80$; $p=0.46$; Fig.5a). All groups also exhibited similar AD threshold currents ($H_{(2,20)}=2.771$; $p=0.25$; Fig.5b), AD durations ($F_{(2,20)}=1.57$; $p=0.232$; Fig.5c), latencies to Grade 4 seizure ($F_{(2,20)}=0.07$; $p=0.93$; Fig.5d) and time spent in high-grade seizures ($F_{(2,20)}=1.124$; $p=0.35$; Fig.5e). While preproSST-treated responders appeared to produce fewer high-grade seizures

than controls or preproSST-treated non-responders, the omnibus ANOVA demonstrated that the effect was not statistically significant ($F_{(2,20)}=2.17$; $p=0.14$; Fig.5f).

4. DISCUSSION

The results of this study showed that vector-mediated ectopic preproSST expression prevented seizures in a significant fraction of kindled rats. The large therapeutic effect size encourages development of SST gene therapy for TLE. Control rats continued to exhibit high-grade evoked seizures despite displaying equivalent kindling acquisition characteristics to preproSST-treated rats. The presence or absence of an effect of the preproSST vector was consistent across repeated kindling sessions. SST was co-labeled in eGFP⁺ neurons in the pyramidal cell layer of the CA1 and CA3 regions and in eGFP⁺ neurons in the granule cell layer. Co-labeling was significantly elevated in these regions following treatment with the preproSST vector relative to treatment with control vector. eGFP expression patterns, behavioral responses and EEG characteristics during kindling acquisition could not explain differences between preproSST-treated responder and non-responder groups.

The relevance of the kindling model to human TLE has been debated but there is agreement that the model approximates early clinical seizure susceptibility with limited neuropathology and has contributed significantly to our understanding of how limbic networks change in TLE and to the development of anti-epileptic drugs (Bertram, 2007; Löscher, 2002b; McNamara et al., 1980; Sato et al., 1990). Repeated kindling stimulations produce robust evoked seizures that are reliable across time and multiple tests (Brandt, Ebert, & Löscher, 2004; Kalynchuk, 2000; Pinel & Rovner, 1978). We add to this paradigm by showing that rats exhibited robust evoked seizures when challenged

even once after a month-long break from kindling sessions, supporting that changes underlying seizure susceptibility are relatively permanent (Goddard et al., 1969). In fact, spontaneous seizure refractoriness was observed in 1 kindled rat, consistent with previous reports (Freeman & Jarvis, 1981; Löscher & Köhling, 2010; Mucha & Pinel, 1977; Stripling & Russell, 1989). Our observation that neither the AAV5 vector nor eGFP expression affected seizure behavior supports the viability of developing gene therapy approaches for epilepsy treatment in TLE models.

The most exciting outcome of our study was that a significant fraction of preproSST-treated rats were seizure-free when challenged during the test session and even across post-test sessions. The clinical relevance of complete seizure freedom in this subset of rats was showcased by the very large effect size produced by the difference in seizure grade between responder (Grade 0) and eGFP-treated controls (~Grade 5) on the test session and even by the encouraging intermediate effect size produced by the difference in seizure grade between preproSST-treated responder and non-responder groups pooled and eGFP-treated controls. These findings expand our previous work showing that sustained preproSST expression prevented the acquisition of a fully kindled state (Zafar et al., 2012) and encourage the pre-clinical development of sustained preproSST expression as an effective non-destructive therapeutic alternative to surgery in antiepileptic drug-resistant TLE. Of course, testing the efficacy of this approach in chronic TLE models (Bertram & Cornett, 1994; Löscher, 2002b; Morimoto, Fahnestock, & Racine, 2004) will be important next steps for advancing the strategy to clinic.

Identifying the side effects of sustained ectopic AAV vector-mediated SST expression is also an important step in translating this strategy to clinic. Indeed, safer alternatives to antiepileptic drugs that can aggravate TLE-associated cognitive decline are desirable (Carreño, Donaire, & Sánchez-Carpintero, 2008; Kwan & Brodie, 2001). We are currently testing the effects of sustained preproSST expression on spatial cognition and neuroinflammation in kindled and control rats. Expanding this work across behavioral tasks sensitive to other cognitive domains will be an important pre-clinical step. Notably, overt effects of sustained preproSST expression on measures of general health (i.e. body mass, porphyrin production and grooming) were not observed in the current study. Although sustained SST expression could lead to the disinhibition of inhibitory interneurons (Pfeffer, 2014), changes in seizure frequency or ADs indicative of hippocampal network disinhibition were not detected. We previously confirmed that sustained AAV vector-mediated preproSST expression did not produce gross neurodegeneration using Fluoro-Jade C (unpublished data), but experiments quantifying cell stress and death markers in this context would validate whether cell viability is compromised. Our data add to the growing picture that AAV vector-mediated gene delivery and sustained neuropeptide expression is relatively safe, although future work should document how variability in injection sites, transduction efficiency, vector titers and survival times impact safety measures.

Our analysis of eGFP expression patterns indicated that preproSST expression in any single hippocampal region could mediate a strong therapeutic response. For example, eGFP expression in 1 responder rat was largely restricted to the hippocampal CA1 region (Table2). The effect of ectopically expressed SST was likely mediated

through 1 or more of the SST receptor subtypes that are expressed throughout the hippocampus (Viollet et al., 2008). Although endogenous SST is largely expressed by a subpopulation of inhibitory neurons (Freund & Buzsáki, 1996), SST modulates excitability across hippocampal regions. For example, the application of SST to CA1 and CA3 regions suppresses epileptiform activity presynaptically by inhibiting glutamate release and postsynaptically by modulating voltage sensitive potassium channels (Boehm & Betz, 1997; Kozhemyakin et al., 2013; Moore, Madamba, Joëls, & Siggins, 1988; Qiu et al., 2008; Tallent & Siggins, 1997, 1999). In addition, SST can modulate lateral perforant path long-term potentiation (Baratta, Lamp, & Tallent, 2002). Testing how the specific transduction of cell subtypes and subregions impacts hippocampal excitability and the therapeutic efficacy of sustained SST expression, as well as identifying the SST subtypes that mediate these effects, could provide insight about how to improve responder rates.

While the all-or-none response of preproSST vector-treated rats is puzzling, non-responder subpopulations are not atypical for antiepileptic therapies (Löscher, 2002a; Löscher, Rundfeldt, & Hönack, 1993). We did find that responders exhibited fewer high-grade seizures than eGFP-treated controls and non-responders during kindling sessions prior to treatment (Fig.5), but this difference was insufficiently powered to achieve statistical significance. Interestingly, individuals with epilepsy who exhibit fewer seizures prior to the onset of antiepileptic drug treatment are more likely to respond to the treatment (Brodie, 2005; Kwan & Brodie, 2000; Sillanpää, 1993). Consistent with this idea, response rates were higher in our previous study that treated rats with AAV-

preproSST *before* kindling sessions (Zafar et al., 2012) than in the current study that treated rats *after* they exhibited 3 consecutive Grade 5 seizures.

A number of factors could have varied between the responder and non-responder groups. It is possible that responders and non-responders exhibited overall differences in levels of mature SST or neuronostatin. Mass spectrometric analyses in progress will compare inter-rat and regional transgene peptide content differences, although qualitative comparison could not discriminate explanatory variations. Apart from potential differences in transcription, translation, and derivation of transgene peptides, the expression of catabolic proteases cleaving SST or even neuronostatin in cells transduced to express preproSST (Billova et al., 2007; Brakch, Galanopoulou, Patel, Boileau, & Seidah, 1995) could have varied across subregions within the hippocampus. Natural variation in SSTR expression changes related to kindling or gene transfer could also be linked to responder rates. Kindled seizures transiently upregulate SST peptide immunoreactivity, which is followed by the transient downregulation of SSTR₂ immunoreactivity in the rat hippocampus (Csaba et al., 2004; Tallent & Qiu, 2008; Vezzani & Hoyer, 1999). However, rats in the current study were perfused weeks after transient kindling-induced changes in SST and SSTR₂ would be observable. Importantly, the onset of our treatment may have been initiated after the critical period for modulating this or other transient mechanisms in some rats. Consistent with this idea, response rates were higher in our previous study that treated rats with AAV-preproSST *before* kindling sessions

There may also be time points after which reversing more permanent changes hypothesized to underlie seizure development and maintenance becomes more difficult.

For example, early SST treatment could prevent the seizure-induced neuronal loss and sprouting of SST-containing neurons in TLE models (Buckmaster & Dudek, 1997; Peng et al., 2013; Sloviter, 1987; Sun et al., 2007; Zhang et al., 2009). Kindling drastically increases neural progenitor cell division that leads to the accumulation of young hyperplastic neurons with aberrant morphologies and connectivity that are hypothesized to contribute to seizure maintenance (Botterill, Brymer, Caruncho, & Kalynchuk, 2015; Parent, Jessberger, Gage, & Gong, 2007). SST could prevent the accumulation of aberrant neurons through anti-proliferative effects on neural progenitor cells (Lamberts, Krenning, & Reubi, 1991). The ability of SST to potentially reverse the chronic inflammatory response that accompanies TLE (Basivireddy et al., 2013; Vezzani, French, Bartfai, & Baram, 2011) is likely less time sensitive. The anticonvulsant properties of preproSST could be byproducts of its modulatory effects on seizure-associated cell death, aberrant neurogenesis or neuroinflammation but the efficacy of SST treatment on these factors could be time sensitive.

Here we show the first preclinical evidence that sustained preproSST expression initiated *after* stable seizures are evoked in experimental TLE is anticonvulsant in a subset of rats. The opportunity to achieve complete seizure-free state with superior responder rates in epilepsy gene therapy intervention also supports development and optimization of better gene delivery strategies like AAV capsid shuffling (Gray et al., 2010), the use of capsid mutated (Petr-Silva et al., 2009; Zhong et al., 2008; Zhong et al., 2007) and self-complementary AAV vectors (McCarty et al., 2003; McCarty, Monahan, & Samulski, 2001) that can possibly enhance transgene expression and provide superior transduction (Srivastava, 2016). Future studies might also test whether

the use of promoters to drive expression selectively in specific cell types might improve responder rates (Nathanson et al., 2009). Nevertheless, our findings demonstrated that sustained preproSST expression treated induced seizures in kindled rats and advance a novel gene therapy intervention using this neuropeptide. Testing therapeutic efficacy in chronic TLE models that exhibit spontaneous recurrent seizures will be an important step forward for translational advancement.

ACKNOWLEDGEMENTS

We thank the Vector Core Lab, Powell Gene Therapy Center at the University of Florida for packaging the AAV5-CBa-preproSST-eGFP and AAV5-CBa-eGFP vectors. We thank Dr. David Stanley and Immanuel B.H. Samuel for help with exporting the EEG data and Dr. Francisco Delgado for technical support and insightful discussions. This research was supported by the Department of Defense Congressionally Directed Medical Research Programs (CDMRP) Grant Number PR121769, the Wilder Center of Excellence for Epilepsy Research, the Children's Miracle Network and NIH Grant #R03 AGO49411. This work was also supported by resources provided by the North Florida/South Georgia Veterans Health System, Gainesville, FL. The contents do not represent the views of the U.S Department of veterans Affairs or the United States Government.

Figure legends

Fig.1 Representation of the a) preproSST schematic b) preproSST amino acid

sequence and c) AAV5-CBa-preproSST-eGFP vector construct a, b) The 116 amino acid preproSST contains an N-terminal region of 24 amino acids. This signaling sequence directs the translocation of the translated preproSST product into the endoplasmic reticulum following which it is cleaved off at positions 24-25 (glycine-alanine) of the preproSST molecule (Goodman et al., 1983). The remaining 92 amino acid prohormone proSST undergoes alternative cleavage by specific proteases (Billova et al., 2007; Brakch et al., 1995; Galanopoulou et al., 1995; Goodman et al., 1983; Winsky-Sommerer et al., 2000) to yield the different mature peptide products SST-14, SST-28 (the N-terminal extension of SST-14) and the more recently discovered neuronostatin (Samson et al., 2008). c) The hybrid CMV early enhancer/CBa promoter was used to drive expression of the preproSST transgene tagged with a downstream eGFP reporter. The CBa-preproSST-eGFP plasmid contained an IRES sequence between the preproSST and the eGFP coding sequences that made eGFP expression an inert reporter of preproSST expression in this vector (Wong et al., 2002). This plasmid construct was packaged into serotype 5 AAV capsids. The final dot blot titer value was 4.19×10^{13} vg/ml. *Adapted with permission from (Phoenix Pharmaceuticals, 2016).*

Fig.2 Sustained preproSST expression prevented induced seizures in a subset of kindled rats AAV5-CBa-preproSST-eGFP-treated rats (n=13) and AAV5-CBa-eGFP-treated rats (n=10) were given a stimulation session 3 weeks after vector injection at the AD threshold current to test whether sustained preproSST expression could prevent seizure behavior. **a)** The average seizure grade produced by the preproSST-treated rats was significantly lower than the average seizure grade produced by eGFP-treated rats ($*p<0.05$, one-tailed). The scatterplot analysis of individual seizure grades revealed that a subset of preproSST-treated rats (preproSST-treated responders, n=6/13, 100% Grade 0 in the 6 rats) was completely seizure-free relative to eGFP controls (n=10, 0% Grade 0, 10% Grade 1, 20% Grade 2-3, 10% Grade 4, 60% Grade 5). The seizure grade remained largely unchanged in the remaining preproSST-treated rats (preproSST-treated non-responders, n=7/13, 14.3% Grade 3, 14.3% Grade 4, 71.4% Grade 5 in the 7 rats). **b)** The seizure grade varied between the eGFP-treated, preproSST-treated responder and preproSST-treated non-responder groups such that the average seizure grade was significantly lower in the preproSST-treated responder ($**p<0.01$) but not in the preproSST-treated non-responder ($p=0.73$) group relative to the eGFP-treated control group. **c)** Severe ADs characteristic of Grade 5 seizures were recorded from the amygdala, of a representative rat in the AAV5-CBa-eGFP-treated group and also an eventual preproSST-treated responder rat from the AAV5-CBa-preproSST-eGFP-treated group. Three weeks after vector expression, during the test session, ADs were absent in the preproSST-treated responder rat relative to the eGFP-treated rat that produced severe ADs. Arrows indicate the onset of ADs. *All data are represented as mean $\pm$ SEM.*

Fig.3 AAV5-CBa-preproSST-eGFP transduction within the hippocampus of a rat in the preproSST vector-treated group Robust eGFP expression was observed bilaterally within the dentate hilus, granule cell layer, CA3 region and CA1 region of the hippocampus. In this rat a relatively small number of thalamic neurons ventral to the hippocampus also showed eGFP expression.

Fig.4 Coincident eGFP and SST localization was consistent with vector

transduction of hippocampal principal neurons a, b) Examples of native eGFP⁺

neurons (in green), eGFP⁺/SST⁺ (both green and red) and SST⁺ (in red) neurons found in the CA3 hippocampal region of rats treated with the control **(a)** or preproSST **(b)**

vector. Few eGFP⁺ neurons in the pyramidal cell layer were immunoreactive for SST in eGFP vector-treated rats whereas most of eGFP⁺ neurons were immunoreactive for SST in the preproSST-treated rats. Scale bar represents 50µm. Arrows indicate co-

labeled neurons. **c)** The percentage of eGFP⁺/SST⁺ neurons increased in the CA3 region (**** $p<0.0001$) and CA1 region (** $p<0.01$) pyramidal cell layers, granule cell layer (GCL, ** $p<0.01$) and hilus (* $p<0.05$) of preproSST vector-treated versus control vector-

treated rats. **d)** The percentage of eGFP⁺/SST⁺ neurons increased in the granule cell layer (GCL, *** $p<0.001$) and hilus (* $p<0.05$) but not the CA3 region and CA1 region pyramidal cell layers of preproSST vector-treated versus control vector-treated rats. **e)**

The percentage of neurons immunoreactive for transduced and/or endogenous SST increased in the CA3 region pyramidal cell layer (**** $p<0.0001$) and CA1 region pyramidal cell layer (** $p<0.01$), the granule cell layer (GCL, *** $p<0.001$) and hilus (**** $p<0.0001$) of the preproSST vector-treated versus control vector-treated rats.

Fig.5 PreproSST-treated responders and non-responders exhibited similar behavioral responses and EEG characteristics during kindling acquisition An omnibus ANOVA did not reveal significant differences between preproSST-treated responders, preproSST-treated non-responders and eGFP-treated rats in **a)** the number of sessions to criterion **b)** the AD threshold current **c)** AD duration **d)** latency to Grade 4 seizure and **e)** time spent in high-grade seizure. Although preproSST-treated responders specifically appeared to produce fewer high-grade (Grade 4/5) seizures during kindling acquisition relative to preproSST-treated non-responders, this was not overall statistically significant between preproSST-treated responders, preproSST-treated non-responders and eGFP-treated rats (**f**).

REFERENCES

- Annegers, J. F., Hauser, W. A., & Elveback, L. R. (1979). Remission of seizures and relapse in patients with epilepsy. *Epilepsia*, 20(6), 729-737.
- Aourz, N., De Bundel, D., Stragier, B., Clinckers, R., Portelli, J., Michotte, Y., et al. (2011). Rat hippocampal somatostatin sst3 and sst4 receptors mediate anticonvulsive effects in vivo: indications of functional interactions with sst2 receptors. *Neuropharmacology*, 61(8), 1327-1333.
- Bankiewicz, K. S., Eberling, J. L., Kohutnicka, M., Jagust, W., Pivrotto, P., Bringas, J., et al. (2000). Convection-enhanced delivery of AAV vector in parkinsonian monkeys; in vivo detection of gene expression and restoration of dopaminergic function using pro-drug approach. *Exp Neurol*, 164(1), 2-14.
- Baraban, S. C., & Tallent, M. K. (2004). Interneuron Diversity series: Interneuronal neuropeptides--endogenous regulators of neuronal excitability. *Trends Neurosci*, 27(3), 135-142.
- Baratta, M. V., Lamp, T., & Tallent, M. K. (2002). Somatostatin depresses long-term potentiation and Ca²⁺ signaling in mouse dentate gyrus. *J Neurophysiol*, 88(6), 3078-3086.
- Basivireddy, J., Somvanshi, R. K., Romero, I. A., Weksler, B. B., Couraud, P. O., Oger, J., et al. (2013). Somatostatin preserved blood brain barrier against cytokine induced alterations: possible role in multiple sclerosis. *Biochem Pharmacol*, 86(4), 497-507.
- Bertram, E. H. (2007). The relevance of kindling for human epilepsy. *Epilepsia*, 48 Suppl 2, 65-74.

Bertram, E. H., & Cornett, J. F. (1994). The evolution of a rat model of chronic spontaneous limbic seizures. *Brain Res*, 661(1-2), 157-162.

Billova, S., Galanopoulou, A. S., Seidah, N. G., Qiu, X., & Kumar, U. (2007). Immunohistochemical expression and colocalization of somatostatin, carboxypeptidase-E and prohormone convertases 1 and 2 in rat brain. *Neuroscience*, 147(2), 403-418.

Bobo, R. H., Laske, D. W., Akbasak, A., Morrison, P. F., Dedrick, R. L., & Oldfield, E. H. (1994). Convection-enhanced delivery of macromolecules in the brain. *Proc Natl Acad Sci U S A*, 91(6), 2076-2080.

Boehm, S., & Betz, H. (1997). Somatostatin inhibits excitatory transmission at rat hippocampal synapses via presynaptic receptors. *J Neurosci*, 17(11), 4066-4075.

Botterill, J. J., Brymer, K. J., Caruncho, H. J., & Kalynchuk, L. E. (2015). Aberrant hippocampal neurogenesis after limbic kindling: Relationship to BDNF and hippocampal-dependent memory. *Epilepsy Behav*, 47, 83-92.

Brakch, N., Galanopoulou, A. S., Patel, Y. C., Boileau, G., & Seidah, N. G. (1995). Comparative proteolytic processing of rat prosomatostatin by the convertases PC1, PC2, furin, PACE4 and PC5 in constitutive and regulated secretory pathways. *FEBS Lett*, 362(2), 143-146.

Brandt, C., Ebert, U., & Löscher, W. (2004). Epilepsy induced by extended amygdala-kindling in rats: lack of clear association between development of spontaneous seizures and neuronal damage. *Epilepsy Res*, 62(2-3), 135-156.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., et al. (1973). Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science*, 179(4068), 77-79.

Brodie, M. J. (2005). Diagnosing and predicting refractory epilepsy. *Acta Neurol Scand Suppl*, 181, 36-39.

Buckmaster, P. S., & Dudek, F. E. (1997). Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol*, 385(3), 385-404.

Buckmaster, P. S., Otero-Corchón, V., Rubinstein, M., & Low, M. J. (2002). Heightened seizure severity in somatostatin knockout mice. *Epilepsy Res*, 48(1-2), 43-56.

Carreño, M., Donaire, A., & Sánchez-Carpintero, R. (2008). Cognitive disorders associated with epilepsy: diagnosis and treatment. *Neurologist*, 14(6 Suppl 1), S26-34.

Cascino, G. D. (2008). When drugs and surgery don't work. *Epilepsia*, 49 Suppl 9, 79-84.

Cliff, N. (1993). Dominance Statistics: Ordinal Analyses to Answer Ordinal Questions (pp. 494-509).

Cramer, J. A. (1994). Quality of life for people with epilepsy. *Neurol Clin*, 12(1), 1-13.

Csaba, Z., Pirker, S., Lelouvier, B., Simon, A., Videau, C., Epelbaum, J., et al. (2005). Somatostatin receptor type 2 undergoes plastic changes in the human epileptic dentate gyrus. *J Neuropathol Exp Neurol*, 64(11), 956-969.

Csaba, Z., Richichi, C., Bernard, V., Epelbaum, J., Vezzani, A., & Dournaud, P. (2004). Plasticity of somatostatin and somatostatin sst2A receptors in the rat dentate gyrus during kindling epileptogenesis. *Eur J Neurosci*, 19(9), 2531-2538.

Epelbaum, J. (1986). Somatostatin in the central nervous system: physiology and pathological modifications. *Prog Neurobiol*, 27(1), 63-100.

Fisher, R., Salanova, V., Witt, T., Worth, R., Henry, T., Gross, R., et al. (2010). Electrical stimulation of the anterior nucleus of thalamus for treatment of refractory epilepsy. *Epilepsia*, 51(5), 899-908.

Freeman, F. G., & Jarvis, M. F. (1981). The effect of interstimulation interval on the assessment and stability of kindled seizure thresholds. *Brain Res Bull*, 7(6), 629-633.

Freese, A., Kaplitt, M. G., O'Connor, W. M., Abbey, M., Langer, D., Leone, P., et al. (1997). Direct gene transfer into human epileptogenic hippocampal tissue with an adeno-associated virus vector: implications for a gene therapy approach to epilepsy. *Epilepsia*, 38(7), 759-766.

Freund, T. F., & Buzsáki, G. (1996). Interneurons of the hippocampus. *Hippocampus*, 6(4), 347-470.

Galanopoulou, A. S., Seidah, N. G., & Patel, Y. C. (1995). Heterologous processing of rat prosomatostatin to somatostatin-14 by PC2: requirement for secretory cell but not the secretion granule. *Biochem J*, 311 (Pt 1), 111-118.

Goddard, G. V., McIntyre, D. C., & Leech, C. K. (1969). A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol*, 25(3), 295-330.

Goodman, R. H., Aron, D. C., & Roos, B. A. (1983). Rat pre-prosomatostatin. Structure and processing by microsomal membranes. *J Biol Chem*, 258(9), 5570-5573.

Gray, S. J., Blake, B. L., Criswell, H. E., Nicolson, S. C., Samulski, R. J., McCown, T. J., et al. (2010). Directed evolution of a novel adeno-associated virus (AAV) vector that crosses the seizure-compromised blood-brain barrier (BBB). *Mol Ther*, 18(3), 570-578.

Haberman, R. P., Samulski, R. J., & McCown, T. J. (2003). Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. *Nat Med*, 9(8), 1076-1080.

Hökfelt, T. (1991). Neuropeptides in perspective: the last ten years. *Neuron*, 7(6), 867-879.

Jobst, B. C., & Cascino, G. D. (2015). Resective epilepsy surgery for drug-resistant focal epilepsy: a review. *JAMA*, 313(3), 285-293.

Kalynchuk, L. E. (2000). Long-term amygdala kindling in rats as a model for the study of interictal emotionality in temporal lobe epilepsy. *Neurosci Biobehav Rev*, 24(7), 691-704.

Kanter-Schlifke, I., Toft Sørensen, A., Ledri, M., Kuteeva, E., Hökfelt, T., & Kokaia, M. (2007). Galanin gene transfer curtails generalized seizures in kindled rats without altering hippocampal synaptic plasticity. *Neuroscience*, 150(4), 984-992.

Klein, R. L., Hamby, M. E., Gong, Y., Hirko, A. C., Wang, S., Hughes, J. A., et al. (2002). Dose and promoter effects of adeno-associated viral vector for green fluorescent protein expression in the rat brain. *Exp Neurol*, 176(1), 66-74.

Kozhemyakin, M., Rajasekaran, K., Todorovic, M. S., Kowalski, S. L., Balint, C., & Kapur, J. (2013). Somatostatin type-2 receptor activation inhibits glutamate release and prevents status epilepticus. *Neurobiol Dis*, 54, 94-104.

Kwan, P., Arzimanoglou, A., Berg, A. T., Brodie, M. J., Allen Hauser, W., Mathern, G., et al. (2010). Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia*, 51(6), 1069-1077.

Kwan, P., & Brodie, M. J. (2000). Early identification of refractory epilepsy. *N Engl J Med*, 342(5), 314-319.

Kwan, P., & Brodie, M. J. (2001). Neuropsychological effects of epilepsy and antiepileptic drugs. *Lancet*, 357(9251), 216-222.

Kwan, P., & Brodie, M. J. (2002). Refractory epilepsy: a progressive, intractable but preventable condition? *Seizure*, 11(2), 77-84.

Kwan, P., Schachter, S. C., & Brodie, M. J. (2011). Drug-resistant epilepsy. *N Engl J Med*, 365(10), 919-926.

Lamberts, S. W., Krenning, E. P., & Reubi, J. C. (1991). The role of somatostatin and its analogs in the diagnosis and treatment of tumors. *Endocr Rev*, 12(4), 450-482.

Lin, E. J., Richichi, C., Young, D., Baer, K., Vezzani, A., & During, M. J. (2003). Recombinant AAV-mediated expression of galanin in rat hippocampus suppresses seizure development. *Eur J Neurosci*, 18(7), 2087-2092.

Löscher, W. (2002a). Animal models of drug-resistant epilepsy. *Novartis Found Symp*, 243, 149-159; discussion 159-166, 180-145.

Löscher, W. (2002b). Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res*, 50(1-2), 105-123.

Löscher, W., & Köhling, R. (2010). Functional, metabolic, and synaptic changes after seizures as potential targets for antiepileptic therapy. *Epilepsy Behav*, 19(2), 105-113.

- Löscher, W., Rundfeldt, C., & Hönack, D. (1993). Pharmacological characterization of phenytoin-resistant amygdala-kindled rats, a new model of drug-resistant partial epilepsy. *Epilepsy Res*, 15(3), 207-219.
- Mazarati, A., & Wasterlain, C. G. (2002). Anticonvulsant effects of four neuropeptides in the rat hippocampus during self-sustaining status epilepticus. *Neurosci Lett*, 331(2), 123-127.
- McCarty, D. M., Fu, H., Monahan, P. E., Toulson, C. E., Naik, P., & Samulski, R. J. (2003). Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo. *Gene Ther*, 10(26), 2112-2118.
- McCarty, D. M., Monahan, P. E., & Samulski, R. J. (2001). Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. *Gene Ther*, 8(16), 1248-1254.
- McCown, T. J. (2004). The clinical potential of antiepileptic gene therapy. *Expert Opin Biol Ther*, 4(11), 1771-1776.
- McCown, T. J. (2006). Adeno-associated virus-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity in vivo. *Mol Ther*, 14(1), 63-68.
- McCown, T. J. (2010). The future of epilepsy treatment: focus on adeno-associated virus vector gene therapy. *Drug News Perspect*, 23(5), 281-286.
- McNamara, J. O., Byrne, M. C., Dasheiff, R. M., & Fitz, J. G. (1980). The kindling model of epilepsy: a review. *Prog Neurobiol*, 15(2), 139-159.
- Moore, S. D., Madamba, S. G., Joëls, M., & Siggins, G. R. (1988). Somatostatin augments the M-current in hippocampal neurons. *Science*, 239(4837), 278-280.

- Morimoto, K., Fahnestock, M., & Racine, R. J. (2004). Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol*, 73(1), 1-60.
- Morrell, M. J., & Group, R. S. i. E. S. (2011). Responsive cortical stimulation for the treatment of medically intractable partial epilepsy. *Neurology*, 77(13), 1295-1304.
- Mucha, R. F., & Pinel, P. J. (1977). Postseizure inhibition of kindled seizures. *Exp Neurol*, 54(2), 266-282.
- Nathanson, J. L., Jappelli, R., Scheeff, E. D., Manning, G., Obata, K., Brenner, S., et al. (2009). Short Promoters in Viral Vectors Drive Selective Expression in Mammalian Inhibitory Neurons, but do not Restrict Activity to Specific Inhibitory Cell-Types. *Front Neural Circuits*, 3, 19.
- Niwa, H., Yamamura, K., & Miyazaki, J. (1991). Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene*, 108(2), 193-199.
- Noè, F., Pool, A. H., Nissinen, J., Gobbi, M., Bland, R., Rizzi, M., et al. (2008). Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. *Brain*, 131(Pt 6), 1506-1515.
- O'Connor, W. M., Davidson, B. L., Kaplitt, M. G., Abbey, M. V., During, M. J., Leone, P., et al. (1997). Adenovirus vector-mediated gene transfer into human epileptogenic brain slices: prospects for gene therapy in epilepsy. *Exp Neurol*, 148(1), 167-178.
- Parent, J. M., Jessberger, S., Gage, F. H., & Gong, C. (2007). Is neurogenesis reparative after status epilepticus? *Epilepsia*, 48 Suppl 8, 69-71.
- Paxinos, G., & Watson, C. (2007). *The Rat Brain In Stereotaxic Coordinates*: Elsevier.

Peng, Z., Zhang, N., Wei, W., Huang, C. S., Cetina, Y., Otis, T. S., et al. (2013). A reorganized GABAergic circuit in a model of epilepsy: evidence from optogenetic labeling and stimulation of somatostatin interneurons. *J Neurosci*, 33(36), 14392-14405.

Petrs-Silva, H., Dinculescu, A., Li, Q., Min, S. H., Chiodo, V., Pang, J. J., et al. (2009). High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. *Mol Ther*, 17(3), 463-471.

Pfeffer, C. K. (2014). Inhibitory neurons: vip cells hit the brake on inhibition. *Curr Biol*, 24(1), R18-20.

Phoenix Pharmaceuticals, I. (2016). *Rat Somatostatin Preprohormone Schematic*, from <http://www.phoenixpeptide.com>

Pinel, J. P., & Rovner, L. I. (1978). Experimental epileptogenesis: kindling-induced epilepsy in rats. *Exp Neurol*, 58(2), 190-202.

Qiu, C., Zeyda, T., Johnson, B., Hochgeschwender, U., de Lecea, L., & Tallent, M. K. (2008). Somatostatin receptor subtype 4 couples to the M-current to regulate seizures. *J Neurosci*, 28(14), 3567-3576.

Racine, R. J. (1972a). Modification of seizure activity by electrical stimulation. I. After-discharge threshold. *Electroencephalogr Clin Neurophysiol*, 32(3), 269-279.

Racine, R. J. (1972b). Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*, 32(3), 281-294.

Riban, V., Fitzsimons, H. L., & During, M. J. (2009). Gene therapy in epilepsy. *Epilepsia*, 50(1), 24-32.

Richichi, C., Lin, E. J., Stefanin, D., Colella, D., Ravizza, T., Grignaschi, G., et al. (2004). Anticonvulsant and antiepileptogenic effects mediated by adeno-associated

virus vector neuropeptide Y expression in the rat hippocampus. *J Neurosci*, 24(12), 3051-3059.

Riekkinen, P. J., & Pitkänen, A. (1990). Somatostatin and epilepsy. *Metabolism*, 39(9 Suppl 2), 112-115.

Robbins, R. J., Brines, M. L., Kim, J. H., Adrian, T., de Lanerolle, N., Welsh, S., et al. (1991). A selective loss of somatostatin in the hippocampus of patients with temporal lobe epilepsy. *Ann Neurol*, 29(3), 325-332.

Samson, W. K., Zhang, J. V., Avsian-Kretchmer, O., Cui, K., Yosten, G. L., Klein, C., et al. (2008). Neuronostatin encoded by the somatostatin gene regulates neuronal, cardiovascular, and metabolic functions. *J Biol Chem*, 283(46), 31949-31959.

Sato, M., Racine, R. J., & McIntyre, D. C. (1990). Kindling: basic mechanisms and clinical validity. *Electroencephalogr Clin Neurophysiol*, 76(5), 459-472.

Schwarzer, C., Sperk, G., Samanin, R., Rizzi, M., Gariboldi, M., & Vezzani, A. (1996). Neuropeptides-immunoreactivity and their mRNA expression in kindling: functional implications for limbic epileptogenesis. *Brain Res Brain Res Rev*, 22(1), 27-50.

Sillanpää, M. (1993). Remission of seizures and predictors of intractability in long-term follow-up. *Epilepsia*, 34(5), 930-936.

Simonato, M., Bregola, G., Beani, L., Vezzani, A., Sala, R., Raiteri, M., et al. (1998). Time- and region-specific variations in somatostatin release following amygdala kindling in the rat. *J Neurochem*, 70(1), 252-259.

Sloviter, R. S. (1987). Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science*, 235(4784), 73-76.

- Sperk, G., Marksteiner, J., Gruber, B., Bellmann, R., Mahata, M., & Ortler, M. (1992). Functional changes in neuropeptide Y- and somatostatin-containing neurons induced by limbic seizures in the rat. *Neuroscience*, 50(4), 831-846.
- Srivastava, A. (2016). Adeno-Associated Virus: The Naturally Occurring Virus Versus the Recombinant Vector. *Hum Gene Ther*, 27(1), 1-6.
- Stripling, J. S., & Russell, R. D. (1989). Twenty-four-hour post-seizure inhibition during limbic kindling requires seizure generalization. *Neurosci Lett*, 99(1-2), 208-213.
- Sun, C., Mtchedlishvili, Z., Bertram, E. H., Erisir, A., & Kapur, J. (2007). Selective loss of dentate hilar interneurons contributes to reduced synaptic inhibition of granule cells in an electrical stimulation-based animal model of temporal lobe epilepsy. *J Comp Neurol*, 500(5), 876-893.
- Sørensen, A. T., Nikitidou, L., Ledri, M., Lin, E. J., During, M. J., Kanter-Schlifke, I., et al. (2009). Hippocampal NPY gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP. *Exp Neurol*, 215(2), 328-333.
- Tallent, M. K., & Qiu, C. (2008). Somatostatin: an endogenous antiepileptic. *Mol Cell Endocrinol*, 286(1-2), 96-103.
- Tallent, M. K., & Siggins, G. R. (1997). Somatostatin depresses excitatory but not inhibitory neurotransmission in rat CA1 hippocampus. *J Neurophysiol*, 78(6), 3008-3018.
- Tallent, M. K., & Siggins, G. R. (1999). Somatostatin acts in CA1 and CA3 to reduce hippocampal epileptiform activity. *J Neurophysiol*, 81(4), 1626-1635.

- Tavianini, M. A., Hayes, T. E., Magazin, M. D., Minth, C. D., & Dixon, J. E. (1984). Isolation, characterization, and DNA sequence of the rat somatostatin gene. *J Biol Chem*, 259(19), 11798-11803.
- Vezzani, A. (2004). Gene therapy in epilepsy. *Epilepsy Curr*, 4(3), 87-90.
- Vezzani, A. (2007). The promise of gene therapy for the treatment of epilepsy. *Expert Rev Neurother*, 7(12), 1685-1692.
- Vezzani, A., French, J., Bartfai, T., & Baram, T. Z. (2011). The role of inflammation in epilepsy. *Nat Rev Neurol*, 7(1), 31-40.
- Vezzani, A., & Hoyer, D. (1999). Brain somatostatin: a candidate inhibitory role in seizures and epileptogenesis. *Eur J Neurosci*, 11(11), 3767-3776.
- Viollet, C., Lepousez, G., Loudes, C., Videau, C., Simon, A., & Epelbaum, J. (2008). Somatostatinergic systems in brain: networks and functions. *Mol Cell Endocrinol*, 286(1-2), 75-87.
- Weinberg, M. S., & McCown, T. J. (2013). Current prospects and challenges for epilepsy gene therapy. *Exp Neurol*, 244, 27-35.
- Weinberg, M. S., Samulski, R. J., & McCown, T. J. (2013). Adeno-associated virus (AAV) gene therapy for neurological disease. *Neuropharmacology*, 69, 82-88.
- Winsky-Sommerer, R., Benjannet, S., Rovère, C., Barbero, P., Seidah, N. G., Epelbaum, J., et al. (2000). Regional and cellular localization of the neuroendocrine prohormone convertases PC1 and PC2 in the rat central nervous system. *J Comp Neurol*, 424(3), 439-460.
- Woldbye, D. P., Angehagen, M., Gøtzsche, C. R., Elbrønd-Bek, H., Sørensen, A. T., Christiansen, S. H., et al. (2010). Adeno-associated viral vector-induced overexpression

of neuropeptide Y Y2 receptors in the hippocampus suppresses seizures. *Brain*, 133(9), 2778-2788.

Wong, E. T., Ngoi, S. M., & Lee, C. G. (2002). Improved co-expression of multiple genes in vectors containing internal ribosome entry sites (IRESes) from human genes. *Gene Ther*, 9(5), 337-344.

Zafar, R., King, M. A., & Carney, P. R. (2012). Adeno associated viral vector-mediated expression of somatostatin in rat hippocampus suppresses seizure development. *Neurosci Lett*, 509(2), 87-91.

Zhang, W., Yamawaki, R., Wen, X., Uhl, J., Diaz, J., Prince, D. A., et al. (2009). Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy. *J Neurosci*, 29(45), 14247-14256.

Zhong, L., Li, B., Mah, C. S., Govindasamy, L., Agbandje-McKenna, M., Cooper, M., et al. (2008). Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses. *Proc Natl Acad Sci U S A*, 105(22), 7827-7832.

Zhong, L., Zhao, W., Wu, J., Li, B., Zolotukhin, S., Govindasamy, L., et al. (2007). A dual role of EGFR protein tyrosine kinase signaling in ubiquitination of AAV2 capsids and viral second-strand DNA synthesis. *Mol Ther*, 15(7), 1323-1330.

Table.1 Seizure grade exhibited by preproSST-treated responder rats on post-test sessions (PTS)

	PTS-1	PTS-2	PTS-3	PTS-4	PTS-5	PTS-6 (+50 μ A)	PTS-7 (+50 μ A)	PTS-8 (+100 μ A)
Rat 1	0	0	0	0	0	0	0	0
Rat 2	0	0	0	0	0	0	4	4
Rat 3	0	0	5	0	0	5	0*	2
Rat 4	0	0	0	5	5	0	2	5
Rat 5	0	0	0	0	0	0	0	0
Rat 6	5	5	5	5	5	0	0	0

*The current employed for this rat during this session was the AD threshold current. Post-test sessions during which the stimulus current was increased above the AD threshold current are shown in grey.

Table.2 eGFP expression in hippocampal and extrahippocampal regions

Rat #	CA1		CA3		GCL		Hilus		Subiculum		Cortex	Thalamus
	Side1	Side2	Side1	Side2	Side1	Side2	Side1	Side2	Side1	Side2		
SST Non-Responders												
Rat 1	++	++	++	-	-	-	-	-	++	-	N	N
Rat 2	++	++	++	+++	-	+	-	+++	+	+	Y	N
Rat 3	++	++	++	++	-	+	-	++	-	-	Y	Y
Rat 4	++	+	+++	+++	++	++	+++	+++	+	-	Y	Y
Rat 5	++	++	+++	++	+++	+++	+++	+++	+	+	Y	N
Rat 6	++	++	+++	+	+	+	+++	+++	+	-	Y	N
SST Responders												
Rat 1	++	++	+++	++	+	-	+++	-	-	+	Y	N
Rat 2	++	+++	++	++	-	++	++	+++	+	-	Y	Y
Rat 3	+++	+++	-	-	-	-	-	-	++	++	Y	N
Rat 4	++	++	+++	++	++	+	+++	+++	++	-	Y	N
Rat 5	++	++	++	+	+	+	+++	++	+	+	N	N
Rat 6	+++	+++	++	++	-	+++	-	-	++	+	Y	N

The presence or absence of eGFP expression is denoted by '+' and '-' symbols respectively. The number of '+' symbols represents the qualitative robustness of eGFP expression in each region, where '+' represents a few scattered eGFP⁺ cells, '++' denotes a moderate number of eGFP⁺ cells and "+++" denotes that most cells were eGFP⁺. Scattered cells were detected in the cortices and thalamic region of some rats. 'N' denotes no eGFP⁺ cells detected and 'Y' denotes eGFP⁺ cells detected. Note: One preproSST-treated non-responder rat was excluded from the analysis because its tissue was damaged during histology.

Fig.1

a. Rat Somatostatin Prehormone Schematic

b. Rat Somatostatin Prehormone Sequence

c. AAV5-CBa-PreproSST-eGFP Schematic

Fig.2

Fig.3

Fig.4

Fig.5