ADENO-ASSOCIATED VIRAL VECTOR DELIVERED SOMATOSTATIN AS A CANDIDATE FOR GENE THERAPY FOR TEMPORAL LOBE EPILEPSY

By

RABIA ZAFAR

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2010

To Papa, Mama, Saira and Sadia

ACKNOWLEDGMENTS

The journey that has led me to this point in my life has not been easy, and there have been a multitude of people who I owe sincere gratitude to. First and foremost, I want to thank my parents, Drs. Zafar Iqbal and Nasim Zafar for their unconditional love and unwavering support. I'm sure the many years of separation were tougher on them than they were on me. I would also like to thank my sisters Saira and Sadia, who have always been one phone call away when I needed them, and who have always been role models.

Many thanks to all the collaborators who have helped me shape my project and learn the many techniques that I have become proficient in over the last 5 years. Special thanks to my mentor Dr. Paul Carney, who gave me the opportunity to do science the way it should be done – with the freedom to ask questions that interested me and the resources to find answers. My gratitude also goes to Dr. William Ogle, Dr. Jason Frazier and Dr. Michael King for serving on my committee. Special thanks go to Dr. King for the many hours he has spent listening to my idle banter and giving me direction. I would also like to thank Dr. Kevin Foust, Dr. Arun Srivastava and Dr. Jayandharan G. Rao for their help and guidance with my first attempt at cloning. I am also thankful to Dr. Alex Cadotte for his help with statistical analysis.

I am eternally grateful to all the wonderful friends I have made through graduate school, especially Ekta Patel who has lent me her shoulder many a times. My deepest gratitude also goes to my lab mates, who at the end of these five years have become very close friends, and the best group of people I could ever hope to work with. Thank you Mansi for always being by my side, literally and figuratively. Without your ears and thoughtful advice, I would be so lost in graduate school. Thank you Stephen for being

my partner in crime and knowing what I didn’t know, so between the two of us we knew everything. Thank you Svetlana for being the radiant person you are, and bringing so much humor into my life. Most importantly, thank you Frank for always being such a source of comfort and support. My experience in graduate school would definitely not have been this smooth had it not been for the unfaltering advice of Dr. Sue Semple-Rowland, who took it upon herself to see us all succeed. I also thank BJ Streetman, who always brought order to my chaos.

TABLE OF CONTENTS

page

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
ACSF	Artificial cerebral spinal fluid
AD	Afterdischarge
AED	Anti-epileptic drug
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
APV	(2R)-amino-5-phosphonovaleric acid
CNS	Central nervous system
DG	Dentate gyrus
DNQX	6,7-dinitroquinoxaline-2,3-dione
EEG	Electroencephalography
GFP	Green fluorescent protein
IP	Intraperitoneal
ITR	Inverted terminal repeat
LTLE	Lateral temporal lobe epilepsy
LTP	Long-term potentiation
MTLE	Mesial temporal lobe epilepsy
NMDA	N-methyl-D-aspartic acid
NPY	Neuropeptide Y
PTX	Picrotoxin
sEPSC	Spontaneous excitatory post-synaptic current
sIPSC	Spontaneous inhibitory post-synaptic current
SQ	Subcutaneous
SST	Somatostatin
TLE	Temporal lobe epilepsy

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

ADENO-ASSOCIATED VIRAL VECTOR DELIVERED SOMATOSTATIN AS A CANDIDATE FOR GENE THERAPY FOR TEMPORAL LOBE EPILEPSY

By

Rabia Zafar

December 2010

Chair: Paul Richard Carney
Major: Medical Sciences – Neuroscience

Epilepsy is a debilitating neurological disorder that affects millions worldwide. Even with severe surgical interventions like hippocampal resection and administration of several antiepileptic drugs, a third of the population affected continues to have seizures. Not only are such therapies costly, they also lend patients susceptible to serious side effects and cognitive impairments. Gene delivered neuropeptides provide a much less invasive alternative to resective surgery as well as a solution to drug resistance. Somatostatin (SST) is an endogenous neuropeptide, and has many functions in the central nervous system. A growing body of evidence now supports the general conclusion that the presence of SST inhibits or reduces seizure severity and its absence makes seizures worse.

Gene therapy therefore provides a promising alternative to treating intractable epilepsy. We packaged the preprosomatostatin gene into serotype 5 of the adeno-associated viral (AAV) vector and delivered it directly into the rat dentate gyrus and CA1 sub-regions of the hippocampus. The efficacy of this gene therapy was assessed in an electrically kindled animal model of limbic epilepsy by monitoring behavioral responses

as well as EEG dynamics. All animals over-expressing SST had a higher threshold to generalized seizures, and seventy percent did not have a single grade 5 (generalized) seizure after 30 electrical stimulations. We also tested learning and memory impairments, which are common co-morbidities associated with epilepsy, using the Morris water maze. Finally, electrophysiological techniques were used to try and unravel the physiological basis for observed behavioral changes between control and gene injected animals.

CHAPTER 1
INTRODUCTION

Epilepsy

Epilepsy is a disorder of diverse etiologies, with over forty different types defined on the basis of location or distribution and cause (http://www.epilepsyfoundation.org/). Although the disorder can be classified as a syndrome with divergent symptoms, the hallmark of all seizures remains aberrant electrical activity in the brain that results in the onset of recurrent, spontaneous seizures (Bennewitz and Saltzman 2009, Sashindranath, et al. 2010). Epilepsy is one of the most common neurological conditions, with over 50 million people affected worldwide, and a prevalence rate of 1 per 118 in the United States (http://www.wrongdiagnosis.com/e/epilepsy/stats.htm). Most epileptic seizures can be characterized as partial, or generalized, depending on the localization and distribution of excitatory activity in the brain (Bancaud, et al. 1981). Partial or focal seizures may be triggered within a small focus of the brain, and later spread in a process known as secondary generalization. Generalized seizures may range from absence seizures (petit mal) to tonic-clonic (grand mal) seizures, all involving the loss of consciousness. The etiology of epilepsy is very diverse. Mutations in several genes including those coding for proteins in voltage-gated and ligand-gated channels have been implicated in the induction of generalized seizures (Meisler and Kearney 2005, Turnbull, et al. 2005). Additionally, childhood brain trauma, prolonged febrile activity (Crompton, et al. 2010) or infection (Akins, et al. 2010, Jequier Gygax, et al. 2010) may also lead to epilepsy later on in life.

Although the causes of epilepsy can be divergent as mentioned above, the basic manifestation of the disease is an offset in the balance between excitation and inhibition

in the brain which leads to altered synchronization and firing patterns of neurons. Although epilepsy may be controlled to an extent, 30% of patients suffering seizures remain refractory to currently available therapies including anti-epileptic drugs (AEDs), the vagus nerve stimulator (VNS), ketogenic diet, and in extreme cases, surgical resection of the epileptic focus (Noe, et al. 2009, Strom, et al. 2010).

The focus of our lab is expanding our knowledge of a specific type of epilepsy known as Temporal Lobe Epilepsy (TLE). TLE is the most common type of the partial epilepsies, and also the most intractable (Cascino 2009). Experiments in our lab cover the entire spectrum of the disorder, from trying to understand the underlying cause of seizures to devising alternative treatment strategies for intractable epilepsy. The specific scope of this dissertation is to describe the neuroprotective effects of the neuropeptide somatostatin (SST) when delivered into highly epileptogenic regions of the brain using the adeno-associated viral (AAV) vector. To this effect, we have described the cloning of three viral vectors that were used in this study to assess neuronal tropism and transduction efficiency using pseudotype 5 of the AAV vector in Chapter 3. Chapter 4 discusses the behavioral outcomes of over-expressing somatostatin *in vivo* in electrically kindled rats, while also assessing the issue of cognitive impairment, which is a common co-morbidity in patients suffering from temporal lobe epilepsy (Chiu, et al. 2010, Jansari, et al. 2010). Possible cellular changes underlying the differences observed across animal groups *in vivo* were discussed in Chapter 5, which delves into preliminary electrophysiological experiments performed on brain slices of animals from control and SST over-expressing groups.

The following subsections of this chapter give a more thorough understanding of the disease, the choice of animal model utilized to assess the efficacy of this gene therapy, as well as a fundamental understanding of what gene therapy entails including state of the art findings.

Temporal Lobe Epilepsy

Temporal Lobe Epilepsy is the single most common form of partial epilepsy causing intractable seizures. TLE is further divided into Lateral Temporal Lobe Epilepsy (LTLE) involving the more lateral structures of the temporal lobe, e.g. the neocortex at the onset of seizures, and Mesial Temporal Lobe Epilepsy (MTLE), with seizures arising from midline structures of the temporal lobe, e.g. the hippocampus, amygdala or parahippocampal gyrus. MTLE, the more common of the two, is poorly controlled with pharmacological intervention and surgical treatment is often necessitated by the nature of these complex partial seizures. Co-morbidities like learning impairment and memory loss are often associated with TLE since the hippocampus is also the seat of memory formation (Cascino 2009). The hallmark pathology of the underlying epileptogenic zone in MTLE is mesial temporal sclerosis (Cascino 2008), which is defined as partial loss of neurons in the CA1 as well as the dentate hilus in addition to granule cell dispersion and glial activation (Sloviter 2008).

The initial response to medication is of prognostic importance and will, in most cases, be paramount to predicting a seizure-free status for the patient (Hauser 1992). Patients experiencing developmental delay, foreign-tissue lesions or remote symptomatic neurological diseases will seldom attain a seizure-free status. The fundamental goal of using anti-epileptic drugs is to achieve this seizure-free status without drug toxicity. The past decade has witnessed the FDA approval of a number of

new AEDs including gabapentin (1993), lamotrigine (1994), tiagabine (1997), topiramate (1996), oxcarbazepine (1999), levetiracetam (1999) and zonisamide (2000). Although these additions to the older generation AEDs have offered seizure control with fewer side effects to some patients, they have not had a major impact on the overall number of patients who achieve seizure-free status.

Approximately 400,000 of the 2 million individuals with partial epilepsy in the United States have a medically refractory partial seizure disorder (Hauser 1990). Despite the development of newer AEDs, almost 50% of these patients will not attain seizure remission with pharmacological intervention (Kwan and Brodie 2003). The need for alternative therapies therefore stems from the inability to manage intractable partial seizure disorders, especially for cases that are not surgically remediable.

Limbic anatomy

Hippocampus. A more thorough understanding of hippocampal cytoarchitecture is important to gain an understanding of which structures are primarily responsible for the generation of seizures in MTLE and how they are connected. It will also serve to illustrate why we chose the amygdala as the focus of seizure induction, and how this structure communicates with the hippocampus, sub-regions of which we chose to inject with SST. The terms “hippocampus” and “hippocampal formation” have been used interchangeably in the scientific community. The hippocampus is considered one of the many related regions of the brain that constitute the hippocampal formation. The hippocampus proper is defined as having three subdivisions: the CA3, CA2 and CA1 (Amaral and Lavenex 2007). The term ‘CA’ comes from the word “cornu ammonis” or “Ammon’s horn”, which was coined in 1742 based on the morphological similarity of this structure with a ram’s horns (De Garengeot 1742). The hippocampal formation includes

neighboring structures as well, like the dentate gyrus, the subiculum, presubiculum, parasubiculum, and entorhinal cortex. The dentate gyrus and CA subfields form two interlocking C-shaped structures, with a predominantly unidirectional flow of information. Early literature emphasized the “trisynaptic” flow of information between the dentate and CA sub-regions (Andersen, et al. 1969). Information comes into the dentate gyrus from the entorhinal cortex through the perforant pathway. The second part of this loop is through mossy fibers, which are axons of dentate granule cells that synapse on CA3 pyramidal cells. The last part of this loop is the connection between CA3 and CA1 pyramidal cells through the Schaffer collaterals. It is now also known that there are direct connections to the CA3 and CA1 regions from the entorhinal cortex. Stimulation of many of these fiber bundles including the perforant pathway (Orban, et al. , Sloviter 1987) and Schaffer collaterals (Ghijsen, et al. 2007, Salmani, et al.) has been used extensively in animal models to evoke seizures both *in vivo* and *in vitro* with robust, reproducible results.

Amygdala. The amygdalae are almond-shaped nuclei embedded in the medial aspect of the temporal lobes. The primary role of the amygdala is to process and recall emotional reactions. The amygdaloid complex is comprised of several nuclei: the basolateral complex, which is further subdivided into the lateral, basal and accessory basal nuclei; the centromedial nucleus and the cortical nucleus (Amunts, et al. 2005, Solano-Castiella, et al. 2010). The amygdala has strong efferent connections to the entorhinal cortex (Amunts, et al. 2005). The majority of these connections stem from the lateral, basal and accessory basal nuclei to layer III of the entorhinal cortex, which in turn projects to the stratum lacunosum moleculare of CA1 (Steward and Scoville 1976).

Tract tracer studies in rats have shown that in addition to the entorhinal cortex, a few of these projections also terminate in a narrow zone between the CA1 and subiculum (Krettek and Price 1977). In MTLE, the epileptic focus often resides in the amygdala, in addition to the hippocampus. In some cases, amygdalectomy alone is sufficient to eliminate seizures (Aroniadou-Anderjaska, et al. 2008). Animal studies have suggested that the amygdala is even more prone to seizure activity than the hippocampus (Goddard 1967, Racine, et al. 1988). Therefore, kindling develops at a faster rate in the amygdala and interictal discharges tend to originate from the amygdala/piriform cortex irrespective of the site of kindling (Kairiss, et al. 1984, Racine, et al. 1988). The basolateral nucleus in particular plays the most fundamental role in the initiation and spread of seizures, even if seizures are initiated in extra-amygdaloid regions (Aroniadou-Anderjaska, et al. 2008). Additionally, pathological alterations are seen most often within the basal and lateral nuclei of the amygdala. Like the dentate hilus, SST-positive interneuronal populations are also particularly sensitive to seizure-induced damage in the amygdala. Somatostatin-containing neurons here exhibit calbindin, but no calretinin or parvalbumin (McDonald and Mascagni 2002), and how this differential expression of calcium-binding proteins affects the amygdala circuitry during epileptogenesis is yet to be determined. It is due to such extensive involvement of the amygdala in the generation and maintenance of epileptic seizures that we chose the amygdala as the site of electrical stimulation to induce seizures in our experimental model.

Animal models

A number of animal models have been suggested and are in use for studying TLE to get a better understanding of the underlying pathophysiology and progression of the

disease. The generation of such robust, translational models becomes imperative not only to further our understanding of the changes occurring in the brain during *epileptogenesis*, but also to potentially lead to novel effective therapies. Epileptogenesis is the process by which a normal brain undergoes structural and functional changes to ultimately develop epilepsy after an initial insult. The choice of both animal species and modality of seizure induction depends on the type of epilepsy desired to be developed and studied.

Rodents have been one of the most common models of choice for developing seizures. They are the lowest phylogenetic species that have brain tissue sufficiently similar to humans for these experiments. The rat provides a very useful animal model to develop a variety of experimental approaches and to provide a first-stage evaluation of their merit. In addition, there is a wealth of accumulated data on the neuroanatomy, pathology and immunology of the rat relevant to the objectives of the study we are undertaking. Furthermore, elicitation of seizures through chemical and electrical means in rodents reflects behavioral and electroencephalographic characteristics of human seizures. The scientific community as a whole has gathered a wealth of information about the underlying mechanisms of epilepsy, epileptogenesis and therapeutics through the use of animal models. The utility of animal models, however, is to be taken with a grain of salt since they require rigorous validation. This validation is more complex than a simple verification of how similar the model is to the human condition. Additionally, there are questions of scaling up from small animal models to non-human primates to humans, a progression crucial to the translation of these findings to human disease.

Kindling. One phenomenon discovered through animal experimentation is that repeated low-level electrical stimulation to some brain sites can lead to permanent increases in seizure susceptibility – in other words, a permanent decrease in seizure threshold. This phenomenon known as kindling was first discovered by Dr. Graham Goddard in 1967 (Goddard 1967). Kindling involves the progressive intensification of both electrographic and behavioral seizures as a result of daily, low-intensity electrical or chemical stimulation to a particular structure of the brain that manifests a permanent progressive functional reorganization of the neuronal network. Characterized almost 40 years ago, kindling has become a well-established model to study epileptogenesis and the mechanisms that maintain an epileptic state.

The electrical kindling model requires the surgical implantation of electrodes stereotactically in a specific brain area like the hippocampus or amygdala, which is a rapidly kindling limbic structure, and then stimulating the region by passing a low-intensity current through it. This lowers the animal's threshold for seizures, and electrographic seizures increase both in duration and complexity. Additionally, there is an orderly progression from mild to severe behavioral seizure activity with successive stimulations. Rats are generally considered "fully kindled" when stimulation of the focus will reliably trigger a motor seizure characterized as grade 5 on the Racine Scale, involving rearing and falling over of the animal (Racine 1972). Most kindling paradigms consider elicitation of 3 consecutive grade 5 seizures as the endpoint.

The mechanisms that lead to the increased susceptibility towards electrical stimulation are many, and it is difficult to attribute these changes to a single structure. It is the harmonious changes in a number of structures and molecular cascades that

render the neuronal network able to produce and maintain an epileptic state. Neuronal systems within the brain are particularly sensitive to inhibitory tone, and the concept of disinhibition therefore becomes an attractive mechanism through which an epileptic state may develop during kindling. High frequency electrical stimulation delivered to limbic sites produces a concurrent increase in glutamate and GABA release into the synaptic clefts from presynaptic terminals (Morimoto, et al. 2004). Glutamate then activates post-synaptic AMPA receptors, but this depolarization is immediately antagonized by $GABA_A$ mediated recurrent inhibition. If the stimulus intensity is sufficiently above threshold, inhibition failure occurs with continuous AMPA receptor activation, which results in burst firing and synchronization which manifests as the onset of afterdischarges (AD) on EEG. The prolonged depolarization also activates NMDA receptors, which mediates calcium influx in a voltage-dependent manner (Morimoto, et al. 2004). The activation of both AMPA and NMDA receptors ultimately leads to the reorganization of glutamate and GABA systems. These changes are both activity dependent and compensatory and may not be directly related to epileptogenesis. In the early stages of kindling, NMDA receptor-dependent potentiation develops in multiple brain regions, thus promoting and amplifying focal ADs to more distal brain sites (Morimoto, et al. 2004). During kindling, the dentate gyrus becomes a substrate for enhancement of NMDA-mediated currents. The hippocampal CA1 sub-region and amygdala experience the highest failure of GABA-mediated inhibition, while recurrent inhibition strengthens in the dentate gyrus and some parts of the piriform cortex, likely as compensatory mechanisms (Morimoto, et al. 2004). Late stages of kindling

encompassing generalized seizures experience more morphological changes like neurogenesis, synaptogenesis, axonal sprouting and astroglial activation.

As with electrical kindling, chemical kindling can also evoke repetitive epileptic spikes (an afterdischarge, or AD), gradually increasing the duration of these spikes and permanently increasing seizure susceptibility. Various agents with diverse pharmacological profiles have been used to induce such chemical kindling, a few of those being carbachol (Saucier and Cain 1996), pentylenetetrazole (Maciejak, et al. 2010, Mehla, et al. 2010), and bicuculline (Bertram, et al. 2008). The disparate nature of all these stimulating agents suggests that the underlying mechanism for kindling is the repeated occurrence of ADs, or episodes of network synchronization.

After a period of acquisition, the responses to stimulation are quite stable for each animal over a number of months. The two measures of seizure activity in the kindling model are: 1) spread to other regions of the brain as measured by an increase in seizure grade as scored on the Racine scale, and 2) length of seizure activity as measured by the duration of afterdischarges on EEG. Kindling has been extensively studied and employed as a model of temporal lobe epilepsy, yet its relevance to human epilepsy is still debated. It is well established that kindling leads to a predictable sequence of evolving cellular and molecular alterations in neural circuits, but the slow progression to spontaneous seizures limits its credibility as a clinical model. Nonetheless, the ability of obtaining the kindled response in multiple species, from amphibians to primates, underscores its clinical relevance. As a chronic model, kindling has broadened our understanding of how repeated brief seizures affect the brain. It has also been very useful for learning about circuits in that seizures can be induced by a

highly focal stimulation. Consequently, the pattern of initiation and the spread throughout the brain can be readily examined. It also offers a tool to evaluate the initiating circuitry as it allows precise selection of the site of stimulation and seizure initiation (Bertram 2007).

Gene Therapy

Gene delivered neuropeptides provide a novel approach to treating patients who are either pharmacoresistant to AEDs or are not candidates for epilepsy surgery. Appropriate gene replacement may enhance or facilitate restoration of limbic network properties through fundamental neuromodulation of a hyperexcitable limbic system. Targeted, cell-specific delivery of therapeutic agents into the brain can be achieved using vectors like adeno-associated viral (AAV) vectors that can deliver the gene of interest into the central nervous system (CNS) with minimum toxicity.

Viral vectors, in addition to other methods of *in vivo* gene transfer, are novel tools for studying gene function in the mammalian central nervous system. Furthermore, such approaches can induce expression of therapeutic molecules that provide potential alternatives for treating nervous system disorders. Neurotropic viral vectors can express single or multiple foreign genes and can be engineered at several levels to induce long-lasting, highly specific gene transfer. Many viral vectors like adenoviruses (Doloff and Waxman 2010, Wilmott, et al. 1994), herpes simplex virus (Manservigi, et al. 2010) and lentiviruses (Dreyer 2010) are currently in use for gene therapy. The use of adeno-associated viral vectors appears to have several advantages over the use of other vectors. AAV vectors are most importantly nonpathogenic, and are capable of infecting many different cell types in a wide variety of host organisms. They lack the machinery for viral replication and hence, infection is limited to the site of injection. Additionally,

they have a high efficiency of infection and minimal induction of host immune and inflammatory responses (Janson, et al. 2001, Mori, et al. 2004). As of 2006, there have been 11 naturally occurring AAV serotypes described, based on distinct variations between subspecies. A serotype is a classification at the subspecies level due to characteristics that differentiate between two members of the same species. These characteristics can be differences in cell surface antigens, virulence or phages. Of the AAV serotypes, serotype 2 is the most excessively studied (Choi, et al. 2005b). However, it has also been shown that other serotypes can be more effective as gene delivery vectors. For instance, AAV6 seems to be more efficient in infecting airway epithelial cells, while AAV7 presents a very high transduction rate of murine skeletal muscle cells similar to AAV1 and AAV5, both of which are also very efficient in gene delivery to vascular endothelial cells (Chen, et al. 2005, Flotte and Berns 2005a, Halbert, et al. 2001, Rabinowitz, et al. 2004). AAV5 has also demonstrated a larger distribution and higher number of neurons transduced than AAV2 in the central nervous system (Sun, et al. 2007), which is why we employed the use of AAV5 in our study to express SST in portions of the hippocampal formation of rats. Additionally, approximately 80% of the population has had prior exposure to AAV2, which incurs the presence of antibodies to this pseudotype of AAV whereas there are no such known complications with the use of AAV5.

AAV5 was originally isolated from a human clinical sample (Bantel-Schaal and zur Hausen 1984, Rutledge, et al. 1998) and contains inverted terminal repeats (ITRs) that are structurally analogous to AAV2, but are only 58% identical at the sequence level (Chiorini, et al. 1999). AAV5 is the most divergent of the AAV serotypes (Chiorini, et al.

1999). Its *rep* nucleotide sequence is 67% identical to AAV2 *rep*, while the *cap* sequence is only 56% identical to AAV2 *cap*. Prior to gene expression, AAV goes through a series of steps to attain transduction (Choi, et al. 2005b). These include binding or attachment to surface receptors. AAV5 specifically binds to the platelet derived growth factor receptor. This is followed by endocytosis and trafficking to the nucleus of the host cell where the virus un-coats to release the genome. The final step involves conversion of the genome from single-stranded to double-stranded DNA which then acts as a template for transcription within the host cell's nucleus.

A number of clinical trials are currently underway using different serotypes of AAV for cystic fibrosis (Flotte 2005), hemophilia (Wang and Herzog 2005), Parkinson's disease (Kaplitt, et al. 2007, Mandel and Burger 2004) and Alzheimer's disease (Mandel 2010) to name a few. There are promising pre-clinical trials underway for epilepsy as well using galanin and neuropeptide Y (NPY) (Noe, et al. 2007, Richichi, et al. 2004, Schlifke, et al. 2006). NPY and galanin are neuropeptides present in the CNS and both are known to have antiepileptic properties, as shown by their direct application to the CNS, or through use of their analogs. It is generally accepted that both these neuropeptides suppress epileptic seizures by antagonizing excitatory neurotransmission and hyperpolarizing neurons (Colmers, et al. 1985, Schlifke, et al. 2006, Sorensen, et al. 2009). However, little is known about the specific mechanisms of either of these neuropeptides. SST and NPY are extensively co-localized (Kohler, et al. 1987, Mikkelsen and Woldbye 2006, Noe, et al. 2007, Sloviter and Nilaver 1987) and results from studies on NPY therefore provide clues about the potential mechanism of action for SST. SST was the focus of our study because of the extensive *in vitro* data available

including precise knowledge about its receptors and corresponding pharmacological analogs (Csaba, et al. 2005, Csaba, et al. 2004, Qiu, et al. 2008). Somatostatin acts through a family of 5 G-protein couples receptors, each of which have been thoroughly characterized. It has also been identified that SST receptor subtype 2 is specifically dominant in rats, and it is through this receptor that most of the neuroprotective functions are carried out (Tallent and Qiu 2008). There is a relative sparing of dentate granule cells despite the loss of SST somatostatinergic interneurons in the hilus after electrical stimulation (Richichi, et al. 2004). Axons of hilar SST-positive interneurons synapse on distal dendrites of granule cells in the outer molecular layer (Amaral, et al. 1988, Csaba, et al. 2004), which may present a target for exogenously applied SST in the electrically kindled animals. Several other studies have addressed the changes in spontaneous and evoked neuronal activity that occurs as a result of loss of somatostatinergic neurons *in vitro* (Gavrilovici, et al. 2006, Shoji, et al. 1998, Stief, et al. 2007, Tallent and Qiu 2008). Based on such findings, it is imperative that the role of SST be further investigated in a pre-clinical setting, and our *in vivo* study is translationally relevant to the development of SST as a novel therapeutic agent for patients.

Pharmacology of Neuropeptides

Neuropeptides are small molecules similar to proteins that are used as signaling molecules within the CNS. The presence of a specific neuropeptide confers a biochemical phenotype to the particular neuron that expresses it. Currently there are approximately 100 different neuropeptides identified, each released by different neuronal populations within the mammalian brain (http://www.neuropeptides.nl/). For the scope of this dissertation, we will focus on neuropeptides found within interneurons.

A number of neuropeptides co-localize with the classical interneuronal neurotransmitter GABA. However, these neuropeptides differ in their size, synthesis, and release pattern when compared to GABA. Most neuropeptides are confined to dense-core vesicles as opposed to synaptic vesicles, and are released at a slower rate (<50 ms) for a sustained duration (Baraban and Tallent 2004). Additionally, contrary to synaptic vesicles that are released at axon terminals, dense-core vesicles are released at more diverse locations like dendrites and axons (Ludwig and Pittman 2003). The release of neuropeptides from these dense-core vesicles is a Ca^{2+} dependent process (Kits and Mansvelder 2000), and their physical distance from Ca^{2+} channels may be why neuropeptide release requires a higher level of activity than neurotransmitter release.

The list of neuropeptides expressed by interneurons is by no means short, yet NPY, SST, galanin and opioid peptides like dynorphin-A-1-13 are of special importance due to their abundance within the hippocampus (Mazarati and Wasterlain 2002). There is much evidence for modulation of excitatory synaptic transmission by these neuropeptides within the hippocampus by antagonizing pre-synaptic glutamate release (Cherubini and North 1985, Tallent and Siggins 1997, Whittaker, et al. 1999), which subsequently also has inhibitory effects on the generation of long-term potentiation (LTP). Evidence for modulation of GABA-mediated synaptic transmission is sparse; nonetheless, such activity does occur and fits with the overall mechanism of neuropeptide-mediated modulation of neurotransmitter release. For example, SST in the subiculum has been shown to reduce inhibitory post-synaptic potentials (IPSPs) (Greene and Mason 1996).

Amongst the numerous interneuronal neuropeptides, SST is the most well studied and well characterized in its effects on post-synaptic neuronal excitability. The two active forms of SST are SST-14 and SST-28, which are produced through alternate cleavage of the preprosomatostatin precursor (Fig 1-1) (Bowen 2002), the full sequence for which has been provided in the appendix. The specific effects of SST on CA1 and CA3 firing properties are described in Chapter 4. Like other neuropeptides in the dentate gyrus, SST is also involved in learning and memory. Deficits in spatial learning and memory have been observed in studies utilizing knockout mice models. It is speculated that SST may facilitate acquisition of spatial maps by filtering out background cues and increasing the "signal-to-noise" ratio (Tallent 2007). SST is also implicated to play a role in aging, and Alzheimer's disease patients suffering from memory loss show a reduction in the levels of cortical SST (Davies, et al. 1980, Davis, et al. 1999).

The role of neuropeptides in epilepsy becomes apparent when considering their antagonistic effects towards excitatory activity within the brain. Additionally, neuropeptide release from interneurons is a sequel to high-frequency stimulation, similar to seizure-like activity. Given that seizures induce changes in neuronal populations expressing these neuropeptides, as well as mRNA and peptide expression, it only follows that this endogenous anti-epileptic system be studied more in depth to understand the precise mode of action for full exploitation of alternative therapeutic agents for temporal lobe epilepsy.

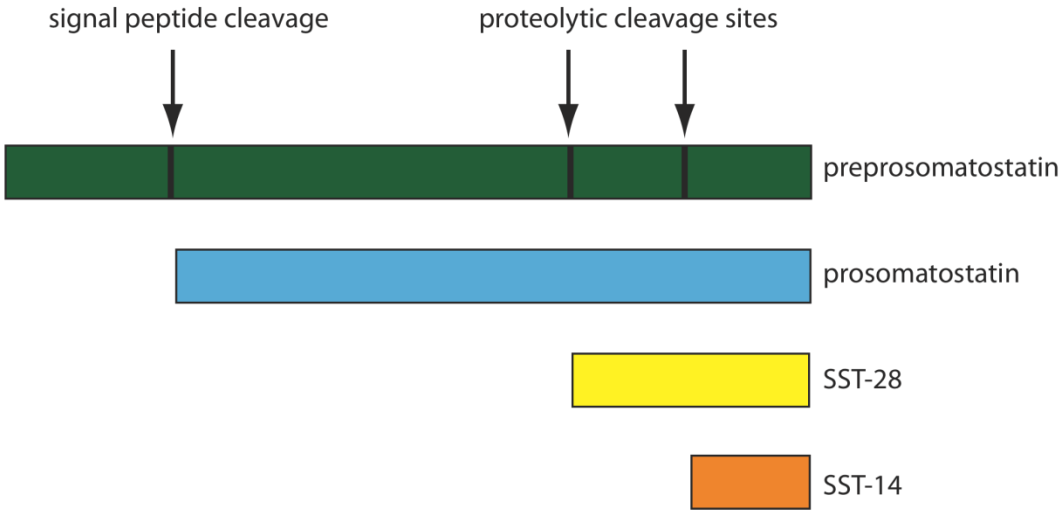

Figure 1-1. Production of active forms of SST through alternate cleavage of its precursors. SST-14 and SST-28 are produced by cleavage of prosomatostatin, which is derived from preprosomatostatin.

CHAPTER 2
EXPERIMENTAL METHODS

Surgical Implantation of Electrodes

All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee (IACUC) and conformed to animal welfare guidelines issued by the National Institutes of Health. Male Sprague-Dawley rats (Harlan) weighing 225-250g were pre-medicated with xylazine (10 mg/kg, SQ) and anesthesia induced with 4% isoflurane in 1 L/min oxygen. Anesthesia was maintained at 1.5% isoflurane and 0.4 L/min oxygen while placed in a Kopf stereotaxic frame. The surgical surface was sterilized using three alternate scrubs of 70% alcohol and Prepodyne. A midline incision was made to expose the landmarks bregma and lambda on the skull surface after loosening the muscle and underlying periosteum. The surface of the skull was then wiped with 3% hydrogen peroxide. Holes for screws were drilled using a rotary drill bit, and a dremmel used to punch holes in the skull for electrode implantation. Care was taken to not puncture through the brain. Bipolar teflon-coated stainless steel electrodes 330 µm in diameter (Figure 2-1) were implanted bilaterally in either amygdala, specifically in the basolateral nuclei of the amygdala (-2.2 mm behind Bregma, ±5 mm lateral to midline, and -8.3 mm ventral from dura). Two screw electrodes were placed, one rostral to bregma and the other caudal to lambda, to serve as ground and reference respectively. All electrodes were attached to male Amphenol pins and secured to the skull with two jeweler's screws, each placed on either side of the midline suture and coated with dental acrylic (Figure 2-2). The rats were allocated to three groups; Group 1 being uninjected controls, Group 2 being AAV-SST injected and Group 3 AAV-GFP

injected. All rats were allowed to recover for 1-3 weeks post-surgery before further experimentation was initiated.

Gene Injection

A 10 µl Hamilton syringe (Model# 80000, 1701N 26s/2”/2) fitted with a 27G steel needle with an internal diameter of ~ 200 µm and attached to an infusion pump was used for injecting all vectors. The plunger on the Hamilton syringe was depressed using two 10 ml airtight water-filled syringes connected by a hose. One of the 10 ml syringes was clamped into the infusion pump, while the other 10 ml syringe was hoisted on the stereotax arm above the Hamilton syringe so the two plungers just touched. Depressing the plunger on the 10 ml syringe placed on the pump caused the plunger on the other to pull out, thereby depressing the plunger on the Hamilton syringe. Two µl of the vector was infused bilaterally into each dentate gyrus (DG) and CA1 sub-region of the hippocampus at the rate of 0.5 µl/min for a total of 8 µl of vector in each animal (-3.72 mm behind Bregma, ±2.2 mm lateral to midline, and -3.4 mm ventral from dura for the DG, and 2.4 mm ventral from dura for CA1). The needle was kept in place for 5 additional minutes after cessation of injection to allow for complete distribution of the vector. The needle was then slowly retracted after each injection and flushed with hydrogen peroxide followed by de-ionized water. Electrode implantation proceeded as normal after cessation of the fourth injection.

Kindling Protocol

One to three weeks after surgery, an afterdischarge threshold was determined for each animal using a 2-s, 50 Hz biphasic square wave. An afterdischarge is described as spikes with a frequency greater than or equal to 1 Hz and an amplitude at least twice greater than the pre-stimulation baseline present in the EEG recorded from the site of

stimulation. The current intensity used for kindling acquisition was the minimum current necessary to evoke an afterdischarge on the EEG for each animal (238 +/- 124 μA). Beginning 24 hours post threshold determination, the rats were stimulated twice daily, with at least 6 hours separating stimulations. The protocol produced consistent Racine grade 5 seizures in all age-matched naïve controls and age-matched AAV-GFP injected animals, and stimulation was terminated once three consecutive Racine grade 5 seizures had been produced. Two scores were used to measure the effect of the gene on seizures: behavioral seizure score and the afterdischarge (EEG seizure) duration. The behavioral seizure score was determined using the standard Racine Scale (1: staring and immobility, 2: head nodding, 3: unilateral forelimb clonus, 4: bilateral forelimb clonus without rearing, 5: bilateral forelimb closure with loss of balance and falling) (Racine 1972). The afterdischarge duration was measured in seconds, with onset measured at the end of stimulation and termination at the last regular clonic spike before EEG suppression. These measures are stable from stimulus to stimulus in untreated animals.

Histology

Twenty-four hours following the last stimulation, animals were deeply anesthetized by an initial injection of xylazine (SQ) followed by ketamine (IP), and continuous exposure to isoflurane in oxygen. Animals were perfused through the ascending aorta with 200-300 ml NaCl followed by 300-350 ml of phosphate buffered formalin. The bodies were refrigerated for 2 hours post-fixation to allow for additional *in situ* fixation after which the brain was dissected out and kept in 30% sucrose solution containing 0.02% sodium azide for 24-48 hours for cryoprotection. The brains were then excised to

obtain a block containing both hippocampi and sectioned with a microtome into 50 µm coronal sections.

Somatostatin Immunohistochemistry

Brain slices were collected in vials and stained for SST as free-floating sections. Prior to primary antibody incubation, sections were subjected to the antigen retrieval step, which involved incubation in 10 mM citrate buffer for 25 minutes at 80^{o}C (Jiao, et al. 1999). The sections were then briefly washed once and incubated in 10% methanol containing 0.5% hydrogen peroxide for 15 minutes. Sections were then washed and incubated with a blocking solution for an hour, after which they were incubated overnight with primary monoclonal somatostatin antibody (Biomeda) at a concentration of 1:100. On day 2, the sections were incubated with biotinylated mouse anti IgG secondary antibody (1:10,000). The tissue was then reacted with extravidin-peroxidase (1:1000) for 2 hours followed by visualization with 0.05% 3,3'-Diaminobenzidine (DAB) in 0.0012% hydrogen peroxide.

Glial Fibrillary Acidic Protein (GFAP) Immunohistochemistry

Astrocytosis was visualized with GFAP staining, using a 1:400 dilution of a primary monoclonal anti-GFAP clone (G-A-5, Sigma, St. Louis, MO) in PBS incubated overnight after the antigen retrieval step mentioned above. Sections were then washed and incubated with biotinylated mouse anti IgG secondary antibody (1:10,000) overnight. The tissue was then reacted with extravidin-peroxidase (1:1000) for 2 hours followed by visualization with 0.05% 3,3'-Diaminobenzidine (DAB) in 0.0012% hydrogen peroxide.

Microglial Immunohistochemistry

Microgliosis was assessed by means of the same general technique using overnight incubation with the primary mouse anti-rat CD68 antibody at a dilution of 1:400 (AbD Serotec, Raleigh, NC).

Fluorojade-C Staining

Neuronal degeneration was analyzed using Fluoro-Jade C (FJC) staining as described in Schmued, et al., 2005 (Schmued, et al. 2005). 50 µm sections were mounted on subbed slides and immersed in a basic alcohol solution consisting of 1% sodium hydroxide in 80% ethanol for 5 minutes. The original protocol was modified and slides were then incubated in 70% ethanol for 20 minutes followed by a 3 minute incubation in 0.02% potassium permanganate. Slides were then rinsed and incubated in a 0.0002% solution of FJC (Histo-Chem Inc., Jefferson, AR) in a 0.1% acetic acid vehicle for 30 minutes to complete staining (modified protocol obtained from Sung J. Lee via personal communication). The slides were then rinsed with water, air dried, and cover slipped with Eukitt mounting medium (Fisher Scientific, Pittsburgh, PA).

Cresyl Violet Staining

Sections stained for GFAP and microglia were submerged in cresyl violet solution for 2-3 minutes, dehydrated by passing through increasing concentrations of alcohol (70%-95%-100%), and finally xylene before cover slipping with Eukitt mounding medium.

Microscopy

Following staining and mounting, slides were examined on an Olympus BH-2 bright field and epifluorescence microscope (Olympus America Inc., Center Valley, PA) with a Hitachi KP-D581 color digital video camera (Hitachi Medical Systems America,

Inc., Twinsburg, OH) interfaced with an Integral Technologies frame grabber (Pelco, Clovis, CA) on a desktop computer. Motorized stage and focus (Prior Scientific, Rockland, MA), and image acquisition were controlled through ImagePro Plus (Media Cybernetics, Silver Springs, MD).

Behavioral Testing Using the Morris Water Maze

The Morris water maze swim task was used to test any learning or memory impairments. The water maze apparatus consisted of a black tank, 1.7 meters in diameter, placed in a well-lit room with walls painted black. Water temperature was maintained at 27 $\pm$ 1^{o}C with the water level maintained at about 8 cm below the edge of the tank. A submerged hidden black platform 29 cm in diameter was placed in the middle of one quadrant, about 1 cm below the water level. The maze was surrounded by several visual cues to allow for navigation to the platform's location.

The Morris water maze swim task consisted of cue, spatial and probe trials (Aenlle, et al. 2009) over a 4-day period. The cue trial began with rats being habituated to the testing conditions by releasing them from 4 different locations within the tank and allowing them to swim freely for 20 seconds. At the end of this period, the rats were gently guided to the platform where they were allowed to rest for about 10 seconds. The cue trial constituted placing the platform 1 cm over the water level with a flag attached to the platform. The rats were released from different quadrants of the water maze across 3 trials and allowed to swim and find the platform in a 60 second time frame, at the end of which they were guided to the platform if they failed to reach it on their own. There was a 20 second interval between each trial and about a 15-20 minute interval between each block of 3 trials, during which the animals were housed in standard cages

lined with towels. On Day 2, the rats went through a spatial trial where they were allowed to navigate to the submerged platform using the visual cues placed around the periphery of the tank. Each trial lasted 60 seconds with 5 blocks of 3 trials each, with a probe trial before the 5th block. The probe trial involved removing the platform and allowing the animal to swim for 60 seconds. The duration of time spent in the goal quadrant and number of times the position of the platform was crossed was noted. The animals were allowed to rest on Day 3 and tested on the probe trial alone on Day 4 again.

Statistical Analysis

All statistical analysis was done in MATLAB (Mathworks, Natick, MA). Significance at a 95% confidence interval was assessed using a combination of the nonparametric one-way analysis of variance (anova1.m) and a Bonferroni correction (multcompare.m) for multiple comparisons.

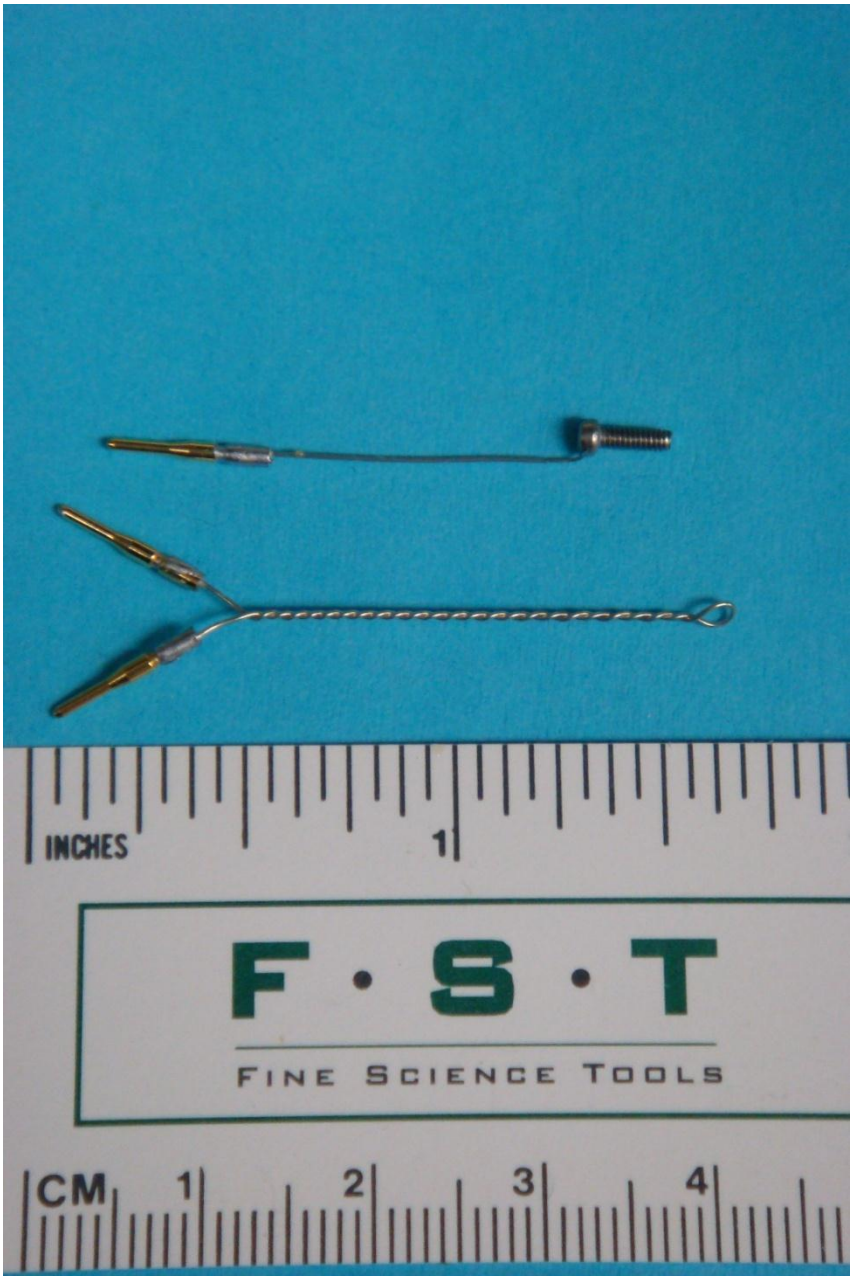

Figure 2-1. Ground/reference electrode (top) and bipolar twist electrode (bottom). The bipolar twist is cut to size before implantation.

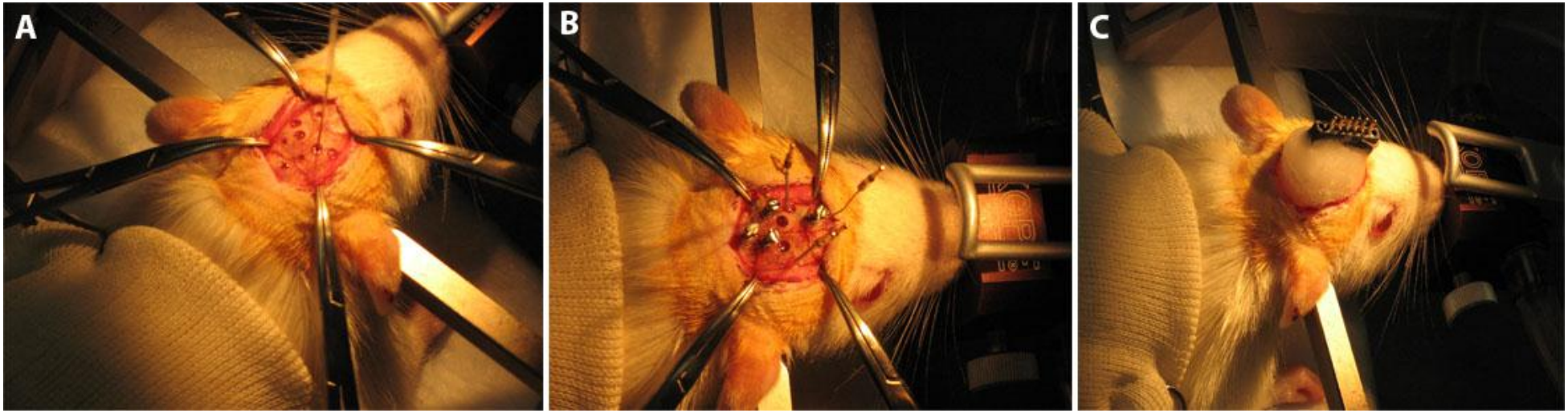

Figure 2-2. Stereotactic surgery for electrode implantation in a rodent. (A) Landmarks showing bregma, lambda and midline suture, along with injection and electrode coordinates. (B) Implantation of ground and reference electrodes, bipolar twist electrodes in the amygdala and anchoring screws on either side of the midline suture. (C) Amphenol pins inserted through plastic casing and enclosed in dental acrylic.

CHAPTER 3
PREPARATION OF VIRAL VECTORS

Background

Viral vectors, in addition to other methods of *in vivo* gene transfer are novel tools for studying gene function in the mammalian central nervous system. Furthermore, such approaches can induce expression of therapeutic molecules that provide potential alternatives for treating nervous system disorders. Neurotropic viral vectors can express single or multiple foreign genes and can be engineered at several levels to induce long-lasting, highly specific gene transfer. Many viral vectors like adenoviruses, retroviruses and lentiviruses are currently in use for gene therapy.

Adeno-Associated Viral Vectors. The adeno-associated virus (AAV) is a single-stranded DNA virus that belongs to the genus *Dependovirus* in the family *Parvovirus*. It is a small virus, with a capsid diameter of 26 nm (Choi, et al. 2005b). The single-stranded genome is flanked by inverted terminal repeats (ITRs) at each end, which is the only cis-acting element required for genome replication and packaging. The genome carries two viral genes: *rep* and *cap*, which generate four replication proteins and three structural capsid proteins respectively, through three promoters and alternate splicing. As a replication deficient virus, AAV requires Adenovirus (Ad) or Herpes Simplex Virus (HSV) as a helper virus to complete its lytic life cycle (Berns and Linden 1995). Recombinant AAV vectors can be produced by removing the two viral genes (*rep* and *cap*) and inserting a transgene between the two ITRs, with *rep* and *cap* provided in trans. Due to a lack of viral genes, minimal toxicity is associated with AAV vectors, which is the most attractive feature of this viral vector for use as a therapeutic agent.

The use of AAV vectors appears to have several other advantages over the use of other vectors as well. Other than being nonpathogenic, these vectors are capable of infecting many different cell types, both dividing and non-dividing in a wide variety of host organisms. Additionally, they have a high efficiency of infection and minimal induction of host immune and inflammatory responses. Long-lasting transduction has been observed with the use of these vectors and a variety of promoters, with gene expression lasting as long as 1.5 years in rodents (Klein, et al. 2002, Niwa, et al. 1991, Peel and Klein 2000, Xiao, et al. 1997) and more than 6 years in primates (Rivera, et al. 2005). As of 2006, there have been 11 naturally occurring AAV serotypes described, with serotype 2 being the most excessively studied. In addition to these, there are numerous engineered, hybrid serotypes that are also currently being used for research purposes.

Methods

Cloning

AAV-CBa-GFP

Standard cloning techniques were used to construct all recombinant AAV-based plasmids. The Green Fluorescent Protein (GFP) vector served as a viral control for animals and had the cytomegalovirus (CMV) immediate early enhancer, the chicken beta-actin (CBa) promoter and a hybrid intron that is part chicken beta-actin and part rabbit beta-globin. The vector was packaged in serotype 5 of the AAV vector by the University of Florida Vector Core as described below. This vector had a titer of $1.02x10^{13}$ vector genomes (vg)/ml at the time of use. The plasmid map is shown in Figure 3-1. For an initial assessment of vector transduction, 8 µl of this vector was injected into the dentate gyrus and CA1 sub-regions of the hippocampus bilaterally in

three animals. These animals were perfused 1, 2 or 3 weeks post injection respectively to assess extent of gene transduction.

AAV-CBa-SST

The AAV-SST vector encoded preprosomatostatin and was a kind gift from the lab of Dr. Terrence Flotte. The entire promoter construct was similar to the GFP vector described above and had the CMV immediate early enhancer, the CBa promoter and a hybrid intron that is part chicken beta-actin and part rabbit beta-globin. The vector was also packaged in serotype 5 of the AAV vector. The vector was kept at -20^{o}C till the time of injection, at which point it was thawed on ice for further use. The viral titer at the time of injection was 4.99x 10^{12} vg/ml. The plasmid map is shown in Figure 3-2. A total of 8 µl of the viral vector was injected into the DG and CA1 sub-regions of the hippocampus bilaterally (n=3) as described in Chapter 2. Animals were perfused 24 hours after the last stimulation and SST immunostaining was compared against naïve (n=3) and uninjected fully kindled animals (n=3).

AAV-CBa-SST-GFP

A plasmid containing a GFP tag on the SST vector was also cloned so as to better identify cells expressing exogenous SST. The backbone used was the CMV-GFP plasmid, a kind gift from the lab of Dr. Arun Srivastava, and was cut open using the EcoRI and XhoI restriction enzymes. This was followed by the end filling Klenow reaction for making blunt ends. The DNA was isolated using phenol-chloroform extraction after which a reaction with T4 polymerase was carried out. The DNA was extracted again using phenol-chloroform. The SST insert was digested out of the CBa-SST plasmid using the EcoRI restriction enzyme. After purification and quantification of digested SST and GFP fragments, the two were ligated and E.coli DH5-alpha cells

transformed. Transformed cells were then plated and colonies were picked out 24 hours later to screen for successfully cloned CMV-SST-GFP plasmids.

The second step of cloning involved swapping out the CMV promoter for the CBa promoter. The CBa promoter was digested out of the CBa-SST using the BglII enzyme and the CMV promoter was deleted from the new CMV-SST-GFP plasmid using MluI and ClaI restriction enzymes. Standard extraction and purification techniques mentioned above were carried out and colonies screened and sequenced for successfully cloned CBa-SST-GFP.

HeLa cells were first transfected in duplicates with both CMV-SST-GFP and CBa-SST-GFP plasmids to assess successful cloning of both plasmids. One successfully cloned colony for CBa-SST-GFP was sent to the University of Florida Vector Core for packaging into serotype 5 of the AAV vector. The titer of this vector at the time of use was 4.24 $x10^{13}$ vg/ml. Two animals were injected with 8 µl total of this viral prep into the DG and CA1 sub-regions as described in Chapter 2 and perfused 3 weeks post injection to allow for maximal gene expression. Following perfusion and sectioning in the brain into 50 µm coronal sections, the sections were incubated with a 1:1000 primary monoclonal antibody to SST (Santa Cruz Biotechnology, Inc) overnight, followed by overnight incubation with Invitrogen Alexa Fluor® 594 goat anti-mouse IgG secondary antibody (Cat. No. A-11005). Once sections were washed, they were mounted on to slides and cover-slipped with Vectashield containing DAPI (Vector Laboratories, Inc).

Viral Vector Packaging

All viral vectors were packaged at the University of Florida Vector Core. Methods for constructing the pXYZ5 helper plasmid for creating the pseudotype with capsid

genes from AAV-5 are described in Potter, Zolotukhin, Byrne et al 2002 (Zolotukhin, et al. 2002). Human kidney epithelial 293 cells from ATCC were transfected with the recombinant virus. The cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% fetal bovine serum and 1% penicillin/streptomycin. In order to attain a confluency of 70-80% or $1x10^9$, 293 cells were split 1:3 in a Nunc cell factory 24 hours prior to transfection. Transfections were performed using the conventional calcium phosphate method. The precipitate was formed by combining 1.8 mg of helper plasmid pXYZ5 mixed with 0.6 mg of the rAAV vector plasmid of interest in a total volume of 50ml of 0.25M $CaCl_2$ followed by 50 ml of 2 x HBS, pH 7.05, to the DNA/$CaCl_2$. This mixture was incubated at room temperature for about 2 minutes at which point precipitate formation was halted by adding 1100 ml of pre-warmed DMEM. This fresh precipitate-containing media was then promptly added to the cells after removal of the culture medium, and allowed to incubate for 60 hours at 37^oC and 5% CO_2. Discarding the culture medium and rinsing the cells with PBS marked the end of the incubation period. The cells were dislodged from the cell factory by using 5 mM EDTA, and pelleted by centrifugation at 1000 g for 10 minutes to be re-suspended with 60 ml lysis solution (150mM NaCl, 50mM Tris, pH 8.4).

Cells were subsequently lysed by three freeze/thaw cycles using dry ice-ethanol and 37^oC water baths. Benzonase (Sigma, St. Louis, MO) was then added to the cell lysates for 30 minutes at 37^oC to remove any nucleic acids. The crude lysate was clarified by centrifugation at 4000 g for 20 minutes, after which the supernatant was loaded onto a discontinuous iodixanol gradient. The tubes were then sealed and the less dense cell lysate displaced by using four gradients going from 60% to 15%

iodixanol. Tubes were then sealed and centrifuged in a Type 70 Ti rotor at 69,000 rpm/350,000g for an hour at 18^{o}C. Five mls of viral particles settled at the 60-40% step interface were then aspirated by side puncturing each tube with a syringe attached to an 18G needle.

In order to purify and concentrate AAV5 vectors, an equilibrated 5 ml HiTrap Q column (Pharmacia) was used. The column was equilibrated at 5 ml/min with 5 column volumes of 25 ml Buffer A (20mM Tris, 15mM NaCl, pH 8.5), then by Buffer B (20mM Tris, 500mM NaCl, pH 8.5), followed by 25 ml of Buffer A using a Pharmacia ATKA FPLC system. The 20 ml iodixanol fraction containing the vector was then diluted 1:1 with Buffer A and passed through the column at the rate of 3-5 ml/min. After loading of the sample, the column was washed with 10 volumes of Buffer A to be eluted with Buffer B. The virus was then desalted and concentrated using Biomax 100K concentrator (Millipore, Bedford, MA) by three cycles of centrifugation. The virus was concentrated into PBS. Vector stocks were analyzed by silver staining to check for protein purity after electrophoresis on 10% SDS-polyacrylamide gels.

To quantify the number of virions containing viral particles, a dot blot assay was performed on the concentrated viral stocks. In order to detect only packaged DNA, the sample was first digested for 1 hour at 37^{o}C with a solution of DNase I (Roche) with 10mM Tris-HCl at pH 7.5 and 1mM $MgCl_2$. This digested any of the unpackaged DNA prior to lysing the virion and releasing the packaged DNA. To release the encapsulated DNA, a solution of 2x proteinase K buffer (20mM Tris–Cl, pH 8.0, 20mM EDTA, pH 8.0, 1% SDS) was used to incubate the sample for 1 hour at 37^{o}C. This degraded the protein capsid and released the single-stranded genomes within the virions. The DNA

was then precipitated with a phenol and ethanol extraction using glycogen as a carrier. The precipitated DNA was dissolved in 40 µl of distilled water. A two-fold dilution series of the plasmid DNA that was packaged was prepared in water and diluted into 0.4N NaOH/10mM EDTA. Denatured vector DNA was then loaded onto a slot blot and immobilized onto a nylon membrane along with a plasmid standard curve. The nylon membrane was then probed for the transgene or the promoter and exposed on film. The signal of the sample was then calculated and compared to the standards, which was then extrapolated to a titer. This titer represented DNAse resistant particles.

Results

AAV-CBa-GFP

Increasing expression of GFP was observed within the entire hippocampal formation from the time of injection to the time of perfusion. GFP expression peaked at 3 weeks post-injection (Fig 3-3). This expression was robust and long lasting, as seen in an animal perfused 2 months post-injection (Fig 3-3 D).

AAV-CBa-SST

Fully kindled animals had a reduced number and immunolabeling intensity of SST-positive cells when compared to baseline levels viewed in a naïve animal (Fig 3-4). However, there were a higher number of SST-positive hilar cells visualized in injected animals when compared to both control and naïve animals. Most SST-positive cells were observed within the dentate hilus and stratum oriens of CA3 and CA1 subfields.

AAV-CBa-SST-GFP

Successful transduction of HeLa cells was obtained with both the CMV (Fig 3-5) and CBa promoters (Fig 3-6). To be consistent with other promoters used, we used the CBa promoter for further experimentation with this vector. Confocal images of sections

from AAV-CBa-SST-GFP injected rodent brains show successful transduction of SST and GFP (Fig 3-7). SST-positive cells (red) tagged with GFP (green) and nuclei stained with DAPI (blue) were seen within the dentate hilus and granule cell layer. Cells not co-expressing green fluorescence were therefore endogenous SST containing cells whereas doubly labeled cells were transduced cells.

Discussion

Efficient cell transduction and gene expression is subject to a combination of the promoter used, as well as the viral serotype. A number of naturally occurring and engineered serotypes exist for AAV and the utility of these various serotypes has been optimized for use in specific regions of the body. AAV5 is of particular interest for gene therapy since it has little to no pathogenicity (Flotte and Berns 2005b), as opposed to AAV2, antibodies to which are present in 80% of the human population (Daya and Berns 2008).

The temporal analysis of GFP expression using AAV5 showed some faint green fluorescing cells in the dentate hilus as early as 1-week post injection, but the expression increased with time to reach a maximum at 3 weeks, after which it remained stable. Robust expression of GFP within the hippocampal formation was observed as far as 2 months post-injection in this study. This is in agreement with other studies using AAV that also show maximal expression occurring at 3-4 weeks post administration (Klein, et al. 1998, Reimsnider, et al. 2007) and stable expression of genes for up to 1.5 years in rodents (Klein, et al. 2002, Niwa, et al. 1991, Peel and Klein 2000) and 6 years or longer in primates (Rivera, et al. 2005). Analysis of expression levels in our animals showed high levels of localized transduction with minimal spread to adjoining limbic structures. This specificity and long-term expression is crucial since SST and its

receptors are widespread in the CNS, and localized, well-targeted expression would limit interruption of endogenous SST effects. Transduction was observed only within neuronal cells and their associated fibers, and this can be attributed to the significant neuronal tropism of these vectors.

SST expression followed a similar temporal profile, with maximal neuronal transduction observed 3 weeks post injection. Fully kindled animals had a marked reduction of SST-positive interneurons within the hilus, which is in accordance with previous studies (Tuunanen, et al. 1997). SST is known to down-regulate with seizures (Sloviter 1987, Tuunanen, et al. 1997), and even with the limited pathology associated with the electrical kindled model, a marked reduction of inhibitory neurons was observed in kindled animals. This could be due to a downregulation of SST mRNA, or degeneration of SST containing cells, both of which have been described as plausible mechanisms of neuropeptide downregulation (Schwarzer, et al. 1996, Tallent 2007) and further explored in Chapter 4 of this dissertation. In AAV-SST injected animals, however, immunolabeling of SST was more reflective of naïve animals in that robust expression was seen within the hilus and surrounding strata of the CA3-CA1. Exogenously expressed SST compensated for the loss of this neuropeptide seen in fully kindled animals most likely due to transduction of previously non-somatostatinergic cells, as well as protection against atrophy that may have resulted from the occurrence of generalized seizures in the same cohort of animals.

Although the over-expression of SST was significant enough to be visualized when compared against a fully kindled animal, the level of expression was not as robust as GFP. This can be attributed to the fact that GFP is an inert protein, which is not

inherently processed, or downregulated within the CNS. SST expression on the other hand, is regulated by the enzymes that catabolize this protein within the brain, as well as saturation profiles of SST receptors within the CNS. It is therefore plausible that a level of expression matching that of GFP is limited by the natural enzymatic cleavage of the protein. Additionally, SST being a much smaller protein than GFP, may also be more sensitive to the perfusion protocol as well as the reagents used throughout the histological procedure. Nonetheless, our results show that expression of viral-vector delivered SST was sufficient to ameliorate the generalization of seizures in rats.

Additionally, by use of the AAV-CBa-SST vector alone, it is impossible to distinguish exogenously expressed SST from endogenous SST. It is for this reason that we cloned the AAV-CBa-SST-GFP plasmid to tag the exogenous SST. The AAV-CBa-SST-GFP vector allowed us to visualize exogenously expressed somatostatin within the hippocampal formation. Since the plasmid construct was a fusion controlled by the single chicken-beta actin promoter, with the SST gene upstream of GFP, any green fluorescence follows the presence of SST as well. We initially tested this construct by transfecting HeLa cells, and saw robust expression of GFP. Confocal microscopy after injection of 8 µl of this construct into adult rats as described above also showed vast expression within the dentate hilus, where most endogenous SST is also expressed.

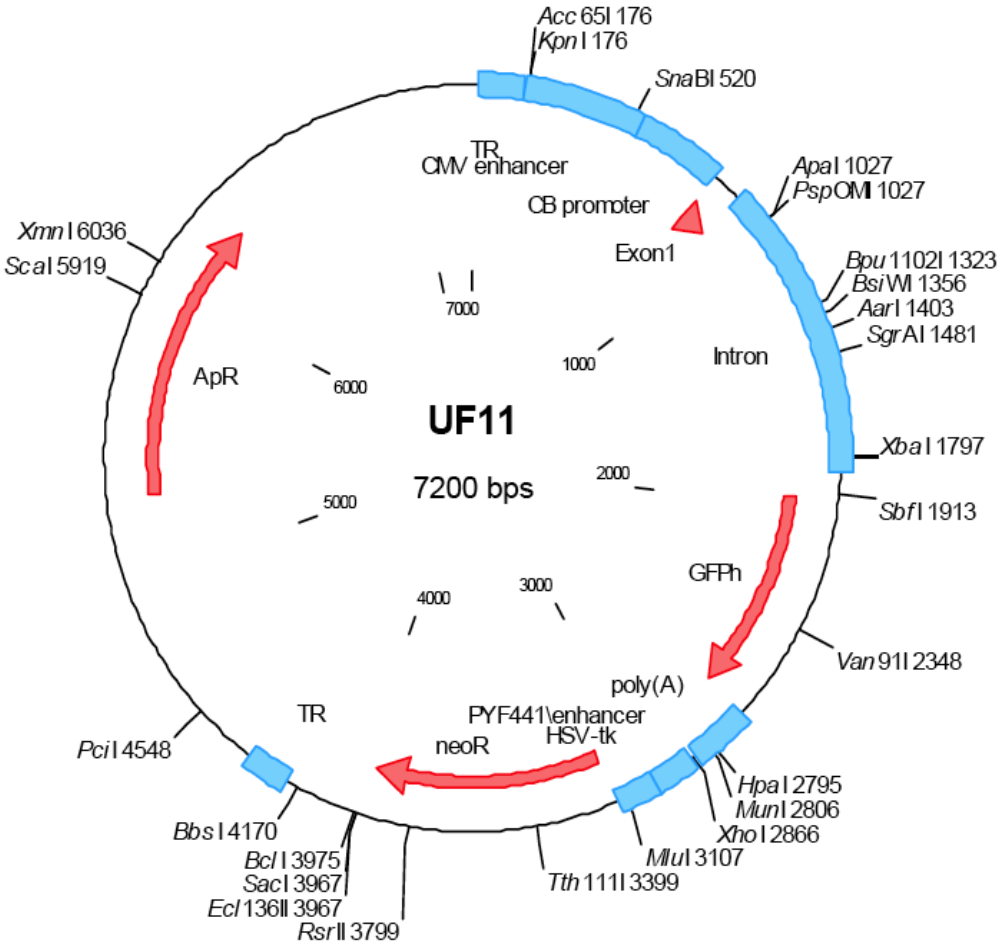

Figure 3-1. Plasmid map for pCBa-GFP (UF-11). ITR= Inverted Terminal Repeat, TR= Terminal repeat, CMV enh= Cytomegalovirus immediate early enhancer, CB promoter= Chicken beta-actin hybrid promoter, GFP= Green Fluorescent Protein, poly(A)= Poly adenylation sequence

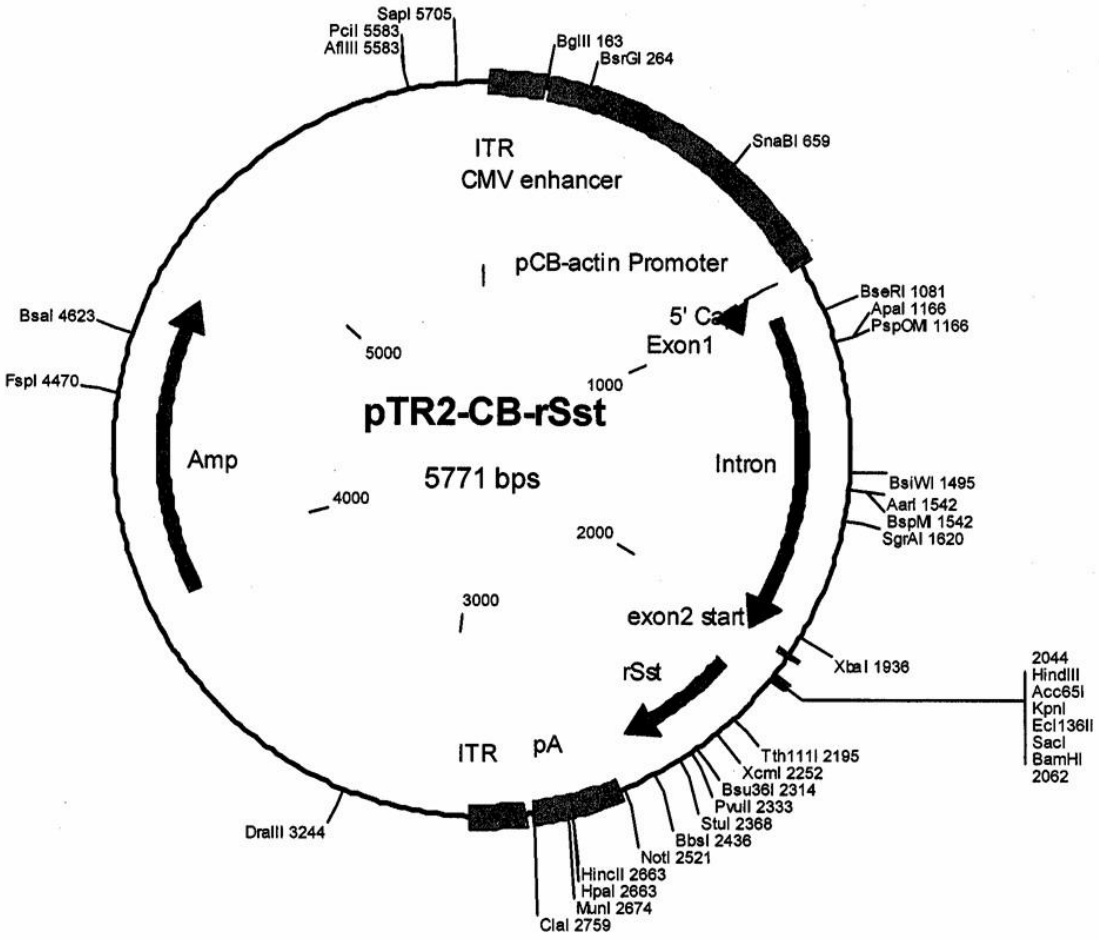

Figure 3-2. Plasmid map for AAV-CBa-SST. rSST= Recombinant somatostatin

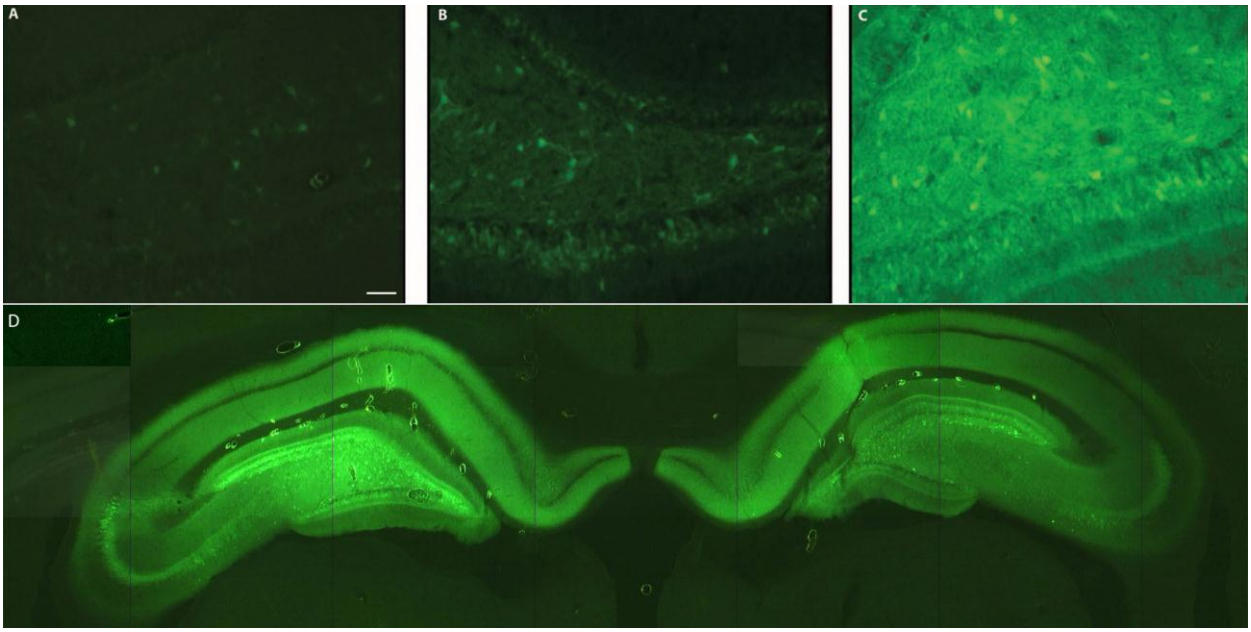

Figure 3-3. Temporal expression of GFP using AAV. Bright green cells show GFP expression. A-C show expression in the dentate hilus 1,2 and 3 weeks post injection respectively. D shows expression 2 months post injection within the entire hippocampal formation bilaterally.

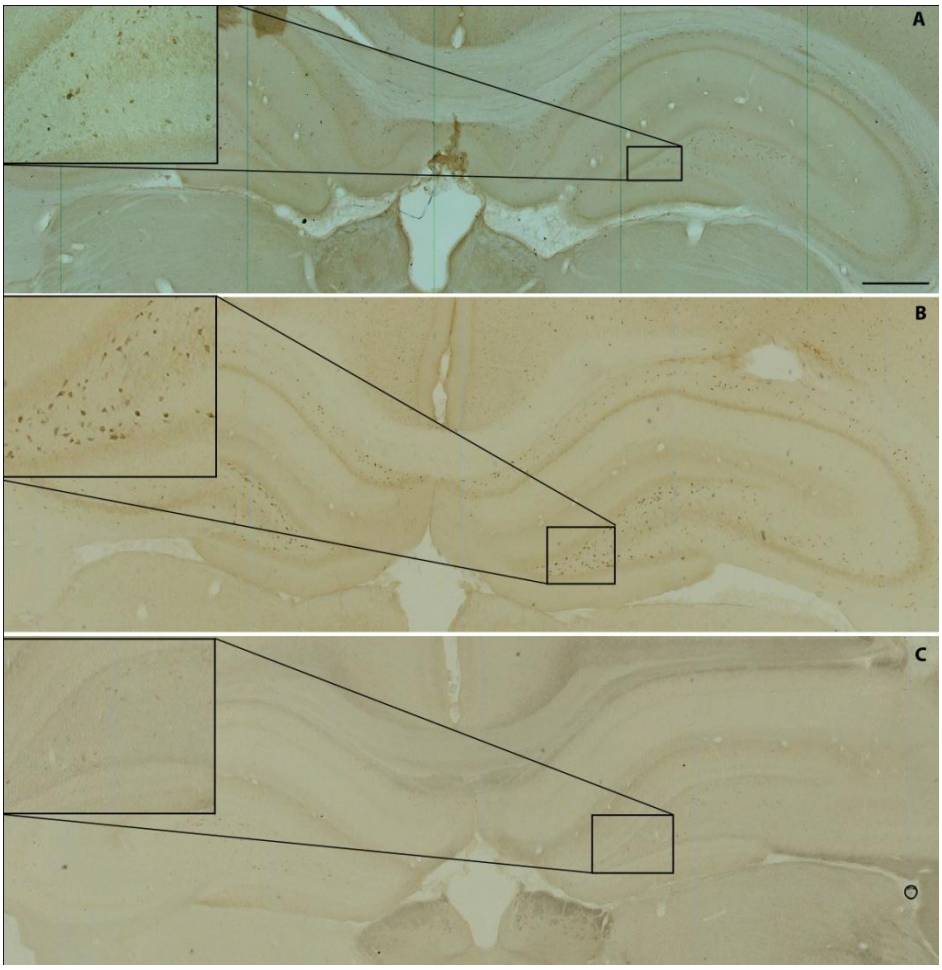

Figure 3-4. Histological analysis of SST in naïve (A), AAV-SST injected (B), and uninjected control (C) animals. A marked reduction of SST levels was seen in uninjected, fully kindled animals (C) as compared to naïve, unstimulated animals (A). Higher level of SST expression was observed in AAV-SST injected animals that did not reach a fully kindled state after 30 stimulations (B). Insets show higher magnification of hilar SST-positive neurons. Scale bar represents 100 um.

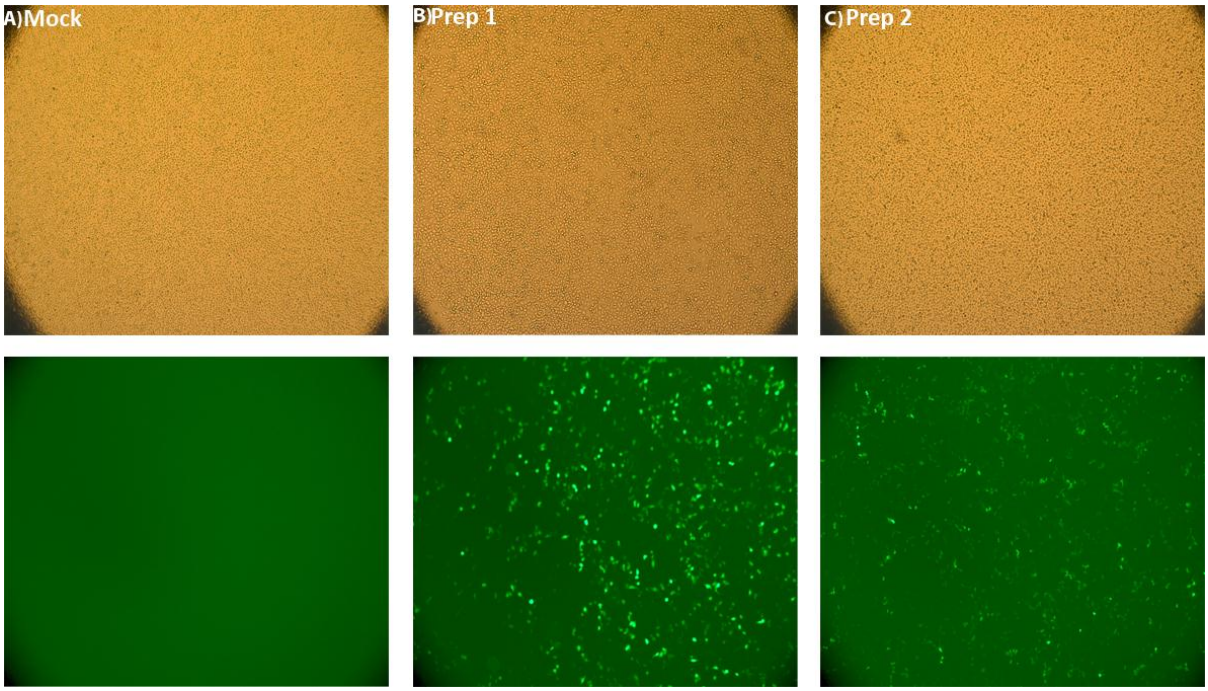

Figure 3-5. Transfection of HeLa cells with AAV-CMV-SST-GFP. Top panels show bright field images. Bottom panels show fluorescent images. (A) Mock infection, (B) Prep 1, (C) Prep 2. Bright green cells are GFP-expressing.

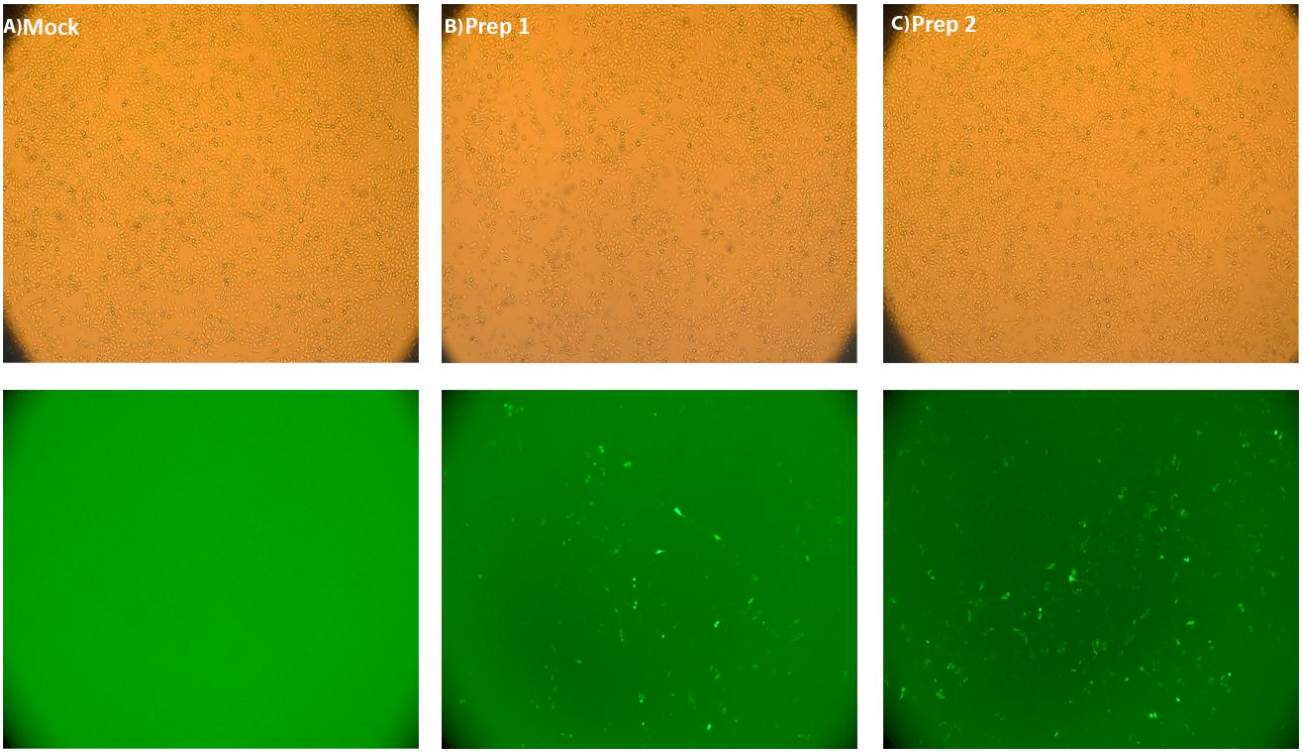

Figure 3-6. Transfection of HeLa cells with AAV-CBa-SST-GFP. Top panels show bright field images. Bottom panels show fluorescent images. (A) Mock infection, (B) Prep 1, (C) Prep 2. Bright green cells are GFP-expressing.

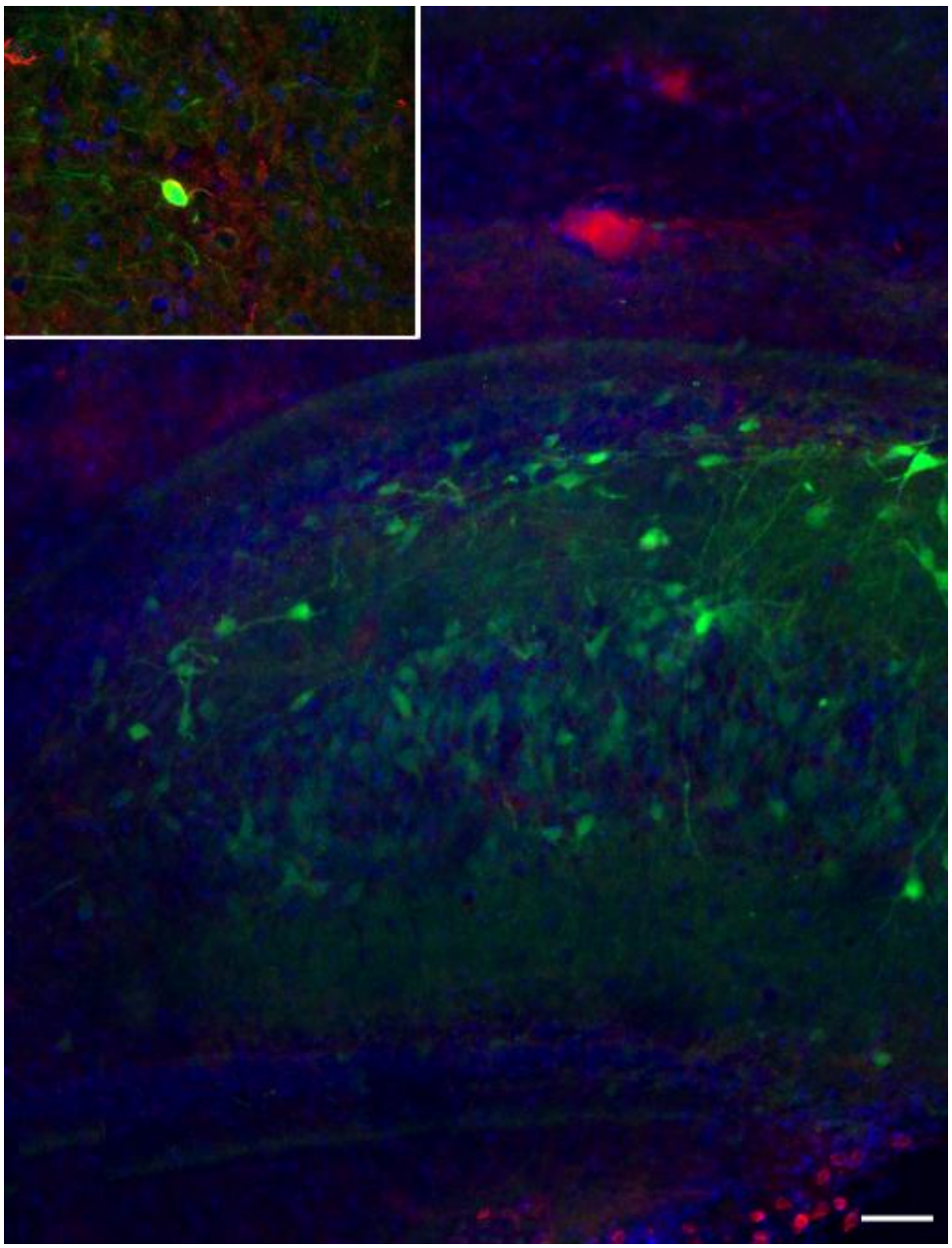

Figure 3-7. Histological verification of the AAV-SST-GFP vector. Bright green neurons are cells expressing GFP clones downstream of SST within the dentate hilus. Higher magnification shows co-localization of a red SST-expressing cell with GFP. Blue denotes nuclear staining using DAPI. Scale bar represents 50 um.

CHAPTER 4
EFFECTS OF SOMATOSTATIN OVER-EXPRESSION *IN VIVO* [1]

Background

Somatostatin (SST)-positive interneurons form the most abundant local circuit type in the hilus and account for 50% of all hilar GABA-ergic neurons (Buckmaster, et al. 2002). SST was initially identified as a hypothalamic cyclic tetradecapeptide in 1973 (Brazeau, et al. 1973) and subsequently described as a neurotransmitter and neuromodulator (Reichlin 1983). There are two biologically active forms of SST; SST-14 and SST-28, both products of alternate cleavage of a single prepropeptide precursor gene. SST-14 is the predominant form of the gene in the nervous system (Epelbaum 1986) and acts through a family of 5 G-coupled receptors (SSTR1-5) (Hoyer, et al. 1994). SST is known to have diverse functions ranging from the control of endocrine and exocrine hormones, inhibition of tumor cells, modulation of cognition, sleep and motor activity. Additionally, levels of SST have seen to be altered in several brain dysfunctions like Alzheimer's disease and temporal lobe epilepsy (Robbins, et al. 1991).

A few studies have alluded to the correlation between reduction in SST levels in the brain and exacerbation of seizures; the first one being the observation of a loss of a selective SST-positive neuronal population in the dentate hilus post seizure induction (Sloviter 1987). The loss of hilar somatostatinergic interneurons reduce functional inhibition of dentate granule cells (de Lanerolle, et al. 1989) and thus may play a role in neuromodulation of the epileptic network. Although the full breadth of SST action is not yet fully understood in relation to epilepsy, its role in the modulation of seizures is becoming more and more apparent. A selective loss of the SST neuronal population is

[1] Parts of this chapter have been submitted for publication.

observed post seizure induction both in humans (Sloviter 1987) and several animal models including kainate (Buckmaster and Dudek 1997), pilocarpine (Choi, et al. 2005a) and electrical stimulation (Sun, et al. 2007, Tuunanen, et al. 1997). Presynaptic somatostatin receptors in the hippocampus have also been shown to selectively inhibit excitatory transmission via G-proteins of the G_i/G_o family and through at least two separate mechanisms; modulation of Ca^{2+} channels, and an effect downstream of Ca^{2+} entry (Boehm and Betz 1997). This reduction in synaptic potentiation in the dentate gyrus is probably what leads to a reduction in long-term potentiation (LTP), manifesting itself as profound impairments in spatial learning and memory observed with over-expression of SST and the related peptide cortistatin (Tallent 2007).

SST has also shown inhibitory actions in the CA3 and CA1 hippocampal sub-fields. SST increases two different types of K^+ currents, the voltage-sensitive M-current (Moore, et al. 1988, Schweitzer, et al. 1990, Tallent and Qiu 2008) and a voltage-independent leak current (Schweitzer, et al. 1990) in the CA1. It also reduces excitatory synaptic input at Schaffer collateral synapses from CA3 (Tallent and Qiu 2008). Based on such convincing evidence of SST's role in epilepsy, we tested the hypothesis that over-expression of SST by use of viral vectors directly into highly epileptogenic areas of the temporal lobe will alleviate seizures by protecting against hyperexcitation of the neuronal network, which is a hallmark of epileptic activity. We found that over-expression of SST in approximately three-fourths of our animals prevented the generalization of seizures, and reduced the rate of kindling in the remaining one-fourth when compared to control and AAV-GFP injected animals. Furthermore, we evaluated activation of micro- and astroglia as markers of inflammation and possible cell death as

a result of gene injections, which could possibly impede the therapeutic benefits of the treatment. These results therefore provide proof-of-principle for the validity of SST as a neuromodulatory agent that increases seizure threshold in an electrical kindling model of epilepsy. Furthermore it justifies further investigation of SST as a novel neuroprotective and therapeutic agent for patients with intractable epilepsy.

Results

After an initial assessment of effective viral transduction, all animals were electrically stimulated. Behavioral seizures were graded on the Racine scale and duration of seizures was noted based on duration of afterdischarges on EEG. All uninjected controls (n=10) and AAV-GFP animals (n=6) reached a fully kindled state (which was set *a priori* as the occurrence of 3 consecutive grade 5 seizures) after 22 ± 0.8 and 19 ± 1 stimulations respectively ($p > 0.05$). 9 of the 13 AAV-SST animals never reached this fully kindled state (Fig 4-1), and kindling was terminated after the 30th stimulation, as this was at least 3 standard deviations higher than the average for uninjected controls ($p < 0.01$ when compared to uninjected and AAV-GFP injected controls). No significant difference was seen in the number of stimulations required to reach the first grade 1-3 seizure across all animal groups. The remaining four AAV-SST animals took 26 ± 1 stimulations to reach a fully kindled state ($p < 0.05$ when compared to uninjected and AAV-GFP injected controls). To take into account both the behavioral severity of the seizure as well as the duration, we measured the “Seizure Quantification Index (SQI)” for all animal groups across successive stimulations. The SQI is defined as follows; SQI = seizure grade x duration of seizure, where the seizure grade was a number between 1-5, as defined by the Racine scale, and the duration of seizure was

calculated in seconds by marking the start and end of electrical stimulation-evoked afterdischarges on EEG.

The trajectory (T) of the SQI was also measured across all animal groups over the entire duration of the kindling protocol. The trajectory of the SQI is quantified by fitting a linear model to the SQI versus sequential stimulations (in temporal order). Specifically, T is quantified by calculating the slope of the linear model fit for each animal (rate of change of SQI/stimulation). All AAV-SST injected animals had a significantly lower ($p < 0.01$) T as compared to uninjected controls and AAV-GFP animals. The value of T for uninjected controls was 18.2 ± 1.98, for AAV-SST injected animals was 4.5 ± 2.09 and for AAV-GFP injected animals was 25.9 ± 2.55 (Fig. 4-2). The average duration of each seizure through the entire kindling protocol for uninjected controls, AAV-SST and AAV-GFP animals was 62.5 ± 6.4s, 55.2 ± 5.6s and 67.9 ± 8.2s respectively (Fig 4-3). No statistically significant difference in the duration of seizures across the three groups ($p > 0.05$) was observed.

To further understand whether the reduction of SST expression seen in Group 1 animals was due to neurodegeneration versus downregulation of SST mRNA, tissue from both Group 1 and 2 animals was stained with Fluoro-jade C and the nucleic marker, DAPI. No neuronal degeneration within the hippocampal formation was observed in fully kindled or AAV-SST injected animals (Fig 4-4 A and B) when compared to a naïve animal (Fig 4-4 C). The full extent of neurodegeneration is seen in Fig 4-4 D, as observed in a spontaneously seizing animal from another study in our lab, to serve as a positive control.

To verify that the over-expression of SST or presence of viral particles did not induce any side effects by way of brain inflammation or neuronal damage, we measured activation of micro- and astroglia. This was assessed by using antibodies against CD68 and GFAP in uninjected control and AAV-SST animals (n=3 each). The same histological procedure was carried out on a naïve control animal (n=1) to serve as negative control (Fig 4-5 A). A representative figure showing positive GFAP staining is shown in panel D of Fig 4-5. No significant difference was observed in microglial or astrocytic activation amongst uninjected and SST-injected animals (Fig 4-5 B and C). A positive control for both micro-and astro-gliosis was included by staining a brain from a spontaneously seizing animal from a separate study in our lab (Fig 4-5 D).

The reduction in the generation of LTP mentioned above may lead to cognitive impairments, and to this effect we also tested naïve, uninjected kindled animals and SST over-expressing animals in the Morris water maze. A preliminary assessment of learning and memory was performed by testing rats in the water maze 24 and 36 hours post-training. We observed no significant difference in the percentage of time spent in the goal quadrant during the probe trial between control and AAV-SST injected rats. Additionally, the number of crossings made over the platform position was also not significantly different between the two animal groups (Fig 4-6).

Discussion

The hypothesis of SST as a neuromodulator for epilepsy stems from the experimental evidence of preferential SST release following elevated neuronal activity (Bartfai, et al. 1988, Hokfelt 1991, Vezzani, et al. 1993), marked alterations in the level of the protein, mRNA and receptors in experimental models of epilepsy (Schwarzer, et al. 1996, Sun, et al. 2007, Tuunanen, et al. 1997), and applications of SST analogs both

in vivo and in *in vitro* preparations to alter seizures and epileptogenesis (Mazarati and Telegdy 1992, Piwko, et al. 1996). It has also previously been described that loss of hilar somatostatinergic interneurons reduces functional inhibition of dentate granule cells (de Lanerolle, et al. 1989). Our data are in agreement with the notion of SST as an important antiepileptogenic and neuroprotective agent for epilepsy and is consistent with results from other studies using inhibitory neuropeptides like galanin and neuropeptide Y (NPY) (McCown 2006, Noe, et al. 2009, Richichi, et al. 2004).

NPY and galanin are neuropeptides present in the CNS and both are known to have antiepileptic properties, as shown by their direct application to the CNS, or through use of their analogs. It is generally accepted that both these neuropeptides suppress epileptic seizures by antagonizing excitatory neurotransmission and hyperpolarizing neurons (Ito 2009). However, little is known about the specific mechanisms of either of these neuropeptides. SST and NPY are extensively co-localized (Kohler, et al. 1987, Mikkelsen and Woldbye 2006, Richichi, et al. 2004, Sloviter and Nilaver 1987), and results from studies on NPY therefore provide clues about the potential mechanism of action for SST. We focused on SST for our study because of the extensive *in vitro* data available on SST including precise knowledge about its receptors and corresponding pharmacological analogs (Csaba, et al. 2005, Csaba, et al. 2004, Qiu, et al. 2008). Somatostatin acts through a family of 5 G-protein coupled receptors, each of which has been thoroughly characterized (Tallent and Qiu 2008, Zeyda and Hochgeschwender 2008). It has also been identified that SST receptor subtype 2 is specifically dominant in rats, and it is through this receptor that most of the antiepleptic functions are carried out (Tallent and Qiu 2008). There is a relative sparing of dentate granule cells despite the

loss of SST somatostatinergic interneurons in the hilus after electrical stimulation (Richichi, et al. 2004). Axons of hilar SST-positive interneurons synapse on distal dendrites of granule cells in the outer molecular layer (Amaral, et al. 1988, Csaba, et al. 2005), which may present a target for exogenously applied SST in the electrically kindled animal. Several other studies have also addressed the changes in spontaneous and evoked neuronal activity that occurs as a result of loss of somatostatinergic neurons *in vitro* (Gavrilovici, et al. 2006, Shoji, et al. 1998, Stief, et al. 2007, Tallent and Qiu 2008). Based on such findings, it is imperative that the role of SST be further investigated in a pre-clinical setting, and our *in vivo* study is hence prudent to the development of SST as a novel therapeutic agent for patients.

Nine out of thirteen AAV-SST injected animals did not experience a single grade 5 seizure after elicitation of 30 electrical stimulations. The remaining four rats did reach a fully kindled state, albeit after a significantly higher number of stimulations when compared to age-matched control and AAV-GFP injected animals. It is expected that not all animals in our experimental cohorts would have the same response to over-expression of SST, similar to the inherent variability in drug responsiveness that exists in the patient population (Ichikou, et al. 1990, Jannetto and Bratanow 2010). Therefore, some rats could have been more responsive to the protective effects of SST over-expression, while leaving the others less susceptible. Additionally, we cannot exclude the possibility that targeting and transduction of SST may have been lower as compared to other animals since the brains of the four AAV-SST injected rats that were fully kindled were used for other experimental procedures and not processed for histology. Finally, the over-expression of SST did not elicit an inflammatory response, which

further underscores the potential of gene-delivered SST as an alternative therapy for epilepsy in humans.

Trajectory analysis is an increasingly common method to study trends across groups of individuals with similar temporal behavioral patterns (Levine, et al., McCoy, et al.). For this study in particular, the trajectory of the Seizure Quantification Index in essence reflects the true measure of the “kindling” effect. By definition, as kindling progresses, seizures become more intense both in behavioral severity as well as duration. The SQI therefore includes both parameters to give an estimate of changes in neuronal networks that lower the threshold for seizures, so that the same amplitude of current evokes a more severe behavioral response from day to day. A lower trajectory of SQI per stimulation for AAV-SST injected animals when compared to control and AAV-GFP injected animals is significant as it reflects protection against the development and recruitment of additional neuronal networks that would intensify both the behavioral seizure grade as well as its duration.

For all animal groups, stimulations required to reach the first grade 1-3 seizures, i.e. the initial insult, did not differ significantly across groups as is reflected by the comparable SQIs across all animal groups during the first 10-13 stimulations. A higher separation between SQIs was apparent as control and AAV-GFP injected animals began to have grade 5 (generalized) seizures, while AAV-SST injected animals did not. This supports the role of SST gene therapy as an antiepileptogenic treatment that does not suppress the severity of the initial insult, which induces epileptogenesis. In other words, no alteration of individual stimulation-evoked seizures leading to the generation of fully kindled animals was observed. This result speaks to one of the main criticisms of

gene therapy which states that therapies shown to alter the rate of kindling may also suppress individual stimulation-evoked seizures (Dudek 2009). In doing so, these therapies suppress the initial insult leading to the final kindled state. Since the rate of kindling depends on the robustness of the evoked seizures, such initial suppressions would no doubt credit the therapy for altering the rate of kindling erroneously. However, since our study did not suppress the initial low-grade seizures that eventually lead up to generalized grade 5 seizures, *we can* attribute the over-expression of SST as the cause for prevention of generalized seizures in a majority of our SST-injected animals and delayed kindling in a few. The duration of each stimulation evoked-seizure was shorter in all AAV-SST animals as compared to controls and AAV-GFP injected animals, although this did not reach statistical significance.

Results from the Morris water maze show that unlike neuropeptide Y, there was no impairment in learning and memory, and SST over-expressing rats performed just as well as control animals. Measures of latency and percentage of time spent in the goal quadrant allow the analysis of a number of variables. Firstly, analysis of time taken to reach the platform over successive trials helps to address the animals' capacity to learn. Ideally, the animals should take less time to reach the submerged platform over successive trials since they learn to navigate to the platform based on navigational cues. Additionally, once the platform is removed in the probe trial, the percentage of time spent in the goal quadrant signifies retention of the platform's location and therefore is a measure of memory.

Our findings are one of the first *in vivo* demonstrations of the antiepileptogenic and neuroprotective roles of SST over-expression in electrically kindled animals. The

degree of protection is not limited to the abatement of seizures, but may also have important restorative cognitive effects. A vast population of patients with epilepsy suffers from impaired memory and learning skills, and it is therefore prudent that this be addressed before the utility of a new therapy is over-stated. A previous study showed promising results with the use of AAV delivered neuropeptide Y in seizing animals, but observed impairments in learning and memory (Sorensen, et al. 2008). Therefore any new therapy that is tested for epilepsy must address these concerns for it to be beneficial to the patient population. Future exploration of the effects of SST in a chronic model of temporal lobe epilepsy will further validate its use as an effective alternative for preventing and treating intractable epilepsy.

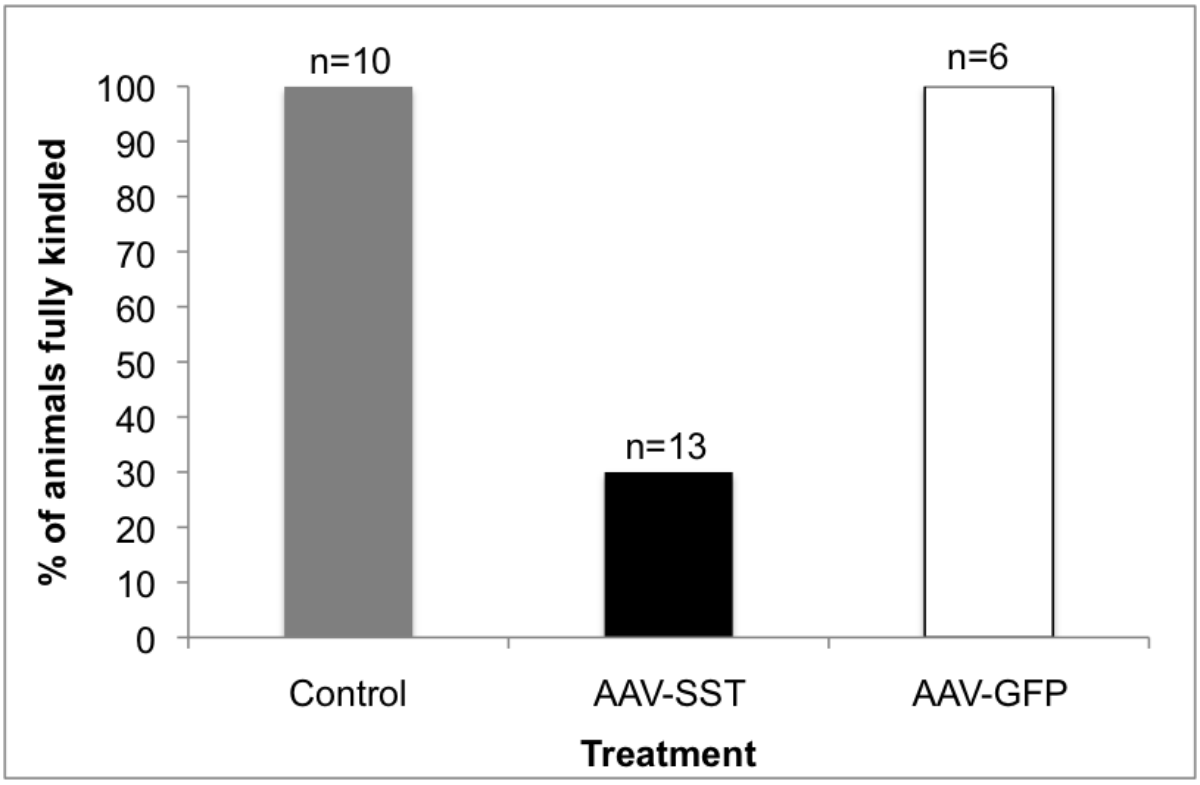

Figure 4-1. Percentage of animals fully kindled to spontaneous recurrent seizures in each treatment group. 100% of all control and AAV-GFP injected animals were fully kindled, whereas only 30% of AAV-SST injected animals reached a fully kindled state, after a significantly higher number of stimulations.

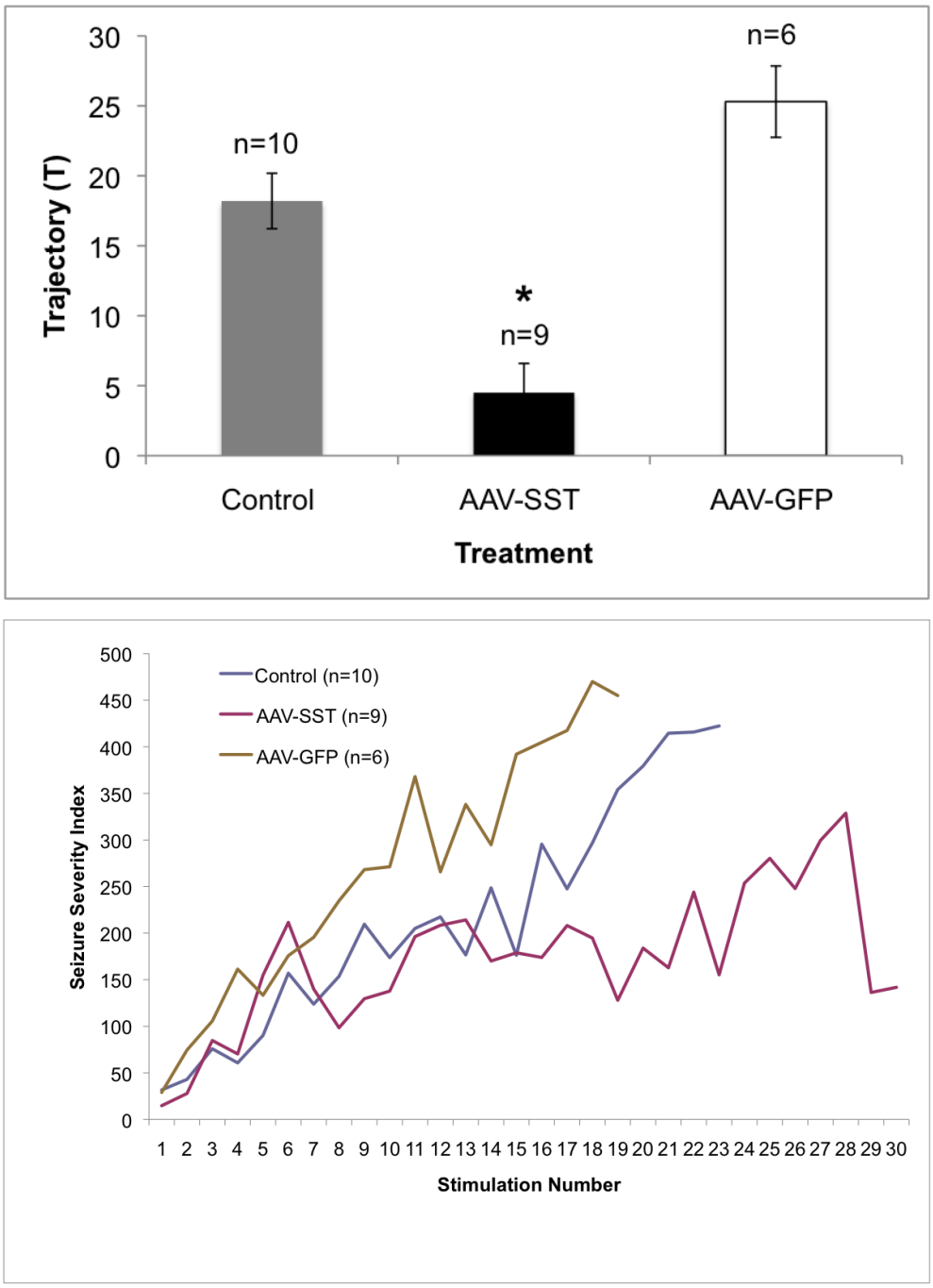

Figure 4-2. Trajectory of Seizure Quantification Index (SQI) calculated for all animal groups. (Top) AAV-SST injected animals had a significantly lower trajectory of SQI per stimulation as compared to control and AAV-GFP injected animals. (Bottom) Control and AAV-GFP injected animals had a progressive increase in seizure grade and duration, as shown by the rising slope of the SQI. AAV-SST injected animals had a lower SQI than other animal groups and eventually reached a plateau.

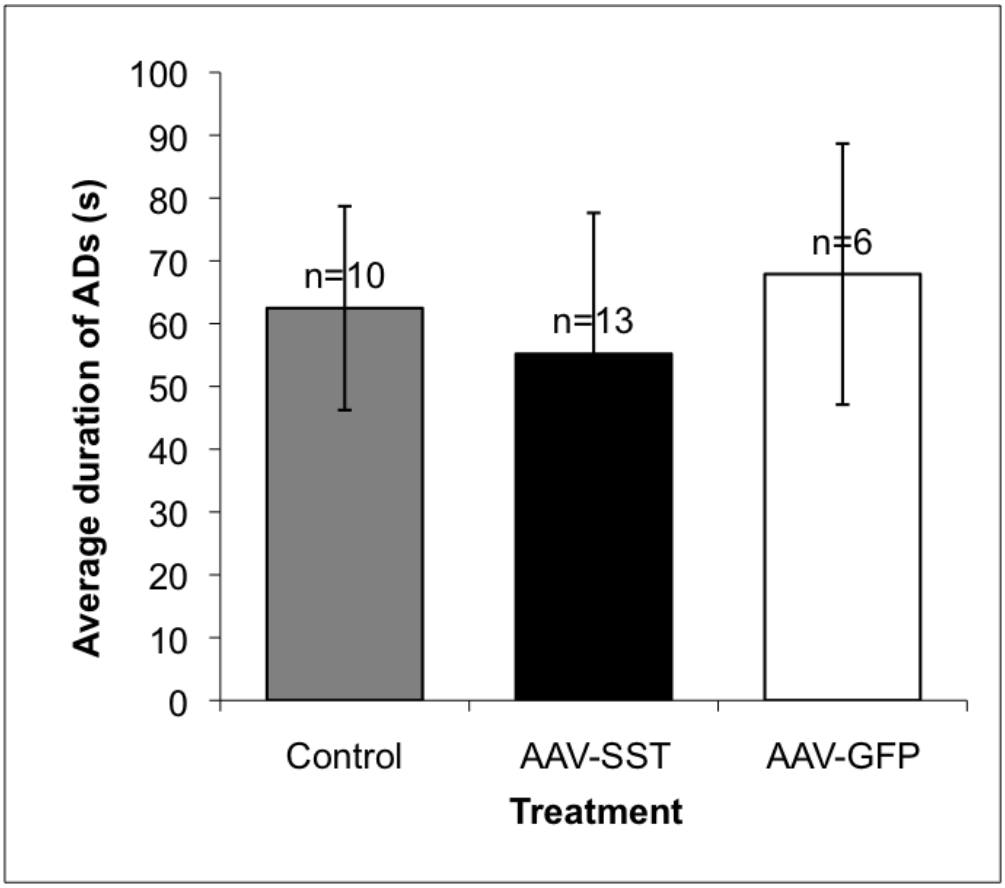

Figure 4-3. Average duration of afterdischarges per seizure in each treatment group. There was no significant difference in the average duration of seizures between control (62.5 ± 6.4s), AAV-SST injected (55.2 ± 5.6s) and AAV-GFP injected (67.9 ± 8.2s) animals ($p>0.05$).

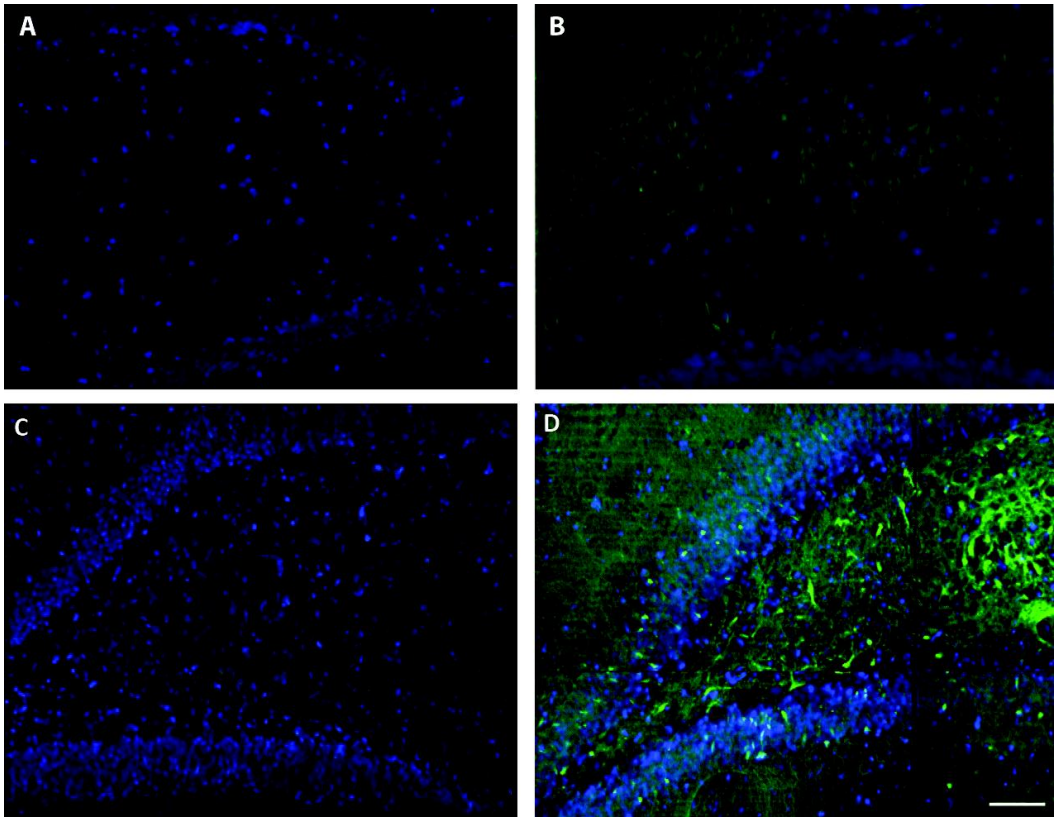

Figure 4-4. Assessment of neurodegeneration in the dentate gyrus as a result of electrical kindling using FJC/DAPI staining. (A) Uninjected kindled animal and (B) AAV-SST injected animal showing no degeneration like a naïve animal (C). (D) shows a positive control from a spontaneously seizing animal from another study with significant neuronal degeneration. Bright green neurons are degenerating. Blue fluorescence shows nuclei stained by DAPI. Scale bar represents 50 µm.

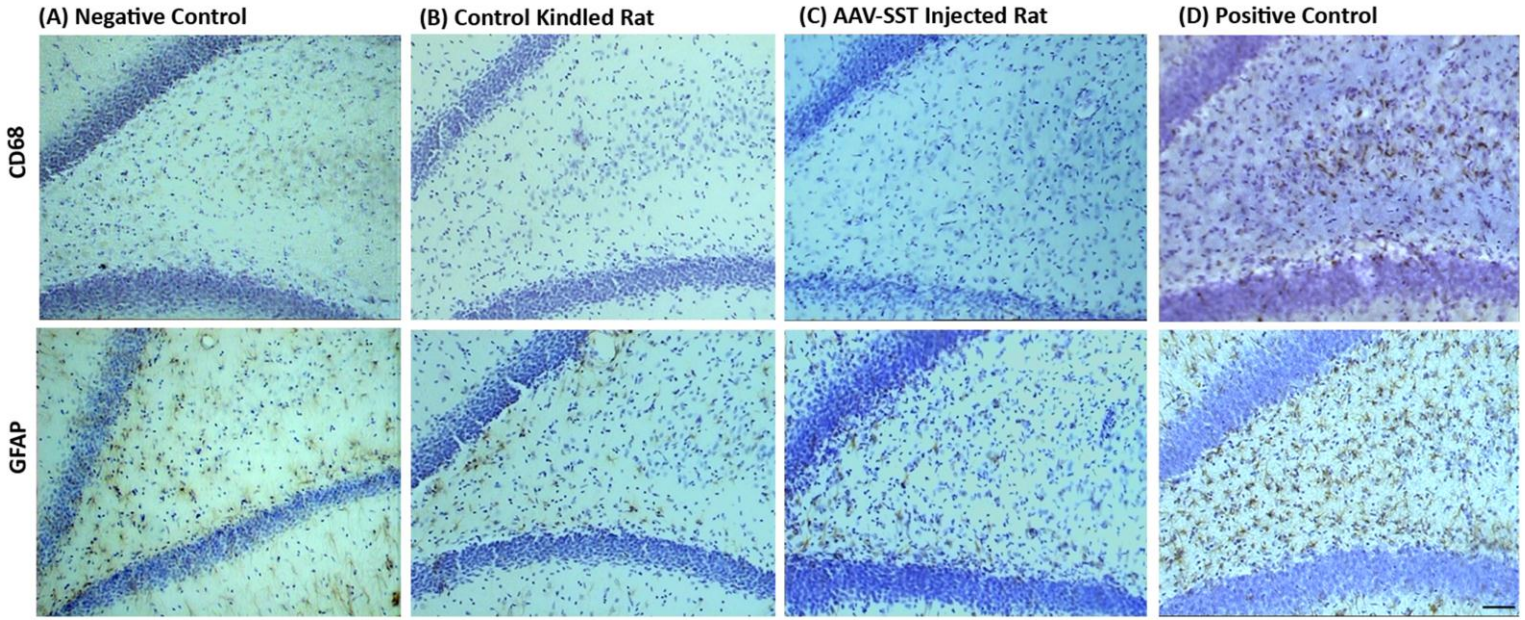

Figure 4-5. Analysis of inflammation in control and AAV-SST injected animals. Upper panel: immunoreactivity for CD68; Lower panel: immunoreactivity for GFAP. A) shows staining in a representative naïve animal. B) and C) show no activation of micro- or astro-glia in uninjected kindled controls and AAV-SST injected animals respectively. D) is a positive control showing activated microglia and astrocytes within the hilus. Scale bar represents 50 µm.

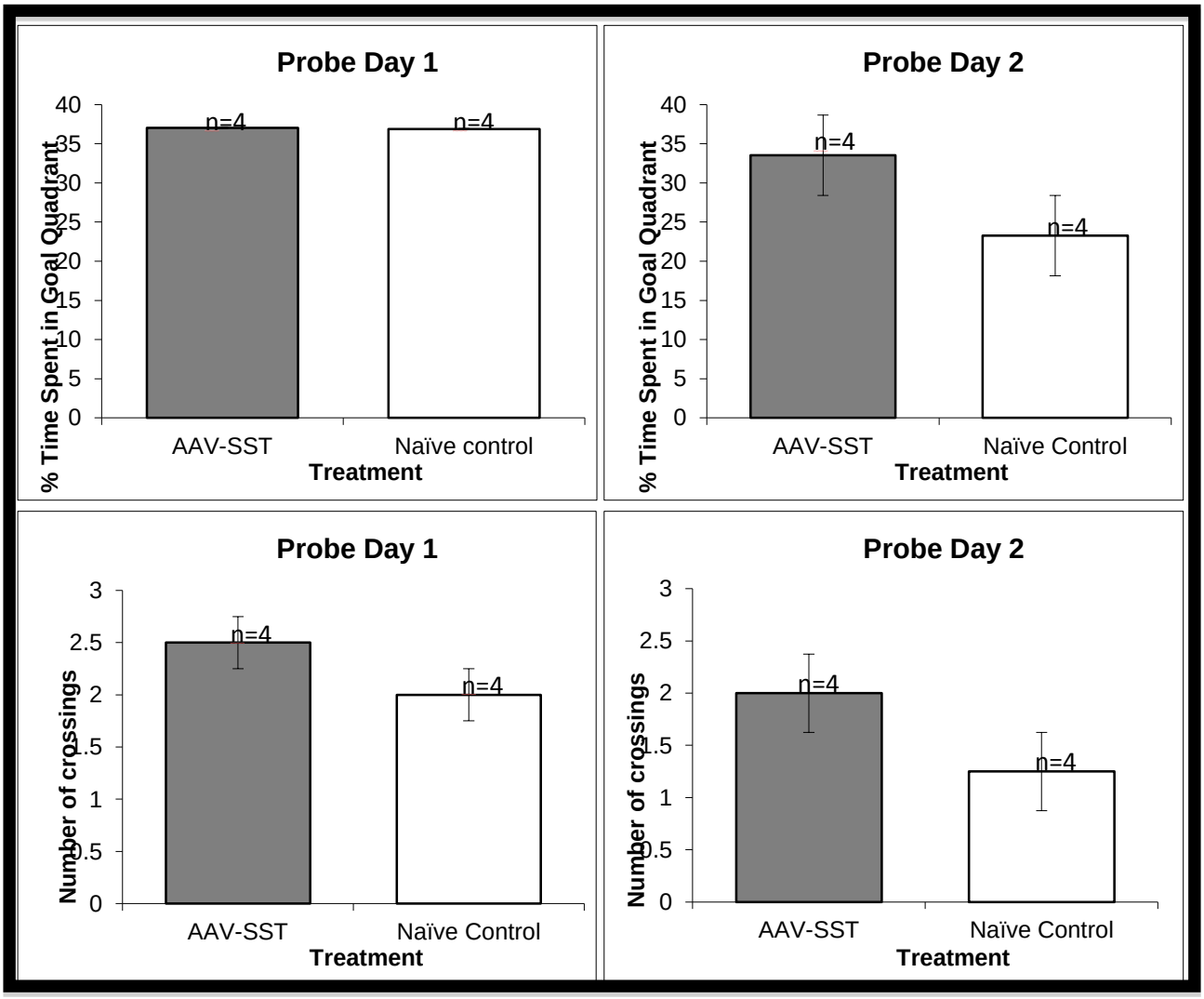

Figure 4-6. Analysis of behavioral effects using the Morris water maze. No signficant difference was observed in percentage of time spent in the goal quadrant and number of crossings over the platform position across control and AAV-SST injected animals. Probe Day 1 signified testing 24h post trainining and probe Day 2 was 48h post training.

CHAPTER 5
IN VITRO ASSESSMENT OF SPONTANEOUS POST-SYNAPTIC EVENTS ACROSS ANIMAL GROUPS

Background

A number of studies have attempted to unravel the mechanism of action of somatostatin using slice physiology to gain a better understanding of its inhibitory effects against seizures. Since the role of SST in the abatement of seizures is becoming increasingly apparent, a better understanding of where this neuropeptide is found and its specific effects in enhancing the inhibitory tone of the brain becomes prudent.

Somatostatin is primarily expressed in GABA-ergic Hilar perforant-path (HIPP) associated cells within the dentate gyrus (Bouilleret, et al. 2000a, Cossart, et al. 2001, Dinocourt, et al. 2003, Ramos, et al. 2006). HIPP cells are long-spined multipolar cells within the dentate gyrus that have long and often branched spines over the soma and dendrites. The axonal plexus can extend as long as 3.5mm along the dorso-ventral axis of the dentate gyrus (Freund and Buzsaki 1996). These morphological features of the HIPP cells make it unique as an interneuron since most interneurons are typically aspiny with local axonal plexuses. Besides SST, these neurons are also known to co-express NPY (Chan-Palay 1987). Additionally hilar somatostatinergic neurons also co-express, mGluR1 and substance P receptors (Freund and Buzsaki 1996). SST-positive interneurons have been reported to form approximately 16% of all GABA-ergic neurons in the dentate gyrus (Kosaka, et al. 1988). Axons of hilar SST-positive interneurons synapse on the distal dendrites of granule cells in the outer molecular layer in both humans and rats. These terminals on granule cells are in close approximation with excitatory synapses formed by perforant path input (Leranth, et al. 1990, Milner and

Bacon 1989). SST-positive interneurons are therefore situated to modulate excitatory afferent connections from the entorhinal cortex into the hippocampus.

Based on *in vivo* data presented in Chapter 4, we wanted to measure the *in vitro* component of SST's protective actions to correlate changes in behavior of SST over-expressing rats to cellular mechanisms within the hippocampal formation. We recorded from granule cells of the dentate gyrus since these are the putative primary targets of SST-positive interneurons. Additionally, the highest level of viral mediated transduction of SST was observed within the hilus, and these neurons presumably synapse on granule cells as mentioned above.

Methods

Slice Preparation

Male Sprague-Dawley rats aged 15-16 weeks after cessation of electrical stimulation as well as age-matched naïve animals were given a 0.5 ml intraperitoneal injection of ketamine and decapitated using a small guillotine. Brains were rapidly dissected out and placed in ice-cold artificial cerebro-spinal fluid (ACSF). Horizontal sections 300 µm thick were obtained using a Pelco Series 3000 Vibratome (Pelco, Redding, CA), after which they were incubated at 30-35^{o}C in a water bath for 30 minutes. The slices were then allowed to equilibrate to room temperature for another 30 minutes, while constantly bubbled with 95%oxygen/5%carbondioxide.

Solutions and Drugs

External solution

A high magnesium, low calcium ACSF was used for all recordings to prevent excitotoxic cell death. The constituents of the ACSF were as follows:

124 mM NaCl, 2.5 mM KCl, 1.23 mM NaH_2PO_4, 2.5 mM $MgSO_4$, 10 mM D-glucose, 1 mM $CaCl_2$, and 25.9 mM $NaHCO_3$.

Internal solution

For whole cell patch clamp recordings, recording pipettes were filled with an internal solution adjusted to pH 7.3 with the following composition:
140 mM K-gluconate, 8 mM KCl, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM EGTA, 2 mM Na_2-ATP, 0.3 mM Na-GTP, 10 mM HEPES, and 0.063 sulforhodamine 101.

Drugs

All drugs were prepared as aliquots and stored at -20^{o}C before diluting for use. 20 µM DNQX was used to block AMPA and kainate receptors while 40 µM APV was used as an NMDA antagonist when recording spontaneous inhibitory post-synaptic currents (sIPSCs). PTX was used to block $GABA_A$ receptors when recording spontaneous excitatory post-synaptic currents (sEPSCs). All chemicals used to prepare internal and external solutions were purchased from Sigma (St. Louis, MO), or Fisher Scientific (Pittsburgh, PA).

Electrophysiological Recordings

Hippocampal slices were placed in a recording chamber and temperature maintained at approximately 30^{o}C. Cells were visualized using an Olympus BX51WI infrared differential interference contrast microscope. Pipettes used for recording were pulled for whole cell patch clamp recordings using a Flaming/Brown electrode puller (Sutter P-97, Sutter Instruments, Novatto, CA). Standard electrode tip resistance was between 3-6 MΩ and was filled with the high chloride internal solution mentioned above. The access resistance was observed throughout the duration of the experiments and ranged between 12-30 MΩ. Recordings were performed from granule cells in the

dentate gyrus in voltage clamp mode using an Axon Multiclamp 700A amplifier (Molecular Devices, Sunnyvale, CA). Data was sampled at 20 kHz and recorded by Digidata 1322A using Clampex 10 (Molecular Devices, Sunnyvale, CA). A holding potential of -70 mV was maintained in all experiments. A 3 minute baseline recording was obtained after successful patching of granule cells, followed by a 16 minute bath application of both DNQX and APV when recording sIPSCs or PTX when recording sEPSCs.

Data Analysis

Spontaneous EPSCs and IPSCs were detected using appropriate parameter based event detection software written in Origin by Dr. Charles Jason Frazier. Further analysis of detected events was then performed using OriginPro 8. Statistical analysis was carried out using Excel 2003/2007 (Microsoft, Seattle, WA) or OriginPro8. Significance was measured using the Student's t-test.

Results

The goal of these experiments was to investigate changes in spontaneous excitatory and inhibitory post-synaptic activity of granule cells within the dentate gyrus of naïve, uninjected fully kindled, and AAV-SST injected animals that were subjected to 30 stimulations but never achieved a grade 5 seizure. Dentate granule cells were patch clamped for whole cell recordings in voltage clamp mode. We recorded both frequency and amplitude of sEPSCs and sIPSCs. For acquisition of sEPSCs, 50 µM PTX was bath applied to block $GABA_A$ receptors 3 minutes post baseline recording. The drug was bath applied for a total of 16 minutes. The first 6 minutes were considered drug wash-in periods, and the following 10 min were used for actual sEPSC acquisition. The frequency (in Hz) of sEPSCs recorded from the three groups was as follows: naïve

animals (n=11): 2.54 ± 0.27, uninjected fully kindled animals (n=12): 2.88 ± 0.21 and AAV-SST (n=7) injected animals: 2.81 ± 0.91. There was no significant difference observed between these frequencies, p >0.05 (Fig. 5-1 B). There was no significant difference in the amplitude (in pA) of these responses either; naïve: 16.43 ± 0.47, uninjected fully kindled animals: 16.6 ± 0.58, and AAV-SST injected animals: 15.4 ± 0.0.49, p >0.05 (Fig. 5-1 C).

Next we recorded sIPSCs from the same three cohorts of animals. 20 µM DNQX and 40 µM APV were bath applied after 3 minutes of baseline recording. Similar to sEPSCs, the last 10 minutes of data segments were used for analysis of sIPSC acquisition. No significant difference was observed in these events when comparing frequency or amplitude of events, p >0.05 (Fig. 5-2 B and Fig. 5-2 C). Frequency of sIPSCs (in Hz) were as follows: naïve (n=17): 3.01 ± 0.23, uninjected fully kindled animals (n=9): 2.67 ± 0.61, and AAV-SST injected animals (n=12): 2.91 ± 0.72. Amplitude of events (in pA) were: naïve: 27.34 ± 1.46, uninjected fully kindled animals: 27.84 ± 3.86, and AAV-SST injected animals: 33.81 ± 4.23.

The cells patched in slices of brains from AAV-SST injected animals did not have a GFP tag on them, and we therefore proceeded to inject animals with the AAV-SST-GFP vector described in Chapter 3. Animals were stimulated as before and brains from those not achieving a single grade 5 seizure were processed as described for sEPSC and sIPSC recordings. We patched only those granule cells that were in close apposition to fluorescing axonal processes, so a more direct effect of SST over-expression could be measured instead of recording from granule cells more distant from transduced cells. Frequency and amplitude of both sEPSCs and sIPSCs were recorded

and compared with data acquired from the previous 3 groups but no significant difference was found between frequency or amplitude of either event, $p > 0.05$ (Fig. 5-1 and Fig. 5-2 respectively). Frequency (in Hz) of sEPSCs recorded from AAV-SST-GFP injected animals (n=6) was: 2.49 ± 0.57, amplitude (pA): 17.04 ± 0.76. Frequency and amplitude of sIPSCs (n=8) was: 3.31 ± 1.13 Hz and 27.23 ± 2.44 pA.

Discussion

There have been conflicting reports on the role of SST in epilepsy, suggesting an increase in both excitatory and inhibitory drive within the brain (Shoji, et al. 1998, Stief, et al. 2007, Tallent and Siggins 1999). Results of our *in vivo* study show an inhibitory effect of SST over-expression in adult rats by increasing the threshold for generalized seizures. We wanted to tie in the behavioral results observed with firing properties of cells intimately associated with SST in the cytoarchitecture of the hippocampus. The rationale for recording from granule cells comes from previous studies showing an innervation of dentate granule cells by the axons of SST-positive hilar interneurons (Tallent and Qiu 2008). An over-expression of SST in the hilus of injected rats would mean an increase in the inhibitory drive on granule cells, thereby decreasing excitatory activity of these cells. Contrary to our expectation, we found no difference in the frequency or amplitude of spontaneous excitatory or inhibitory post-synaptic currents recorded from dentate granule cells of naïve, fully kindled and AAV-SST injected animals.

Our initial result prompted the use of a vector that co-expressed both SST and GFP, so better visualization of SST containing cells and axonal processes could be attained. This would ensure that only those granule cells would be patched and

subsequently recorded from that were directly synaptically innervated by SST-positive interneurons. Even with the use of the AAV-SST-GFP vector, we found no substantial differences in excitatory or inhibitory activity of granule cells. Some *in vitro* studies have shown that EPSCs generated at mossy fiber synapses, which are the output of dentate granule cells are insensitive to SST (Tallent and Siggins 1999). This study also showed that SST inhibits epileptiform activity specifically in the CA1 and CA3 sub-regions of the hippocampus. Augmentation of the K^+ M-current is identified as the major mechanism for the manifestation of SST's inhibitory actions (Moore, et al. 1988, Schweitzer, et al. 1990, Schweitzer, et al. 1998). The M-current (I_M) has been identified as a sustained voltage-dependent potassium conductance in a wide variety of peripheral and central neurons (Womble and Moises 1992).

It is therefore possible that the output of granule cells is not substantially affected by the over-expression of SST, and that most effects are dominant in either CA3 or CA1. SST has also been implicated in memory impairments experienced in Alzheimer's disease, and most of these are in part due to an inhibition of LTP. The major mechanism of LTP inhibition is postulated to be a blockage of voltage-gated calcium channels on dentate granule cells (Tallent 2007). It is therefore plausible that most effects of SST are dendritic and not somatic.

Studies observing cellular responses from the basolateral amygdala neurons of kindled rats have showed an increase in the amplitude of evoked excitatory post-synaptic potentials (EPSPs), with no change in inhibitory post-synaptic potentials (IPSPs) (Shoji, et al. 1998). Another study recorded from layers II and III of the anterior piriform cortex bilaterally in kindled rats, miniature IPSCs (mIPSCs) were recorded from

pyramidal and non-pyramidal neurons. They found an increase in the amplitude of mIPSCs from non-pyramidal cells on the side that was stimulated, suggesting an increased inhibitory synaptic strength on interneurons as a result of kindling (Gavrilovici, et al. 2006). It is therefore possible that granule cells are not the targets of SST's actions and therefore our analysis of the very broad spontaneous activity of granule cells may not have been specific enough to pick up differences in cellular alterations as a result of SST over-expression. Additionally, as mentioned previously, neuropeptide release from interneurons is a result of high-frequency stimulation. The SST gene contains a cyclic AMP response element (CRE). The presence of this regulatory element confers activity dependence to the gene, switching it on when neuronal activity is high (Tallent 2007). Consequently, somatostatin may not be released under basal conditions unless tetanus is applied to the slice. Hence, spontaneous excitatory and inhibitory activity may not change as a result of somatostatin over-expression without some degree of perturbation to the system.

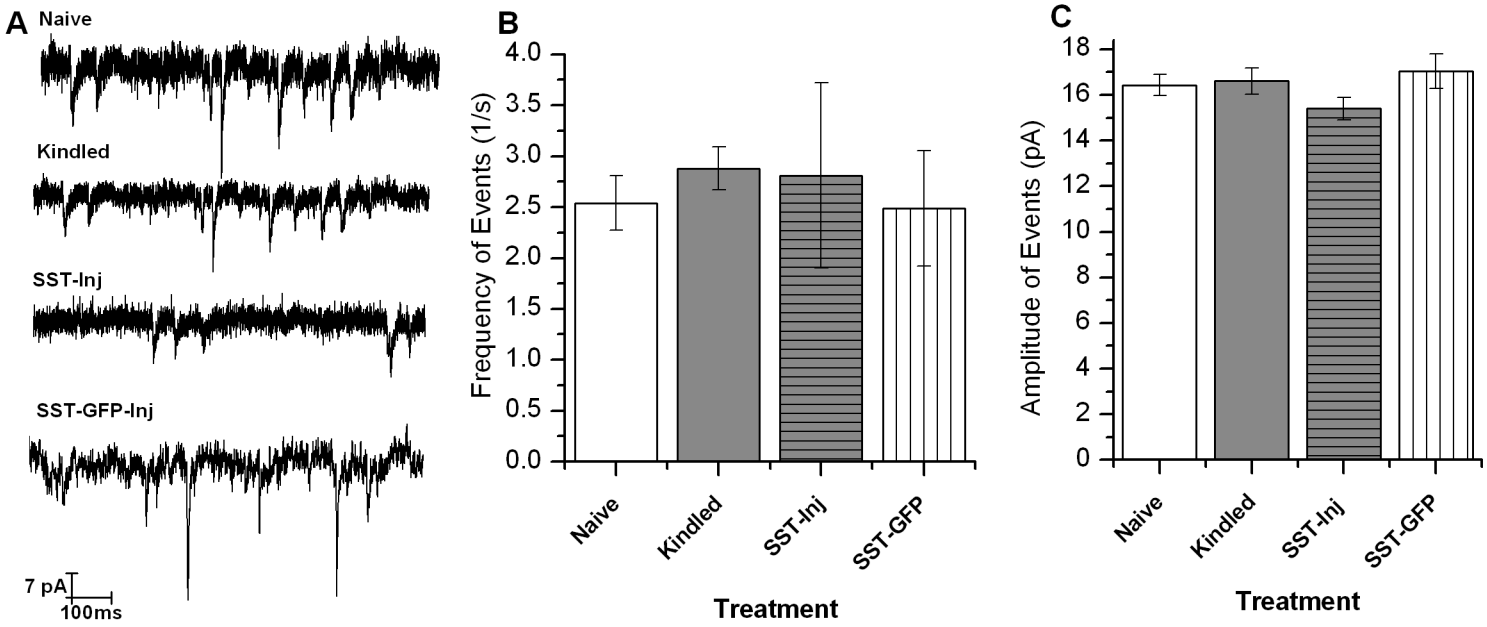

Figure 5-1. Frequency and amplitude of sEPSCs. A) shows a 1s epoch of raw data traces recorded from naïve (n=11), kindled (n=12), AAV-SST (n=7) and AAV-SST-GFP (n=6) injected rats. B) shows frequency of events recorded from the same animal groups, and C) shows amplitude of events. No significant difference was observed in frequency or amplitude of sEPSCs ($p>0.05$, n denotes number of cells).

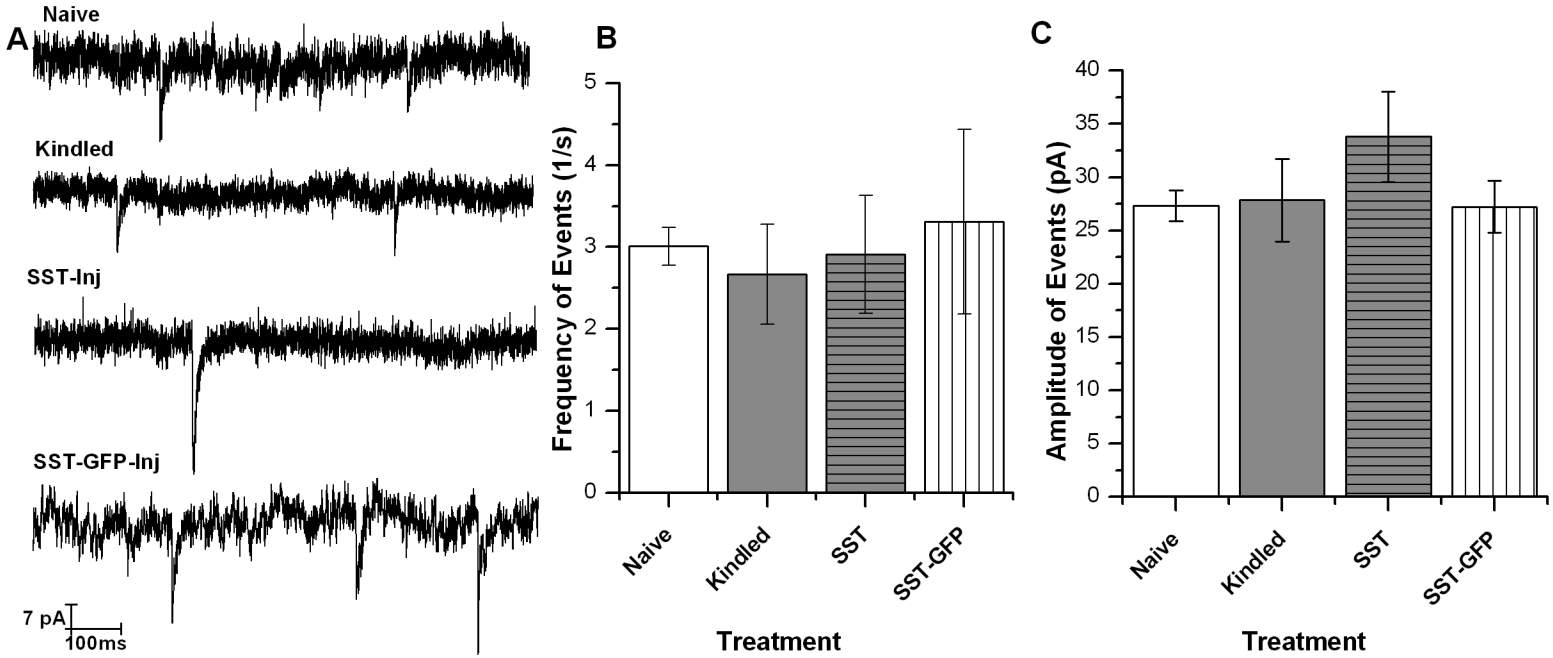

Figure 5-2. Frequency and amplitude of sIPSCs. A) shows a 1s epoch of raw data traces recorded from naïve (n=17), kindled (n=9), AAV-SST (n=12) and AAV-SST-GFP (n=8) injected rats. B) shows frequency of events recorded from the same animal groups, and C) shows amplitude of events for the same. No significant difference was observed in frequency or amplitude of sIPSCs ($p>0.05$, n denotes number of cells).

CHAPTER 6
CONCLUSIONS

Findings and Future Directions

Although epilepsy is one of the oldest conditions to be described in ancient Babylonian texts dating 3000 years ago, the mechanisms that cause seizures still elude us. Due to the complex etiology of epilepsy, no one therapeutic paradigm has been able to achieve a seizure-free status in patients. At best, there has been some success in controlling seizures with anti-epileptic drugs, but these have also not been without caveats. Supplementary to the usage of medication, the Vagus Nerve Stimulator was approved by the FDA in 1997 (Schachter 2002) to be used as an adjunct therapy for patients whose seizures were refractory. In addition, there has been developing interest in Deep Brain Stimulation (DBS) to be used for epilepsy therapy as well. Although DBS has been approved for Parkinson's disease patients and essential tremor (Collins, et al. 2010), it's utility for epilepsy is yet to be fully exploited.

Given the significant population of people afflicted with epilepsy in the United States as well as world-wide, and the 30% from this pool that currently have medically refractory epilepsy, the need for an alternate, safe therapy becomes imminent. The rationale for the project described in this dissertation was not only to devise an alternative treatment for intractable temporal lobe seizures, but also to encompass all the NIH benchmarks in the field of epilepsy research. We utilized gene therapy to construct viral vectors coding for the SST gene to be tested in a rodent model of epilepsy. The details of the construction of three viral vectors we used in our study are discussed in Chapter 3. We saw robust transduction of hippocampal neurons with the use of AAV5 vectors, with sustained temporal and spatial gene expression. The next

step in our approach was to introduce viral vector-delivered SST into hippocampal regions of the rodent brain and then induce seizures by use of electrical stimulation. We observed that 70% of the SST over-expressing animal group did not have a single grade 5 (generalized) seizure compared to control animals. Additionally, the seizure quantification index, calculated by taking into account the behavioral grade as well as seizure length at each stimulation was lower in SST over-expressing rats as compared to controls. This shows a protective effect of SST against the progression of epilepsy. We next performed preliminary testing of these animals in the Morris water maze to assess any impairment in learning and memory based on SST's involvement in the modulation of such cognitive tasks (Gahete, et al.). We observed that SST over-expressing animals performed just as well as naïve and control animals. There was a trend in SST over-expressing animals to perform slightly better in the testing phase 24 hours post training in the water maze, but it is difficult to say this unequivocally due to low sample size. Impairments in memory and learning are also common co-morbidities in patients with epilepsy, so our preliminary assessment is promising for use of SST with this patient population. Lastly, we also attempted to corroborate our *in vivo* findings with *in vitro* mechanisms by using whole patch-clamp techniques to record spontaneous excitatory and inhibitory post-synaptic currents in gene-injected animals versus controls. We did not see a difference in the frequency or amplitude of sEPSCs or sIPSCs. We plan to parse out the *in vitro* effects more in detail by more stringent identification of the substrates that may be responsible for the behavioral changes observed and discussed in Chapter 4.

This study is the first undertaking to identify the *in vivo* effects of SST over-expression by use of viral vectors in an electrical kindling model of temporal lobe epilepsy. There has been a plethora of information obtained from the use of *in vitro* models and how SST may play a role in modulating seizure activity, yet no attempt to demonstrate its neuroprotective effects has thus far been made which would validate its use as a therapeutic agent. We used the electrical kindling model not only because it provides a nice transition from low-grade to high-grade seizures, but also makes it easier to quantify the severity of seizures. We utilized gene therapy as the approach to deliver SST as a therapeutic agent within the CNS, since the technique utilizes viral vectors to deliver the gene without itself having pathological effects within the CNS. Additionally, using a vector with a constitutively active, non-specific promoter allows maximal transgene expression with limited deleterious effects to the host. Future exploration of the effects of SST in a chronic model of temporal lobe epilepsy will further validate its use as an effective alternative for preventing and treating intractable epilepsy. Additionally, assessment of how potassium M-currents and other putative mechanisms are modulated in SST gene-injected animals will also help validate our *in vivo* findings with the current knowledge of the mechanisms of SST.

Highlights in Epilepsy Research – How Far We Have Come

Traditionally, epileptic seizures or “fits” were associated with possession by evil spirits, witchcraft, or poisoning and believed by many to be contagious. Later on, seizures were believed to arise from an imbalance in bodily humors and trepanation and cauterization of the skull was used to “treat” this disorder. The complexity of this disorder was recognized as early as about 400 BC, reflected by Hippocrates remarking that epilepsy would cease to be considered divine the day it was understood.

Since then, much work has been done to unravel the underlying cause of seizures as well as identification of electroencephalographic biomarkers that could point to the onset of an imminent seizure. The understanding of the complex nature of seizures is important since they are a common phenotypic feature not just of epilepsy but also a myriad of other neurological diseases like multiple sclerosis (Catenoix, et al. 2010), Alzheimer's disease (Jayadev, et al. 2010), tuberous sclerosis (Zamponi, et al. 2010) etc. Additionally, the cost of epilepsy is estimated to be about $15.5 billion annually in the U.S. in direct and indirect costs (http://www.epilepsyfoundation.org/). The adverse effects of epilepsy and its treatments are often highlighted among the patient population that is refractory to current medical treatments. About 50% of patients diagnosed with epilepsy become seizure-free after their first antiepileptic drug (Kwan and Brodie 2001). However, it is generally accepted that 30% of patients with epilepsy never achieve a seizure-free status with AEDs (Brodie 2005, Kwan and Brodie 2000). Refractory epilepsy is generally diagnosed after two AEDs fail to control seizures, either because of low efficacy or serious side-effects that make the drug intolerable (Kwan and Brodie 2002). Current treatments for this patient population that does not achieve remission with AEDs include the use of the vagus nerve stimulator (VNS), the ketogenic diet and surgical resection of the seizure focus in severe instances. The United States Food and Drug Administration (FDA) approved the use of the VNS in 1997 as an adjunctive therapy for partial-onset epilepsy. It has been reported that about 50% of patients with the VNS observe a seizure reduction of greater than 50%, but less than 10% become completely seizure-free (Jobst 2010). The exact mechanism of the VNS is currently unknown, but it is believed that stimulation of the vagus nerve increases the inhibitory

GABA-ergic tone and inhibition of abnormal cortical activity due to activation by the reticular system (Ghanem and Early 2006). The ketogenic diet is a specialized diet with a high fat and low carbohydrate content. Although the mechanism of action of this therapy is also unknown, it is believed that the high fat diet metabolizes to produce fatty acids and ketone bodies. These ketone bodies pass into the brain and replace glucose as the major energy source. The resulting ketosis, or high level of ketones in the blood leads to a reduction in the frequency of seizures (Freeman, et al. 2007). The option of surgical resection is intuitively the most invasive and also carries the most risk. Surgical resection is only an option for patients whose seizure focus can be identified with the use of imaging and EEG techniques. Surgical resection, no doubt has the highest risk due to the invasiveness of the procedure. Nonetheless, even this treatment is not without caveats, and a percentage of the population will go into remission for a period of time before the epileptic focus shifts and seizures emerge again.

It is therefore prudent that new treatment options be evaluated for the vast population of patients suffering from refractory epilepsy. Not only should the treatment be effective, but also have limited side effects. The National Institute of Health has laid benchmarks for epilepsy to guide future research. The first benchmark area is ‘prevention of epilepsy and its progression.’ The second to ‘develop new therapeutic strategies and optimize current approaches to cure epilepsy’ and the third being to ‘prevent, limit, and reverse the co-morbidities associated with epilepsy and its treatment.’ Our study encompasses portions, if not all of these three benchmarks in trying to optimize gene-delivery with the use of viral vectors and promoters that will achieve best transgene expression into specific regions of the brain. We have also

shown that SST lends protection against the generalization of seizures in our animal model, with no exacerbation of potential co-morbidities.

The Future of Gene Therapy

The use of gene therapy has not been without controversy. The first reports of using exogenous “good” DNA to replace defective DNA for curing genetic diseases were made in 1972 (Friedmann and Roblin 1972). The first human gene therapy trial in the United States took place in 2002, and since then there have been many subsequent ones for the treatment of a plethora of diseases. The successes of gene therapy have been received with mixed reviews, both due to medical as well as ethical reasons. The death of Jesse Gelsinger in 1999 after a gene therapy trial for ornithine transcarbamylase deficiency (Wilson 2009) using the adenovirus vector was a big setback for scientists. It raised concerns about the safety of using viral vectors as vehicles for delivering therapeutic genes to the human body.

There have been tremendous advances in the development of safer and more efficient viral vectors since then. The development of self-complementary AAV (scAAV) vectors has been a milestone in increasing the efficiency of AAV vectors, since the conversion of single stranded DNA to double-stranded DNA is the rate-limiting step in gene expression (McCarty, et al. 2001). Furthermore, engineering viral capsids to alter surface-exposed tyrosine residues has also increased the efficiency of transgene expression by many-fold due to the avoidance of ubiquitination that leads to degradation (Markusic, et al. 2010). AAV also infects a wide variety of cell types within the body, thereby being a vector of choice for treating many diseases with disparate phenotypes. Many human gene therapy trials involving the use of AAV are currently in Phase I/II clinical trials for the treatment of hemophilia (Manno, et al. 2003), cystic fibrosis (Flotte

and Laube 2001), Parkinson's disease (Kaplitt, et al. 2007), Batten's disease (Worgall, et al. 2008) and muscular dystrophy (Mandel, et al. 2006) to name a few.

The intractable nature of temporal lobe seizures has also spurred research involving the use of neuropeptides in gene therapy. The use of endogenous neuropeptides like galanin and NPY for epilepsy has recently shown beneficial effects by prolonging the latent period following administration of proconvulsants (McCown 2006, Noe, et al. 2009, Noe, et al. 2007, Schlifke, et al. 2006). Pre-clinical successes of these neuropeptides have further strengthened the rationale behind human clinical trials that are currently under evaluation by the FDA (Vezzani 2007). Our study with SST has further enhanced this field by reporting protective effects *in vivo* that have thus far been unexplored. With the use of such peptides that are endogenous to the CNS, the looming threat of an immune response can be minimized. This same property however, makes the use of such peptides a little more risky since they have a variety of functions within the body and alterations in the levels of the molecules and their cognate receptors may have disastrous outcomes. The use of neuron-specific promoters has made this problem trivial to some extent. The only drawback of using AAV has thus far been the limited size of transgene that can be packaged into the vector since most therapeutic genes require complete replacement of the virus's ~4.7 kilobase genome. Experimentation is currently underway to overcome the problem of limited cloning capacity.

The introduction of transgenes can also be facilitated with the use of non-viral vectors. The use of naked DNA and lipoplexes was deemed favorable because of the low cost of production, as well as low immunogenicity. However, a major drawback for

using such vectors has been low levels of transfection and gene expression in addition to risk of *in vivo* toxicity. Furthermore, direct application of exogenous peptides like SST may also not be a viable option due to the short half-life of the peptide (~3 minutes). Additionally, peptides and peptide analogs may not be able to cross the blood brain barrier to reach the site of interest, which makes the usage of largely benign viral vectors attractive as tools for delivering these therapeutic agents.

SST in the CNS

All somatostatin positive neurons in the hippocampus have been classified as interneurons based on morphology, distribution and frequency of occurrence (Freund and Buzsaki 1996). They are all also immunoreactive for GAD, the enzyme responsible for the conversion of glutamate to GABA. SST-containing interneurons in the dentate hilus are synonymous with HIPP cells (Freund and Buzsaki 1996). The dendritic arbors of practically all major “dendritic inhibitory cells” were described initially by Ramon y Cajal (Cajal 1893) and Amaral (Amaral 1978) using Golgi impregnation. HIPP cells were first identified with dendritic arborization limited exclusively to the dentate hilus with extensive axonal innervations to the outer two-thirds of the outer molecular layer of the hippocampus (Han, et al. 1993). HIPP cells largely form symmetrical (inhibitory) synapses on dentate granule cells in conjunction with entorhinal afferents. Dendrites of HIPP cells are also recurrently innervated by axons of granule cells called mossy fibers. Thus, there exists a feedback inhibition of granule cells through these HIPP cells. The laminar distribution of SST-positive axons is consistent from animal to animal within the dentate hilus. Another subset of neurons within the hippocampal CA1 subfield that contains SST are the O-LM cells; interneurons with soma and dendrites in the stratum oriens and axons in lacunosum-moleculare. SST-containing cells are more widespread

in the CA3, with multipolar dendritic trees and frequently occur in stratum pyramidale, stratum lucidum and stratum radiatum. Some cells can also be found in the myelinated axon bundles of the alveus in both regions, but especially in the CA1. SST-positive cells are also immunoreactive for mGluR1 and substance P receptors. Similar to the hilus, SST-positive cells receive excitatory inputs from principal cells in the CA3 and CA1, and thus are likely to mediate feedback inhibition of these principal innervating neurons. SST-containing interneurons in the hilus do not contain calcium binding proteins like parvalbumin, calretinin, or calbindin (Tallent 2007), on the contrary most CA3 and CA1 SST interneurons contain at least one of these (Bouilleret, et al. 2000b).

Dentate hilar interneurons containing SST have gained interest due to studies showing upregulation as a result of mild seizures and vulnerability as a result of severe seizures (Tallent 2007). Additionally, a novel role of SST with implications on Alzheimer's disease has also been reported. It has been shown that SST upregulates neprilysin activity, which is an Aß degrading peptidase. SST knockout models have shown an increase in Aß-42 levels, which is the amyloid fragment most closely associated to Alzheimer's pathology (Saito, et al. 2005). Changes in CNS SST levels have also been reported in other neurodegenerative diseases associated with cognitive impairment. Reduced levels of SST in the CSF were reported in people suffering from depression (Agren and Lundqvist 1984), Parkinson's disease with an onset of cognitive decline (Epelbaum, et al. 1983), multiple sclerosis (Vecsei, et al. 1990) and schizophrenia (Gabriel, et al. 1996).

The contribution of this dissertation has been to highlight the novel role of SST as a potentially new therapeutic agent for temporal lobe epilepsy through a proof-of-

principle, pre-clinical study. Our data adds an *in vivo* dimension to the existing *in vitro* literature highlighting the role of this ubiquitous neuropeptide in the modulation of an epileptic network. Our proof-of-principle findings necessitate further testing of SST in more robust, translational models of epilepsy before it can be considered a valid candidate for testing in human patients. Nonetheless, the protection rendered by somatostatin against the generalization of seizures in a rodent model is a significant first step towards addressing the need for alternative therapies for intractable epilepsy, which may ultimately also be utilized for other diseases.

APPENDIX
SEQUENCE OF PREPROSOMATOSTATIN

ATGCTGTCCTGCCGTCTCCAGTGCGCGCTGGCCGCGCTCTGCATCGTCCTGGCTT

TGGGCGGTGTCACCGGGGCGCCCTCGGACCCCAGACTCCGTCAGTTTCTGCAGA

AGTCTCTGGCGGCTGCCACCGGGAAACAGGAACTGGCCAAGTACTTCTTGGCAGA

ACTGCTGTCCGAGCCCAACCAGACAGAGAACGATGCCCTGGAGCCTGAGGATTTG

CCCCAGGCAGCTGAGCAGGACGAGATGAGGCTGGAGCTGCAGAGGTCTGCCAAC

TCGAACCCAGCCATGGCACCCCGGGAACGCAAAGCTGGCTGCAAGAACTTCTTCT

GGAAGACATTCACATCCTGTTAGCTTTAATATTGTTGTCTCAGCCAGACCTCTGAAG

GGCGAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTAGCG

GATCCGTCGACTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGC

CATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCC

ACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAT

TCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAC

AATAGCAGGCATGCTGGGGAGAGATCTAGGAACCCCTAGTGATGGAGTTGGCCAC

TCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCG

TCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGG

GAGTGGCCAACCCCCCCCCCCCCCCCCCTGCAGCCCTGCATTAATGAATCGGCCA

ACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACT

GACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAG

GCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAG

CAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTT

CCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGG

TGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCC

TCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTC

CCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGT

GTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGAC

CGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTT

ATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGC

GGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAG

TATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGC

TCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCA

GCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGG

GGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTA

TCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCT

AAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCAC

CTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGT

AGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACC

GCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGA

AGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTA

ATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTT

GTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT

CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAA

AAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGT

GTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGT

AAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTAT

GCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT

AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTC

AAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACT

GATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGG

CAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACT

CTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATAC

ATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGA

AAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAAT

AGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACC

TCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGG

GAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCT

GGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTG

TGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGAAATTGTAAACGT

TAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAAT

AGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTG

AGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGT

CAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCC

TAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGG

GAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGA

AGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCAC

GCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTC

GCGCCATTCGCCATTCAGGCTACGCAACTGTTGGGAAGGGCGATCGGTGCGGGC

CTCTTCGCTATTACGCCAGGCTGCA

LIST OF REFERENCES

Aenlle KK, Kumar A, Cui L, Jackson TC, Foster TC. (2009) Estrogen effects on cognition and hippocampal transcription in middle-aged mice. *Neurobiol Aging* 30:932-945.

Agren H, Lundqvist G. (1984) Low levels of somatostatin in human CSF mark depressive episodes. *Psychoneuroendocrinology* 9:233-248.

Akins PT, Belko J, Uyeki TM, Axelrod Y, Lee KK, Silverthorn J. (2010) H1N1 Encephalitis with Malignant Edema and Review of Neurologic Complications from Influenza. *Neurocrit Care*.

Amaral DG. (1978) A Golgi study of cell types in the hilar region of the hippocampus in the rat. *J Comp Neurol* 182:851-914.

Amaral DG, Insausti R, Campbell MJ. (1988) Distribution of somatostatin immunoreactivity in the human dentate gyrus. *J Neurosci* 8:3306-3316.

Amaral DG, Lavenex P. (2007) *Hippocampal Neuroanatomy. In: The Hippocampus Book*. Oxford University Press, New York.

Amunts K, Kedo O, Kindler M, Pieperhoff P, Mohlberg H, Shah NJ, Habel U, Schneider F, Zilles K. (2005) Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. *Anat Embryol (Berl)* 210:343-352.

Andersen P, Bliss TV, Lomo T, Olsen LI, Skrede KK. (1969) Lamellar organization of hippocampal excitatory pathways. *Acta Physiol Scand* 76:4A-5A.

Aroniadou-Anderjaska V, Fritsch B, Qashu F, Braga MF. (2008) Pathology and pathophysiology of the amygdala in epileptogenesis and epilepsy. *Epilepsy Res* 78:102-116.

Bancaud J, Henriksen O, Rubio-Donnadieu F, Seino M, Dreifuss FE, Perry JK. (1981) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia* 22:489-501.

Bantel-Schaal U, zur Hausen H. (1984) Characterization of the DNA of a defective human parvovirus isolated from a genital site. *Virology* 134:52-63.

Baraban SC, Tallent MK. (2004) Interneuron Diversity series: Interneuronal neuropeptides--endogenous regulators of neuronal excitability. *Trends Neurosci* 27:135-142.

Bartfai T, Iverfeldt K, Fisone G, Serfozo P. (1988) Regulation of the release of coexisting neurotransmitters. *Annu Rev Pharmacol Toxicol* 28:285-310.

Bennewitz MF, Saltzman WM. (2009) Nanotechnology for delivery of drugs to the brain for epilepsy. *Neurotherapeutics* 6:323-336.

Berns KI, Linden RM. (1995) The cryptic life style of adeno-associated virus. *Bioessays* 17:237-245.

Bertram E. (2007) The relevance of kindling for human epilepsy. *Epilepsia* 48 Suppl 2:65-74.

Bertram EH, Zhang D, Williamson JM. (2008) Multiple roles of midline dorsal thalamic nuclei in induction and spread of limbic seizures. *Epilepsia* 49:256-268.

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

Bouilleret V, Loup F, Kiener T, Marescaux C, Fritschy JM. (2000a) Early loss of interneurons and delayed subunit-specific changes in GABA(A)-receptor expression in a mouse model of mesial temporal lobe epilepsy. *Hippocampus* 10:305-324.

Bouilleret V, Schwaller B, Schurmans S, Celio MR, Fritschy JM. (2000b) Neurodegenerative and morphogenic changes in a mouse model of temporal lobe epilepsy do not depend on the expression of the calcium-binding proteins parvalbumin, calbindin, or calretinin. *Neuroscience* 97:47-58.

Bowen R. (2002) Hypertexts for Biomedical Sciences.

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

Brodie MJ. (2005) Diagnosing and predicting refractory epilepsy. *Acta Neurol Scand Suppl* 181:36-39.

Buckmaster PS, Dudek FE. (1997) Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol* 385:385-404.

Buckmaster PS, Otero-Corchon V, Rubinstein M, Low MJ. (2002) Heightened seizure severity in somatostatin knockout mice. *Epilepsy Res* 48:43-56.

Cajal Ry. (1893) Estructura del asfa de Ammon y fascia dentata. *Ann SOC Esp Hist Nat* 22.

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

Cascino GD. (2009) Temporal lobe epilepsy is a progressive neurologic disorder: Time means neurons! *Neurology* 72:1718-1719.

Catenoix H, Marignier R, Ritleng C, Dufour M, Mauguiere F, Confavreux C, Vukusic S. (2010) Multiple sclerosis and epileptic seizures. *Mult Scler*.

Chan-Palay V. (1987) Somatostatin immunoreactive neurons in the human hippocampus and cortex shown by immunogold/silver intensification on vibratome sections: coexistence with neuropeptide Y neurons, and effects in Alzheimer-type dementia. *J Comp Neurol* 260:201-223.

Chen S, Kapturczak M, Loiler SA, Zolotukhin S, Glushakova OY, Madsen KM, Samulski RJ, Hauswirth WW, Campbell-Thompson M, Berns KI, Flotte TR, Atkinson MA, Tisher CC, Agarwal A. (2005) Efficient transduction of vascular endothelial cells with recombinant adeno-associated virus serotype 1 and 5 vectors. *Hum Gene Ther* 16:235-247.

Cherubini E, North RA. (1985) Mu and kappa opioids inhibit transmitter release by different mechanisms. *Proc Natl Acad Sci U S A* 82:1860-1863.

Chiorini JA, Kim F, Yang L, Kotin RM. (1999) Cloning and characterization of adeno-associated virus type 5. *J Virol* 73:1309-1319.

Chiu MJ, Lin CW, Chen CC, Chen TF, Chen YF, Liu HM, Chu CP, Liou HH, Hua MS. (2010) Impaired gist memory in patients with temporal lobe epilepsy and hippocampal sclerosis. *Epilepsia* 51:1036-1042.

Choi IS, Cho JH, Lee MG, Choi BJ. (2005a) Pilocarpine-induced seizure susceptibility in rats following prenatal methylazoxymethanol treatment. *Biol Pharm Bull* 28:1408-1413.

Choi VW, McCarty DM, Samulski RJ. (2005b) AAV hybrid serotypes: improved vectors for gene delivery. *Curr Gene Ther* 5:299-310.

Collins KL, Lehmann EM, Patil PG. (2010) Deep brain stimulation for movement disorders. *Neurobiol Dis* 38:338-345.

Colmers WF, Lukowiak K, Pittman QJ. (1985) Neuropeptide Y reduces orthodromically evoked population spike in rat hippocampal CA1 by a possibly presynaptic mechanism. *Brain Res* 346:404-408.

Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben-Ari Y, Esclapez M, Bernard C. (2001) Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nat Neurosci* 4:52-62.

Crompton DE, Scheffer IE, Taylor I, Cook MJ, McKelvie PA, Vears DF, Lawrence KM, McMahon JM, Grinton BE, McIntosh AM, Berkovic SF. (2010) Familial mesial temporal lobe epilepsy: a benign epilepsy syndrome showing complex inheritance. *Brain*.

Csaba Z, Pirker S, Lelouvier B, Simon A, Videau C, Epelbaum J, Czech T, Baumgartner C, Sperk G, Dournaud P. (2005) Somatostatin receptor type 2 undergoes plastic changes in the human epileptic dentate gyrus. *J Neuropathol Exp Neurol* 64:956-969.

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* 19:2531-2538.

Davies P, Katzman R, Terry RD. (1980) Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementa. *Nature* 288:279-280.

Davis KL, Mohs RC, Marin DB, Purohit DP, Perl DP, Lantz M, Austin G, Haroutunian V. (1999) Neuropeptide abnormalities in patients with early Alzheimer disease. *Arch Gen Psychiatry* 56:981-987.

Daya S, Berns KI. (2008) Gene therapy using adeno-associated virus vectors. *Clin Microbiol Rev* 21:583-593.

De Garengeot RJC. (1742) *Splanchnologie ou l'anatomie des viscères*, Paris.

de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. (1989) Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res* 495:387-395.

Dinocourt C, Petanjek Z, Freund TF, Ben-Ari Y, Esclapez M. (2003) Loss of interneurons innervating pyramidal cell dendrites and axon initial segments in the CA1 region of the hippocampus following pilocarpine-induced seizures. *J Comp Neurol* 459:407-425.

Doloff JC, Waxman DJ. (2010) Dual E1A oncolytic adenovirus: targeting tumor heterogeneity with two independent cancer-specific promoter elements, DF3/MUC1 and hTERT. *Cancer Gene Ther*.

Dreyer JL. (2010) Lentiviral Vector-Mediated Gene Transfer and RNA Silencing Technology in Neuronal Dysfunctions. *Mol Biotechnol*.

Dudek FE. (2009) Commentary: a skeptical view of experimental gene therapy to block epileptogenesis. *Neurotherapeutics* 6:319-322.

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

Epelbaum J, Ruberg M, Moyse E, Javoy-Agid F, Dubois B, Agid Y. (1983) Somatostatin and dementia in Parkinson's disease. *Brain Res* 278:376-379.

Flotte TR. (2005) Adeno-associated virus-based gene therapy for inherited disorders. *Pediatr Res* 58:1143-1147.

Flotte TR, Berns KI. (2005a) Adeno-Associated Viral Vectors for Gene Therapy. Laboratory Techniques in Biochemistry and Molecular Biology 31.

Flotte TR, Berns KI. (2005b) Adeno-associated virus: a ubiquitous commensal of mammals. *Hum Gene Ther* 16:401-407.

Flotte TR, Laube BL. (2001) Gene therapy in cystic fibrosis. *Chest* 120:124S-131S.

Freeman JM, Kossoff EH, Hartman AL. (2007) The ketogenic diet: one decade later. *Pediatrics* 119:535-543.

Freund TF, Buzsaki G. (1996) Interneurons of the hippocampus. *Hippocampus* 6:347-470.

Friedmann T, Roblin R. (1972) Gene therapy for human genetic disease? *Science* 178:648-649.

Gabriel SM, Davidson M, Haroutunian V, Powchik P, Bierer LM, Purohit DP, Perl DP, Davis KL. (1996) Neuropeptide deficits in schizophrenia vs. Alzheimer's disease cerebral cortex. *Biol Psychiatry* 39:82-91.

Gahete MD, Rubio A, Duran-Prado M, Avila J, Luque RM, Castano JP. Expression of Somatostatin, cortistatin, and their receptors, as well as dopamine receptors, but not of neprilysin, are reduced in the temporal lobe of Alzheimer's disease patients. *J Alzheimers Dis* 20:465-475.

Gavrilovici C, D'Alfonso S, Dann M, Poulter MO. (2006) Kindling-induced alterations in GABAA receptor-mediated inhibition and neurosteroid activity in the rat piriform cortex. *Eur J Neurosci* 24:1373-1384.

Ghanem T, Early SV. (2006) Vagal nerve stimulator implantation: an otolaryngologist's perspective. *Otolaryngol Head Neck Surg* 135:46-51.

Ghijsen WE, Zuiderwijk M, Lopes da Silva FH. (2007) Electrically evoked GABA release in rat hippocampus CA1 region and its changes during kindling epileptogenesis. *Brain Res* 1135:69-76.

Goddard GV. (1967) Development of epileptic seizures through brain stimulation at low intensity. *Nature* 214:1020-1021.

Greene JR, Mason A. (1996) Effects of somatostatin and related peptides on the membrane potential and input resistance of rat ventral subicular neurons, in vitro. *J Pharmacol Exp Ther* 276:426-432.

Halbert CL, Allen JM, Miller AD. (2001) Adeno-associated virus type 6 (AAV6) vectors mediate efficient transduction of airway epithelial cells in mouse lungs compared to that of AAV2 vectors. *J Virol* 75:6615-6624.

Han ZS, Buhl EH, Lorinczi Z, Somogyi P. (1993) A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurons in the dentate gyrus of the rat hippocampus. *Eur J Neurosci* 5:395-410.

Hauser AH, D. . (1990) *Epilepsy: frequency, causes and consequences*. Demos, New York.

Hauser WA. (1992) The natural history of drug resistant epilepsy: epidemiologic considerations. *Epilepsy Res Suppl* 5:25-28.

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

Hoyer D, Lubbert H, Bruns C. (1994) Molecular pharmacology of somatostatin receptors. *Naunyn Schmiedebergs Arch Pharmacol* 350:441-453.

http://www.epilepsyfoundation.org/.

http://www.neuropeptides.nl/.

http://www.wrongdiagnosis.com/e/epilepsy/stats.htm.

Ichikou N, Ieiri I, Higuchi S, Hirata K, Yamada H, Aoyama T. (1990) Analysis of the factors influencing anti-epileptic drug concentrations--carbamazepine. *J Clin Pharm Ther* 15:337-349.

Ito M. (2009) Functional roles of neuropeptides in cerebellar circuits. *Neuroscience* 162:666-672.

Jannetto PJ, Bratanow NC. (2010) Pharmacogenomic considerations in the opioid management of pain. *Genome Med* 2:66.

Jansari AS, Davis K, McGibbon T, Firminger S, Kapur N. (2010) When "long-term memory" no longer means "forever": analysis of accelerated long-term forgetting in a patient with temporal lobe epilepsy. *Neuropsychologia* 48:1707-1715.

Janson CG, McPhee SW, Leone P, Freese A, During MJ. (2001) Viral-based gene transfer to the mammalian CNS for functional genomic studies. *Trends Neurosci* 24:706-712.

Jayadev S, Leverenz JB, Steinbart E, Stahl J, Klunk W, Yu CE, Bird TD. (2010) Alzheimer's disease phenotypes and genotypes associated with mutations in presenilin 2. *Brain* 133:1143-1154.

Jequier Gygax M, Deonna T, Maeder P, Mayor-Dubois C, Roulet-Perez E. (2010) Acute infantile encephalopathy predominantly affecting the frontal lobe (AIEF): A European case. *Eur J Paediatr Neurol*.

Jiao Y, Sun Z, Lee T, Fusco FR, Kimble TD, Meade CA, Cuthbertson S, Reiner A. (1999) A simple and sensitive antigen retrieval method for free-floating and slide-mounted tissue sections. *J Neurosci Methods* 93:149-162.

Jobst BC. (2010) Electrical stimulation in epilepsy: vagus nerve and brain stimulation. *Curr Treat Options Neurol* 12:443-453.

Kairiss EW, Racine RJ, Smith GK. (1984) The development of the interictal spike during kindling in the rat. *Brain Res* 322:101-110.

Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, Bland RJ, Young D, Strybing K, Eidelberg D, During MJ. (2007) Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet* 369:2097-2105.

Kits KS, Mansvelder HD. (2000) Regulation of exocytosis in neuroendocrine cells: spatial organization of channels and vesicles, stimulus-secretion coupling, calcium buffers and modulation. *Brain Res Brain Res Rev* 33:78-94.

Klein RL, Hamby ME, Gong Y, Hirko AC, Wang S, Hughes JA, King MA, Meyer EM. (2002) Dose and promoter effects of adeno-associated viral vector for green fluorescent protein expression in the rat brain. *Exp Neurol* 176:66-74.

Klein RL, Meyer EM, Peel AL, Zolotukhin S, Meyers C, Muzyczka N, King MA. (1998) Neuron-specific transduction in the rat septohippocampal or nigrostriatal pathway by recombinant adeno-associated virus vectors. *Exp Neurol* 150:183-194.

Kohler C, Eriksson LG, Davies S, Chan-Palay V. (1987) Co-localization of neuropeptide tyrosine and somatostatin immunoreactivity in neurons of individual subfields of the rat hippocampal region. *Neurosci Lett* 78:1-6.

Kosaka T, Wu JY, Benoit R. (1988) GABAergic neurons containing somatostatin-like immunoreactivity in the rat hippocampus and dentate gyrus. *Exp Brain Res* 71:388-398.

Krettek JE, Price JL. (1977) Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. *J Comp Neurol* 172:723-752.

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

Kwan P, Brodie MJ. (2001) Effectiveness of first antiepileptic drug. *Epilepsia* 42:1255-1260.

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

Kwan P, Brodie MJ. (2003) Clinical trials of antiepileptic medications in newly diagnosed patients with epilepsy. *Neurology* 60:S2-12.

Leranth C, Malcolm AJ, Frotscher M. (1990) Afferent and efferent synaptic connections of somatostatin-immunoreactive neurons in the rat fascia dentata. *J Comp Neurol* 295:111-122.

Levine SZ, Lurie I, Kohn R, Levav I. Trajectories of the course of schizophrenia: From progressive deterioration to amelioration over three decades. *Schizophr Res*.

Ludwig M, Pittman QJ. (2003) Talking back: dendritic neurotransmitter release. *Trends Neurosci* 26:255-261.

Maciejak P, Szyndler J, Lehner M, Turzynska D, Sobolewska A, Bidzinski A, Plaznik A. (2010) The differential effects of protein synthesis inhibition on the expression and reconsolidation of pentylenetetrazole kindled seizures. *Epilepsy Behav* 18:193-200.

Mandel RJ. (2010) CERE-110, an adeno-associated virus-based gene delivery vector expressing human nerve growth factor for the treatment of Alzheimer's disease. *Curr Opin Mol Ther* 12:240-247.

Mandel RJ, Burger C. (2004) Clinical trials in neurological disorders using AAV vectors: promises and challenges. *Curr Opin Mol Ther* 6:482-490.

Mandel RJ, Manfredsson FP, Foust KD, Rising A, Reimsnider S, Nash K, Burger C. (2006) Recombinant adeno-associated viral vectors as therapeutic agents to treat neurological disorders. *Mol Ther* 13:463-483.

Manno CS, Chew AJ, Hutchison S, Larson PJ, Herzog RW, Arruda VR, Tai SJ, Ragni MV, Thompson A, Ozelo M, Couto LB, Leonard DG, Johnson FA, McClelland A, Scallan C, Skarsgard E, Flake AW, Kay MA, High KA, Glader B. (2003) AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B. *Blood* 101:2963-2972.

Manservigi R, Argnani R, Marconi P. (2010) HSV Recombinant Vectors for Gene Therapy. *Open Virol J* 4:123-156.

Markusic DM, Herzog RW, Aslanidi GV, Hoffman BE, Li B, Li M, Jayandharan GR, Ling C, Zolotukhin I, Ma W, Zolotukhin S, Srivastava A, Zhong L. (2010) High-efficiency Transduction and Correction of Murine Hemophilia B Using AAV2 Vectors Devoid of Multiple Surface-exposed Tyrosines. *Mol Ther*.

Mazarati A, Wasterlain CG. (2002) Anticonvulsant effects of four neuropeptides in the rat hippocampus during self-sustaining status epilepticus. *Neurosci Lett* 331:123-127.

Mazarati AM, Telegdy G. (1992) Effects of somatostatin and anti-somatostatin serum on picrotoxin-kindled seizures. *Neuropharmacology* 31:793-797.

McCarty DM, Monahan PE, Samulski RJ. (2001) Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. *Gene Ther* 8:1248-1254.

McCown TJ. (2006) Adeno-associated virus-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity in vivo. *Mol Ther* 14:63-68.

McCoy SI, Jewell NP, Hubbard A, Gerdts CE, Doherty IA, Padian NS, Minnis AM. A trajectory analysis of alcohol and marijuana use among latino adolescents in san francisco, california. *J Adolesc Health* 47:564-574.

McDonald AJ, Mascagni F. (2002) Immunohistochemical characterization of somatostatin containing interneurons in the rat basolateral amygdala. *Brain Res* 943:237-244.

Mehla J, Reeta KH, Gupta P, Gupta YK. (2010) Protective effect of curcumin against seizures and cognitive impairment in a pentylenetetrazole-kindled epileptic rat model. *Life Sci*.

Meisler MH, Kearney JA. (2005) Sodium channel mutations in epilepsy and other neurological disorders. *J Clin Invest* 115:2010-2017.

Mikkelsen JD, Woldbye DP. (2006) Accumulated increase in neuropeptide Y and somatostatin gene expression of the rat in response to repeated electroconvulsive stimulation. *J Psychiatr Res* 40:153-159.

Milner TA, Bacon CE. (1989) Ultrastructural localization of somatostatin-like immunoreactivity in the rat dentate gyrus. *J Comp Neurol* 290:544-560.

Moore SD, Madamba SG, Joels M, Siggins GR. (1988) Somatostatin augments the M-current in hippocampal neurons. *Science* 239:278-280.

Mori S, Wang L, Takeuchi T, Kanda T. (2004) Two novel adeno-associated viruses from cynomolgus monkey: pseudotyping characterization of capsid protein. *Virology* 330:375-383.

Morimoto K, Fahnestock M, Racine RJ. (2004) Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol* 73:1-60.

Niwa H, Yamamura K, Miyazaki J. (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 108:193-199.

Noe F, Frasca A, Balducci C, Carli M, Sperk G, Ferraguti F, Pitkanen A, Bland R, Fitzsimons H, During M, Vezzani A. (2009) Neuropeptide Y overexpression using recombinant adeno-associated viral vectors. *Neurotherapeutics* 6:300-306.

Noe F, Nissinen J, Pitkanen A, Gobbi M, Sperk G, During M, Vezzani A. (2007) Gene therapy in epilepsy: the focus on NPY. *Peptides* 28:377-383.

Orban G, Volgyi K, Juhasz G, Penke B, Kekesi KA, Kardos J, Czurko A. Different electrophysiological actions of 24- and 72-hour aggregated amyloid-beta oligomers on hippocampal field population spike in both anesthetized and awake rats. *Brain Res* 1354:227-235.

Peel AL, Klein RL. (2000) Adeno-associated virus vectors: activity and applications in the CNS. *J Neurosci Methods* 98:95-104.

Piwko C, Thoss VS, Samanin R, Hoyer D, Vezzani A. (1996) Status of somatostatin receptor messenger RNAs and binding sites in rat brain during kindling epileptogenesis. *Neuroscience* 75:857-868.

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

Rabinowitz JE, Bowles DE, Faust SM, Ledford JG, Cunningham SE, Samulski RJ. (2004) Cross-dressing the virion: the transcapsidation of adeno-associated virus serotypes functionally defines subgroups. *J Virol* 78:4421-4432.

Racine RJ. (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32:281-294.

Racine RJ, Paxinos G, Mosher JM, Kairiss EW. (1988) The effects of various lesions and knife-cuts on septal and amygdala kindling in the rat. *Brain Res* 454:264-274.

Ramos B, Baglietto-Vargas D, del Rio JC, Moreno-Gonzalez I, Santa-Maria C, Jimenez S, Caballero C, Lopez-Tellez JF, Khan ZU, Ruano D, Gutierrez A, Vitorica J. (2006) Early neuropathology of somatostatin/NPY GABAergic cells in the hippocampus of a PS1xAPP transgenic model of Alzheimer's disease. *Neurobiol Aging* 27:1658-1672.

Reichlin S. (1983) Somatostatin. *N Engl J Med* 309:1495-1501.

Reimsnider S, Manfredsson FP, Muzyczka N, Mandel RJ. (2007) Time course of transgene expression after intrastriatal pseudotyped rAAV2/1, rAAV2/2, rAAV2/5, and rAAV2/8 transduction in the rat. *Mol Ther* 15:1504-1511.

Richichi C, Lin EJ, Stefanin D, Colella D, Ravizza T, Grignaschi G, Veglianese P, Sperk G, During MJ, Vezzani A. (2004) Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. *J Neurosci* 24:3051-3059.

Rivera VM, Gao GP, Grant RL, Schnell MA, Zoltick PW, Rozamus LW, Clackson T, Wilson JM. (2005) Long-term pharmacologically regulated expression of erythropoietin in primates following AAV-mediated gene transfer. *Blood* 105:1424-1430.

Robbins RJ, Brines ML, Kim JH, Adrian T, de Lanerolle N, Welsh S, Spencer DD. (1991) A selective loss of somatostatin in the hippocampus of patients with temporal lobe epilepsy. *Ann Neurol* 29:325-332.

Rutledge EA, Halbert CL, Russell DW. (1998) Infectious clones and vectors derived from adeno-associated virus (AAV) serotypes other than AAV type 2. *J Virol* 72:309-319.

Saito T, Iwata N, Tsubuki S, Takaki Y, Takano J, Huang SM, Suemoto T, Higuchi M, Saido TC. (2005) Somatostatin regulates brain amyloid beta peptide Abeta42 through modulation of proteolytic degradation. *Nat Med* 11:434-439.

Salmani ME, Fathollahi Y, Mirnajafizadeh J, Semnanian S. Epileptogenic insult alters endogenous adenosine control on long-term changes in synaptic strength by theta pattern stimulation in hippocampus area CA1. *Synapse*.

Sashindranath M, McLean KJ, Trounce IA, Cotton RG, Cook MJ. (2010) Early hippocampal oxidative stress is a direct consequence of seizures in the rapid electrical amygdala kindling model. *Epilepsy Res* 90:285-294.

Saucier D, Cain DP. (1996) Competitive NMDA receptor antagonists do not block cholinergic kindling with carbachol. *Epilepsy Res* 24:9-18.

Schachter SC. (2002) Vagus nerve stimulation therapy summary: five years after FDA approval. *Neurology* 59:S15-20.

Schlifke I, Kuteeva E, Hokfelt T, Kokaia M. (2006) Galanin expressed in the excitatory fibers attenuates synaptic strength and generalized seizures in the piriform cortex of mice. *Exp Neurol* 200:398-406.

Schmued LC, Stowers CC, Scallet AC, Xu L. (2005) Fluoro-Jade C results in ultra high resolution and contrast labeling of degenerating neurons. *Brain Res* 1035:24-31.

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* 22:27-50.

Schweitzer P, Madamba S, Siggins GR. (1990) Arachidonic acid metabolites as mediators of somatostatin-induced increase of neuronal M-current. *Nature* 346:464-467.

Schweitzer P, Madamba SG, Siggins GR. (1998) Somatostatin increases a voltage-insensitive K+ conductance in rat CA1 hippocampal neurons. *J Neurophysiol* 79:1230-1238.

Shoji Y, Tanaka E, Yamamoto S, Maeda H, Higashi H. (1998) Mechanisms underlying the enhancement of excitatory synaptic transmission in basolateral amygdala neurons of the kindling rat. *J Neurophysiol* 80:638-646.

Sloviter RS. (1987) Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science* 235:73-76.

Sloviter RS. (2008) Hippocampal epileptogenesis in animal models of mesial temporal lobe epilepsy with hippocampal sclerosis: the importance of the "latent period" and other concepts. *Epilepsia* 49 Suppl 9:85-92.

Sloviter RS, Nilaver G. (1987) Immunocytochemical localization of GABA-, cholecystokinin-, vasoactive intestinal polypeptide-, and somatostatin-like immunoreactivity in the area dentata and hippocampus of the rat. *J Comp Neurol* 256:42-60.

Solano-Castiella E, Anwander A, Lohmann G, Weiss M, Docherty C, Geyer S, Reimer E, Friederici AD, Turner R. (2010) Diffusion tensor imaging segments the human amygdala in vivo. *Neuroimage* 49:2958-2965.

Sorensen AT, Kanter-Schlifke I, Carli M, Balducci C, Noe F, During MJ, Vezzani A, Kokaia M. (2008) NPY gene transfer in the hippocampus attenuates synaptic plasticity and learning. *Hippocampus* 18:564-574.

Sorensen AT, Nikitidou L, Ledri M, Lin EJ, During MJ, Kanter-Schlifke I, Kokaia M. (2009) Hippocampal NPY gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP. *Exp Neurol* 215:328-333.

Steward O, Scoville SA. (1976) Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. *J Comp Neurol* 169:347-370.

Stief F, Zuschratter W, Hartmann K, Schmitz D, Draguhn A. (2007) Enhanced synaptic excitation-inhibition ratio in hippocampal interneurons of rats with temporal lobe epilepsy. *Eur J Neurosci* 25:519-528.

Strom LA, Koh S, Frey L. (2010) New antiepileptic drugs: lacosamide, rufinamide, and vigabatrin. *Curr Treat Options Neurol* 12:287-299.

Sun C, Mtchedlishvili Z, Bertram EH, 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* 500:876-893.

Tallent MK. (2007) Somatostatin in the dentate gyrus. *Prog Brain Res* 163:265-284.

Tallent MK, Qiu C. (2008) Somatostatin: An endogenous antiepileptic. *Mol Cell Endocrinol* 286:96-103.

Tallent MK, Siggins GR. (1997) Somatostatin depresses excitatory but not inhibitory neurotransmission in rat CA1 hippocampus. *J Neurophysiol* 78:3008-3018.

Tallent MK, Siggins GR. (1999) Somatostatin acts in CA1 and CA3 to reduce hippocampal epileptiform activity. *J Neurophysiol* 81:1626-1635.

Turnbull J, Lohi H, Kearney JA, Rouleau GA, Delgado-Escueta AV, Meisler MH, Cossette P, Minassian BA. (2005) Sacred disease secrets revealed: the genetics of human epilepsy. *Hum Mol Genet* 14 Spec No. 2:2491-2500.

Tuunanen J, Halonen T, Pitkanen A. (1997) Decrease in somatostatin-immunoreactive neurons in the rat amygdaloid complex in a kindling model of temporal lobe epilepsy. *Epilepsy Res* 26:315-327.

Vecsei L, Csala B, Widerlov E, Ekman R, Czopf J, Palffy G. (1990) Lumbar cerebrospinal fluid concentrations of somatostatin and neuropeptide Y in multiple sclerosis. *Brain Res Bull* 25:411-413.

Vezzani A. (2007) The promise of gene therapy for the treatment of epilepsy. *Expert Rev Neurother* 7:1685-1692.

Vezzani A, Ruiz R, Monno A, Rizzi M, Lindefors N, Samanin R, Brodin E. (1993) Extracellular somatostatin measured by microdialysis in the hippocampus of freely moving rats: evidence for neuronal release. *J Neurochem* 60:671-677.

Wang L, Herzog RW. (2005) AAV-mediated gene transfer for treatment of hemophilia. *Curr Gene Ther* 5:349-360.

Whittaker E, Vereker E, Lynch MA. (1999) Neuropeptide Y inhibits glutamate release and long-term potentiation in rat dentate gyrus. *Brain Res* 827:229-233.

Wilmott RW, Whitsett JA, Trapnell B, Wert S, Baughman R, Cuppoletti J, Tolstohev P. (1994) Gene therapy for cystic fibrosis utilizing a replication deficient recombinant adenovirus vector to deliver the human cystic fibrosis transmembrane conductance regulator cDNA to the airways. A phase I study. *Hum Gene Ther* 5:1019-1057.

Wilson JM. (2009) Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. *Mol Genet Metab* 96:151-157.

Womble MD, Moises HC. (1992) Muscarinic inhibition of M-current and a potassium leak conductance in neurones of the rat basolateral amygdala. *J Physiol* 457:93-114.

Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure MV, Neyzi N, Dyke JP, Ballon D, Heier L, Greenwald BM, Christos P, Mazumdar M, Souweidane MM, Kaplitt MG, Crystal RG. (2008) Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther* 19:463-474.

Xiao X, Li J, McCown TJ, Samulski RJ. (1997) Gene transfer by adeno-associated virus vectors into the central nervous system. *Exp Neurol* 144:113-124.

Zamponi N, Petrelli C, Passamonti C, Moavero R, Curatolo P. (2010) Vagus nerve stimulation for refractory epilepsy in tuberous sclerosis. *Pediatr Neurol* 43:29-34.

Zeyda T, Hochgeschwender U. (2008) Null mutant mouse models of somatostatin and cortistatin, and their receptors. *Mol Cell Endocrinol* 286:18-25.

Zolotukhin S, Potter M, Zolotukhin I, Sakai Y, Loiler S, Fraites TJ, Jr., Chiodo VA, Phillipsberg T, Muzyczka N, Hauswirth WW, Flotte TR, Byrne BJ, Snyder RO. (2002) Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. *Methods* 28:158-167.

BIOGRAPHICAL SKETCH

Rabia Zafar was born in Islamabad, Pakistan in 1982. She resided in Islamabad till completion of high school at Frobel's International School in 2001. Following this, she moved to Gainesville, Florida in August 2001 to pursue a bachelor's degree at the University of Florida. She graduated *summa cum laude* in 2005 after obtaining a Bachelor of Science in microbiology with a minor in chemistry. She enrolled in the neuroscience concentration of the Interdisciplinary Program in Biomedical Sciences for a Doctor of Philosophy in 2005. Since then, she worked in the lab of Dr. Paul R. Carney to develop a novel gene therapy approach to treating intractable temporal lobe epilepsy using animal models. The skills she obtained during her graduate school career include development of animal models, cloning of viral vectors, analysis of behavioral seizure data and *in vitro* electrophysiological techniques. Her work in Dr. Carney's lab culminated in a Ph.D. from the University of Florida in the fall of 2010.