

Award Number: **W81XWH-12-1-0258**

TITLE: Neuroprotection and Anti-Epileptogenesis with a Mitochondria-Targeted Antioxidant

PRINCIPAL INVESTIGATOR: Jeffrey H. Goodman, Ph.D.

CONTRACTING ORGANIZATION: Research Foundation for Mental Hygiene
Staten Island, New York 10314

REPORT DATE: December 2013

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE Dember2013		2. REPORT TYPE Annual Report		3. DATES COVERED 30September2012-29September2013	
4. TITLE AND SUBTITLE Pe~ rotection anã AntiEpileptogenesis with a T itochonãriaE~argeteã Antioçiaant				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-12-1-0258	
				5c. PROGRAM ELEMENT NUMBER	
E-Mail: jeffrey.goodman@downstate.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Research Foundation For Mental Hygiene Institute for Basic Research Staten Island, New York 10314				8. PERFORMING ORGANIZATION REPORT NUMBER NIIMRFR	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The goals of the project were to assess the neuroprotective and antiepileptogenic properties of a mitochondrial-targeted antioxidant, SS-31 using the pilocarpine (Pilo) model of status epilepticus (SE), the kindling seizure model and the tetanus toxin (Tx) model. Progress focused on Aim#1 due to limited access to SS-31. For Aim #2 the kindling model was established in the laboratory in preparation for drug testing. For Aim#1, adult male Sprague-Dawley rats (260-405g) were pretreated with SS-31 (3 or 10mg/kg, sc; n=14) or saline (n=10) 45min before induction of SE with Pilo (365mg/kg, sc). The latency to SE onset was measured for both groups. The brains were fixed 1-3 days after SE, sectioned through the hippocampus and stained for: Nissl, Fluoro-jade C (FJ), NeuN and heat shock protein (HSP). Three rats from each group died during SE. There was no significant difference in the latency to SE between the two groups. There was also no difference in Nissl, FJ, or NeuN staining between the groups. However one day after SE there was an increase in HSP staining in the granule cells of the dentate gyrus. These results suggest that SS-31 is not neuroprotective in the Pilo model but may be effective against less severe insults to the brain.					
15. SUBJECT TERMS Neuroprotection, antiepileptogenesis, antioxidant					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			UU
					19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusion.....	5
References.....	6
Appendices.....	6
Supporting Data.....	6

INTRODUCTION:

A number of studies have provided evidence that reactive oxygen species play a role in the induction of seizures and seizure-induced neuronal death. The goals of this project are to test the efficacy of a novel, mitochondrial-targeted antioxidant SS-31, as a neuroprotective and antiepileptogenic agent in three experimental models of epilepsy. The pilocarpine-induced model of status epilepticus will be used to test SS-31 as a neuroprotectant, the kindling model will be used to test SS-31 as an antiepileptogenic and anticonvulsant agent, and the tetanus toxin model will be used to test SS-31 as an anticonvulsant. If SS-31 proves to be effective in these studies future experiments will test SS-31 in models of traumatic brain injury.

Body:

SS-31 was created by Dr. Szeto but the rights to the drug are controlled by Stealth Peptides. Progress on the project has been limited due to limited access to the test agent, SS-31. An MTA for SS-31 was executed between the Research Foundation for Mental Hygiene, Weill Cornell Medical College and Stealth. I was offered an initial shipment of **750mg**. I requested the initial shipment contain a reduced amount of SS-31 to limit the potential that it could lose potency as we performed the initial experiments. Stealth agreed to provide **50mg** of SS-31 with a commitment to provide additional drug as needed. At the time that I requested a smaller amount I was told it would be no problem to obtain more SS-31. When I requested more drug my e-mails to Stealth and Dr. Szeto were not answered. It was not until the Research Foundation for Mental Hygiene contacted Cornell that we found out that a report was required to receive more drug. Since I had received less than 10% of the originally offered amount of SS-31, I was not aware that a progress report was required given the limited results that could be obtained with **50mg** of drug. Also, given Cornell's role in a grant whose specific goal is to test the efficacy of SS-31, I felt there was an inherent commitment that the drug would be available to complete the experiments. Below is a description of the limited results we have obtained testing SS-31. These initial experiments focused on **Aim #1** of the grant. The results from these experiments are preliminary and the interpretation of the results is limited by the number of animals examined and the amount of drug we had to work with. A progress report has been submitted to Cornell and Stealth to obtain more drug and it is our intention to complete the project once additional drug is in hand. It is likely that a no-cost extension will be necessary to complete the project.

Aim #1 – Test the neuroprotective and anticonvulsant properties of SS-31 in the pilocarpine model of status epilepticus (SE) in the rat. In this model, prolonged seizure activity causes neuronal cell death in specific neuronal populations in the rodent hippocampus. Adult male Sprague-Dawley rats (260-405g) were used. Experimental animals were pretreated with SS-31 (3 or 10mg/kg, sc) 45min before induction of SE with pilocarpine (365mg/kg, sc). One hour after the onset of SE each animal received an injection of diazepam (5mg/kg, ip) to attenuate SE and to improve survival. Control animals received an injection of saline instead of SS-31. To determine whether treatment with SS-31 affected the development of SE, the time to the onset of SE was measured for control and SS-31 treated animals. To examine neuroprotection the animals were perfusion-fixed with 4% paraformaldehyde 1-3 days after SE. The brains were sectioned on a vibratome through the dorsal hippocampus. Sections were processed for the following histochemical and immunohistochemical stains: Nissl, Fluoro-jade C (FJ), NeuN and heat shock protein 70-72 (HSP). Nissl, FJ and NeuN stains were used to assess neuroprotection. HSP was used to detect neuronal stress but under some conditions has been shown to be neuroprotective. SE was induced in a total of 10 controls and 14 experimental rats. Three rats from each group died during SE and were not available for anatomical assessment leaving a total of 7 controls and 11 drug-treated rats.

RESULTS:

1) Effect of SS-31 on the latency to the onset of SE. There was no significant difference in the latency to the onset of SE between control and SS-31 treated rats.

2) SS-31 as a neuroprotectant in SE. When we compared the tissue from SS-31-treated rats to the controls we did not detect any evidence of neuroprotection in any of the stains except for the expression of HSP. In SS-31 (10mg/kg) treated rats, sacrificed 1 day after SE we detected an increase in HSP

expression in the granule cells and a decrease in hilus of the dentate gyrus. Figure 1 compares HSP-immunoreactivity in the dentate gyrus between a control **(A)** and an SS-31 (10mg/kg, sc) treated rat **(B)**. Granule cells are usually resistant to seizure-induced cell death. It is intriguing that in this limited sample that SS-31 altered the granule cell response to prolonged seizure activity. The failure of SS-31 to delay the onset of SE and to protect vulnerable neurons suggests that it is ineffective in this model. The insult induced by the prolonged seizure activity associated with SE may be too great for SS-31 to be effective. However, the SS-31-induced change in HSP expression suggests that SS-31 is having an effect on the network and suggests that it could be protective against less severe insults to the brain.

Aim #2 –Test the antiepileptogenic properties of SS-31 in the kindling seizure model. At the beginning of the grant the kindling model was not established in the laboratory. The PI has extensive experience with the kindling model but we had to adapt our current, newly designed EEG recording system to deliver the kindling stimulus and record the resultant electrical afterdischarge (AD) and the technician working on the project had to be trained to perform the model. Kindling requires the stereotaxic implantation of depth and screw electrodes to allow for delivery of the kindling stimulus and recording of the resulting AD in awake, free-moving rats. Kindling is a seizure model where repeated, spaced delivery of an initially subconvulsive stimulus to a limbic structure results in a permanent change in brain function such that eventually the kindling stimulus regularly elicits a limbic seizure. The progression through the kindling process can be assessed by measurement of the severity of the behavioral seizure and by measurement of the threshold and duration of the AD. Behavioral seizures are scored on a 1-5 scale with stages 1-2 being equivalent to partial seizures and stages 3-5 equivalent to generalized convulsions. The time to reach a given stage, AD threshold and AD duration can be measured to assess the epileptogenic process. These metrics will be measured after treatment with SS-31 during kindling acquisition to determine whether SS-31 delays or alters the epileptogenic process. Once control animals have exhibited 3 consecutive stage 5 seizures the kindling process is considered complete the animals are considered to be fully kindled. SS-31 will also be assessed in fully-kindled animals to test its efficacy as an anticonvulsant. Figure 2 illustrates an electrographic seizure detected in the EEG of a kindled rat stimulated in the dorsal hippocampus. We have now established the kindling model in the laboratory and just need a sufficient quantity of SS-31 to complete these experiments. To test epileptogenesis SS-31 will be injected before each kindling stimulus to determine whether SS-31 delays the kindling process and alters the amount of current required to elicit an AD.

Aim #3 – Test the anticonvulsant properties of SS-31 in the Tetanus Toxin (Tx) model of mesial temporal lobe epilepsy. The Tx model is established and run on a regular basis in the laboratory. Tx when injected into the hippocampus induces spontaneous seizures without causing a lesion. Seizure detection software can be used to quantify the number of seizures in the presence and absence of drug, Figure 3 illustrates detection of an electrographic seizure from a Tx-treated rat. We will test the anticonvulsant efficacy of SS-31 in this model. These experiments can also proceed with sufficient a quantity of drug.

KEY RESEARCH ACCOMPLISHMENTS:

- Treatment with SS-31 did not delay the onset of status epilepticus in the pilocarpine model
- SS-31 was not neuroprotective in the pilocarpine model of status epilepticus
- Treatment with SS-31 altered the expression of HSP in the granule cells of the dentate gyrus one day after status epilepticus. Although this is not evidence of neuroprotection it does suggest that the drug had an effect on the network.
- The kindling and tetanus toxin models are in place and ready for drug testing.

REPORTABLE OUTCOMES:

None at this time.

CONCLUSIONS:

Progress on the project has been limited due to limited access to the test compound. There was a breakdown in communication between the PI and manufacturer of the drug which is in the process of being resolved. Initial experiments focused on Aim#1, an examination of the neuroprotective properties of SS-31 and whether treatment with SS-31 could delay the onset of SE. In both cases the data were negative. These data are interpreted to suggest that the prolonged seizure activity associated with SE was

too severe for SS-31 have an effect. Treatment with SS-31 did alter the expression of HSP, a marker of neuronal stress but has also been shown to be neuroprotective, in the granule cells of the dentate gyrus. This change in HSP expression suggests that SS-31 is interacting with the network and could have a positive effect against less severe neuronal insults. Additional progress was made establishing the kindling model in the laboratory using a state of the art 24/7 Video/EEG monitoring system. Moving forward everything is in place to complete the project.

References: None

APPENDICES: None

SUPPORTING DATA:

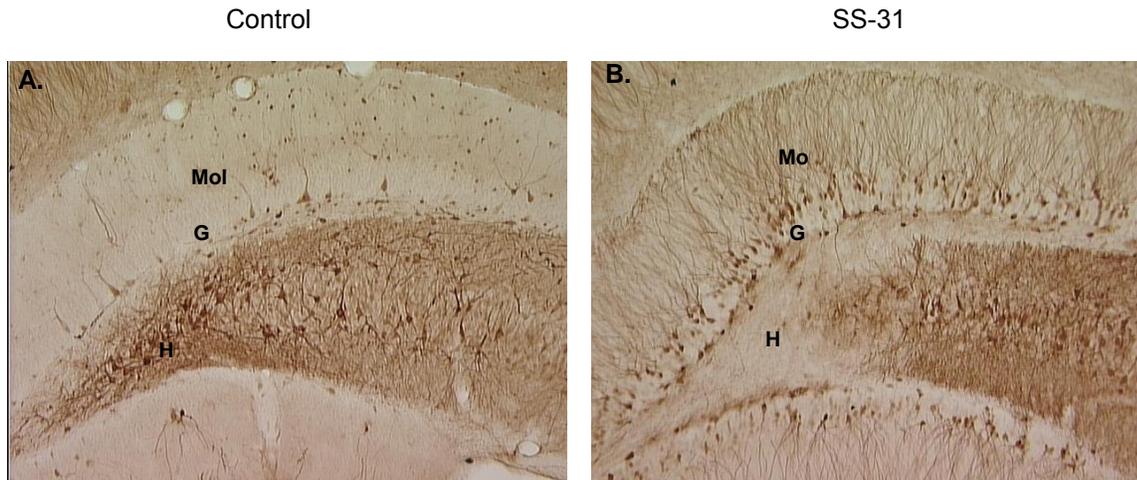


Figure 1 – Expression of HSP in the dentate gyrus 1 day after SE. A) Control; B) SS-31. Note the increased expression of HSP in the granule cell somas (G) and dendrites in the molecular layer (Mo). There appears to be less staining in the dentate hilus (H). Magnification 10x.

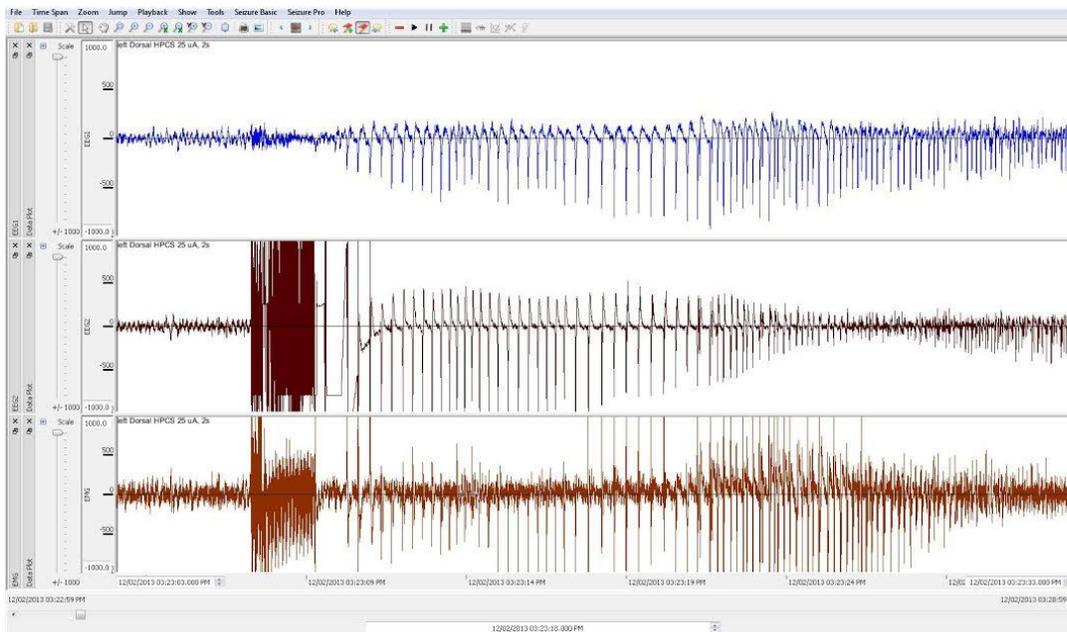


Figure 2 – Example of an awake recording of a kindled afterdischarge from a rat stimulated in the left hippocampus (60Hz, 25 μ A, 2sec). Top trace is taken from screw electrodes implanted in the skull. Middle trace from a depth electrode implanted in the left hippocampus and the bottom trace is from a depth electrode implanted in the right hippocampus. Duration of the recording is 30sec.

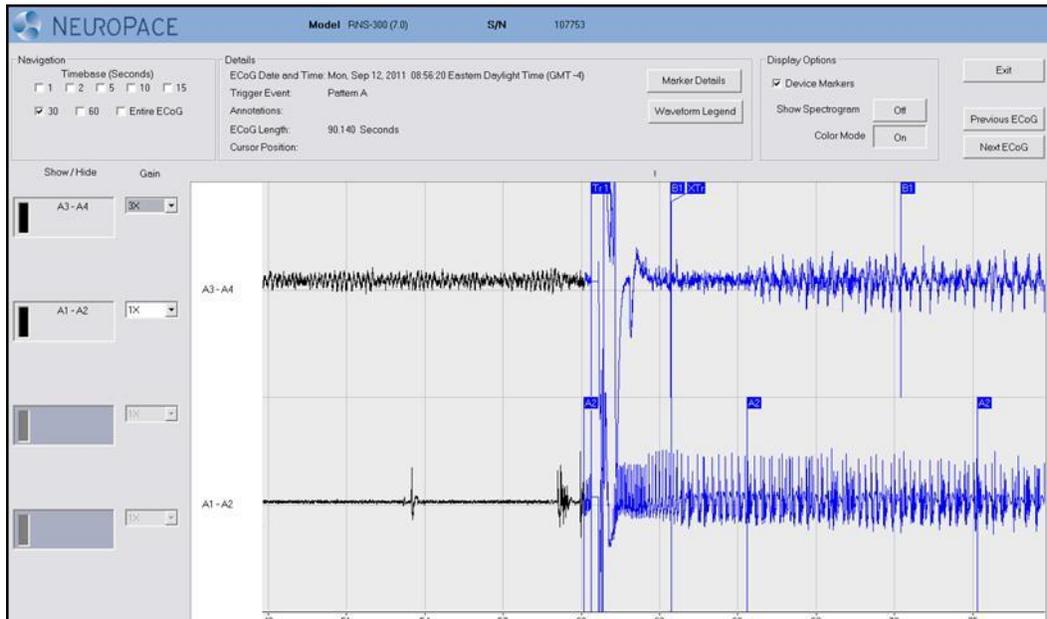


Figure 3 – Detection of a spontaneous seizure in an awake rat treated with Tx. Top trace from a depth electrode in the left hippocampus and the bottom trace is from a depth electrode in the right hippocampus. Tx was injected into the right hippocampus.