Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 - 12/31/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100054865

6. **Project Number and Title of Research Project:** 7. *Neurogenic Actions of Dietary Salt on Sympathetic-Cardiovascular Function*

7. **Start and End Date of Research Project:** 1/1/2011 – 12/31/2012

8. **Name of Principal Investigator for the Research Project:** Sean D. Stocker, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 250,000

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Wang, Xianming	Postdoctoral Scholar	100% Effort	$18,150 (sal+fr)
Bardgett, Megan	Postdoctoral Scholar	100% Effort	$13,624 (sal+fr)
Frielle, Jennifer	Research Technician	72% Effort	$1,958 (sal+fr)
Lang, Kate	Research Tech Wage	100% Effort	$6,351 (sal+fr)
Klinger, Zach	Research Tech Wage	100% Effort	$3,482 (sal+fr)

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Stocker, Sean D, PhD	PI	5
Peterson, Blaise, PhD	Co-I	5
Sinoway, Lawrence, MD	Collaborator	1
Urs Leuenberger, MD	Collaborator	1
Kevin Monahan	Collaborator	1

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
WPI A365R Stimulator	The stimulator was necessary to activate various afferent nerves in Specific Aim 2 experiments	$1,541
Kent Micro-temp LT heating therapy system	The heating system was necessary to maintain the body temperature of animals during any experimental surgical procedure (Specific Aims 1 and 2).	$1,560
Columbus Instruments Microcapnograph Sample Adaptor	The adaptor is a necessary component to a machine that measures end-tidal or expired carbon dioxide levels (Specific Aim 2).	$327
VWR Waterbath	The waterbath is necessary to maintain brain slices used for in vitro patch clamp experiments at optimal temperature (Specific Aim 1).	$804
Thor Labs HeadStage Bracket	This item is used to mount electrodes for patch-clamp recordings onto the headstage and amplifier (Specific Aim 1).	$1,529
Data Sciences International	This item is implanted in rodents to measure	$9,473

Calibrated Pressure Analog Implants	blood pressure and heart rate every day and throughout the day to measure the impact of dietary salt intake on blood pressure and blood pressure variability (Specific Aim 2).	
Kent Scientific Genie Touch Infusion Pump	The infusion pump is used in experiments to routinely and accurately infuse drugs or fluids into animals (Specific Aim 2)	$1,768
Data Sciences International Calibrated Pressure Analog Implants	This item is implanted in rodents to measure blood pressure and heart rate every day and throughout the day to measure the impact of dietary salt intake on blood pressure and blood pressure variability (Specific Aim 2).	$4,978

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
1R01 HL113270 Central Osmosensory Mechanisms in Salt-Sensitive Hypertension PI: Stocker, Sean D	■NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	June 2012	$1,912,500	$1,530,000
12EIA8230000 American Heart Association Established Investigator Award Neurogenic Actions of Dietary Sodium in Sympathetic-Cardiovascular Regulation PI: Stocker, Sean D	☐NIH ☐ Other federal (specify:_____) ■ Nonfederal source (specify: _American Heart Association)	July 2011	$400,000	$400,000
12GRNT10220000 Detrimental Effects of Excess Dietary Sodium Intake on Neurocirculatory Control in Humans PI: Monahan, Kevin	☐NIH ☐ Other federal (specify:_____) ■ Nonfederal source (specify: _American Heart Association)	July 1, 2012	$160,000	$160,000
14PRE195300011 Central Sodium-Sensing in Salt-Sensitive Hypertension PI: Kinsman, Brian	☐NIH ☐ Other federal (specify:_____) ■ Nonfederal source (specify: _American Heart Association)	January 2014	$52,000	$52,000

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

Three additional grants were recently submitted and are described below.

<u>NIH NHBLI R01 HL128388-01</u>
Title: Adverse Neurogenic Actions of Dietary Salt
Multi-PI Application: Sean D. Stocker (Penn State) & Bill Farquhar (Delaware)

Total Costs Requested: $3,825,000
The overall goal of this multi-PI R01 between Penn State University and the University of Delaware is to investigate underlying mechanisms for adverse cardiovascular effects of dietary salt intake in salt-resistant subjects using both animals and humans.
Submitted: October 5, 2014
Score: 44, Percentile: 31%
Planned Resubmission: July 5, 2015

American Heart Association Collaborative Sciences Award 15CSA241800016
Title: Adverse Effects of Dietary Salt on Neurohumoral Cardiovascular Regulation
PI Application: Sean D. Stocker
Total Costs Requested: $750,000
The overall goal of this multi-PI project is to identify novel effects of dietary salt intake on neurohumoral control of cardiovascular function in salt-resistant animals and humans.
Letter of Intent: Accepted 12/29/14 after >90% downselect
Full Application Submitted: 2/10/15

NIH NHLBI HL113270 Supplement
Title: Sex Differences in Central Osmosensory Mechanisms in Salt-Sensitive Hypertension
PI Application: Sean D. Stocker
Total Costs Requested: $100,000
The goal of this supplement is to examine how gender and circulating estradiol alters sodium-sensing and/or the intrinsic osmosensitivity of hypothalamic neurons.
Submitted: 1/13/15

12. Future of Research Project. What are the future plans for this research project?

As outlined above, we are actively performing experiments (with funding from NIH and American Heart Association) but also actively expanding the scope of the project. In this regard, we have initiated a collaboration with human physiologists at the University of Delaware (William Farquhar and Megan Wenner). These investigators have a strong history to perform controlled-feeding studies in human subjects and measure various cardiovascular endpoints. This collaboration has already resulted in numerous abstracts presented at national meetings such as the Experimental Biology Meeting and new grant submission (see NIH NHBLI R01 HL128388-01 and American Heart Association Collaborative Sciences Award 15CSA241800016). We were strongly encouraged to resubmit the grant by NIH and are currently revising the NIH application for a resubmission in July 2015.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	1			1
Female	1			1
Unknown				
Total	**2**			**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	2			2
Unknown				
Total	**2**			**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White	2			1
Black				
Asian				1
Other				
Unknown				
Total	**2**			**2**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

As noted above, the preliminary data that we obtained in this project laid the foundation for several other externally-funded grants that have already attracted $2.5 million in sponsored research support to Penn State Hershey.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a

performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Our long term goal is to identify the neural pathways and cellular mechanisms that increase sympathetic nerve activity (SNA) and total peripheral resistance in salt-sensitive hypertension. Both preliminary and recently published data from our laboratories demonstrate that the ingestion of excess dietary salt enhances sympathetic-cardiovascular function, increases arterial blood pressure (ABP) variability, and contributes to the development of neurogenic salt-sensitive hypertension. The overall goal of this project is to determine how the brain senses changes in dietary salt intake to result in exaggerated sympathetic-cardiovascular regulation and ultimately contribute to the pathogenesis of salt-sensitive hypertension in humans.

Specific Aim 1 will determine whether dietary salt intake elevates plasma sodium concentration to activate "sodium-sensing" neurons in the forebrain hypothalamus via transient receptor potential vanilloid (TRPV) and/or benzamil-sensitive channels and subsequently enhance sympathetic-cardiovascular function, increase ABP variability, and salt-sensitive hypertension. These experiments will use "state-of-the-art" electrophysiological and molecular approaches in rodents to investigate this hypothesis.

Electrophysiological Recordings of OVLT Neurons. Male Sprague-Dawley rats (200-225 g) were deeply anesthetized with 5% isoflurane, decapitated, and the brain harvested. A block of tissue (2 x 2 cm) containing the OVLT was placed into a vibratome chamber containing artificial cerebrospinal fluid (in mM): 125 NaCl, 2 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 10 D-glucose, and 0.4 ascorbic acid (osmolality, 300-305 mOsm/L, pH 7.4). The brain was cut into 200-300 µm slices. These slices were incubated at room temperature (24-26ºC) for 1 h artificial cerebrospinal fluid. Then, slices were transferred to a glass-bottomed recording chamber and viewed through an upright microscope (FN1, Nikon) equipped with DIC optics, epi-fluorescence, an infrared (IR) filter and an IR-sensitive video camera. An appropriate filter was used to visualize retrogradely labeled neurons with rhodamine beads.

Patch electrodes were pulled from borosilicate glass and polished to a tip resistance of 4-5 MΩ. Electrodes will be filled with a solution containing (in mM) 135 K-gluconate, 10 HEPES, 0.1 EGTA, 1.0 MgCl, 1.0 NaCl, 2.0 Na_2ATP, and 0.5 Na_2GTP. After achieving a GΩ seal and whole cell configuration, cell capacitance, access resistance, and resting membrane potential was monitored until stable. Recordings were made using an Axopatch 200B amplifier and pCLAMP

software. Signals are filtered at 1kHz, digitized at 10kHz, and saved on a computer for off-line analysis. Cells that meet the following criteria will be used in experiments: action potential amplitude ≥ 50 mV from threshold to peak, input resistance >0.5 GΩ (determined by injection of -20 pA from a holding potential of -80 mV), resting V_m negative to -50mV, and <20% change in series resistance during the recording.

Experimental Protocol and Drug Application. After a stable recording was achieved, baseline discharge in current-clamp mode was recorded for 2 min. Then, bath osmolality was increased approximately 10-20 mOsm/L for 2 min through the addition of NaCl to the bath solution, and then returned to normal artificial cerebrospinal fluid (i.e. "wash-out"). If an OVLT neuron exhibited an increase in cell discharge (30%) in response to the increased bath osmolality, the protocol was repeated but with benzamil (1-50µM).

Figure 1 illustrates experimental recordings of neurons located in the organum vasculosum of the lamina terminalis (OVLT). Figure 1A illustrates step-wise increases in bath osmolality or [NaCl] produce concentration dependent increases in cell discharge (summary data in Figure 1C). Figure 1B illustrates a step increase in bath [NaCl] produces a sustained increase in cell discharge (summary data in Figure 1D). There were no differences in the electrophysiological characteristics of osmosensitive versus non-osmosensitive neurons (i.e, input resistance, resting membrane potential, after hyperpolarization, etc). These data have important implications: 1) hypothalamic neurons of the organum vasculosum of the lamina terminalis (OVLT) are extremely sensitive to changes in NaCl or osmolality, and 2) OVLT neurons do not adapt to changes in bath [NaCl]. Since OVLT neurons do not adapt to the increase in bath [NaCl] (Figure 1B), we are now using this protocol to identify the novel ion channel that mediates these responses.

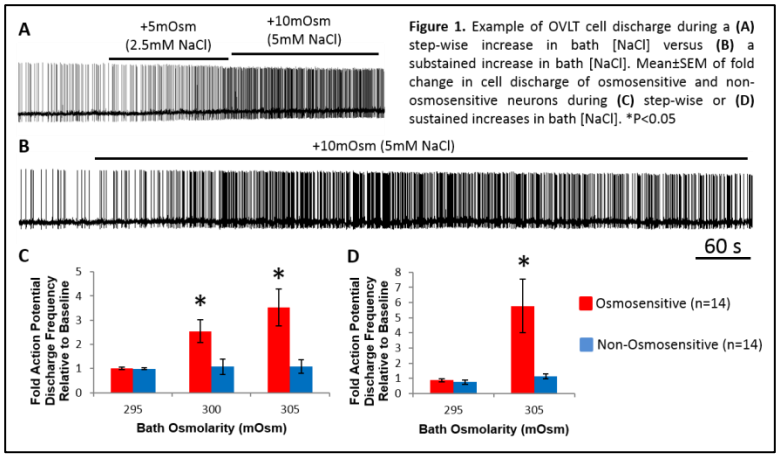

Figure 1. Example of OVLT cell discharge during a **(A)** step-wise increase in bath [NaCl] versus **(B)** a substained increase in bath [NaCl]. Mean±SEM of fold change in cell discharge of osmosensitive and non-osmosensitive neurons during **(C)** step-wise or **(D)** sustained increases in bath [NaCl]. *P<0.05

Using the protocol outlined in Figure 1B, we then asked whether a benzamil-sensitive ion channel mediated this response. Benzamil is an amiloride analog that will block a number of non-voltage-dependent sodium channels including Na+/H+ exchanger, Na+-Ca++ pump, acid-sensing ion channels, and epithelial sodium channel (ENaC). Figure 2 illustrates bath application of benzamil reversed the increased discharge during a 15mOsm step-wise increase in bath osmolality. Since benzamil does block all of the above-mentioned channels, we have recently utilized a smaller dose of benzamil to selectively block ENaC. Figure 1B illustrates that a smaller dose of benzamil similarly reversed the increased discharge frequency produced by hyperosmolality. These findings suggest that a brain ENaC mediates brain osmosensing. Since these experiments, our laboratory has developed mouse lines with selective deletion of the ENaC subunits from brain. These animals are currently

being used in electrophysiological experiments to identify the putative ENaC subunit involved in brain osmosensing.

A second goal of Specific Aim 1 was to perform a parallel set of experiments to investigate the role of the transient receptor vanilliod type 1 (TRPV1) in brain osmosensing. However, we did

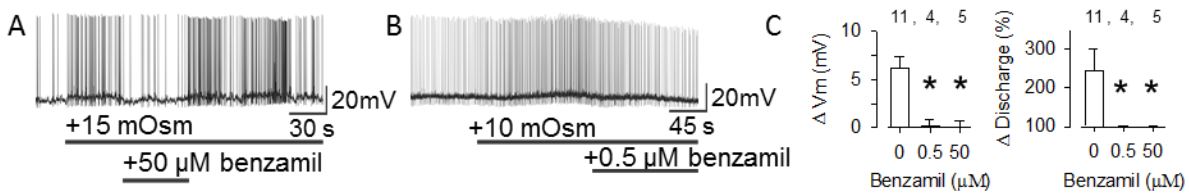

Figure 2. Application of the ENaC antagonist benzamil at **(A)** 50μM or **(B)** 0.5μM reversed the membrane depolarization and incresed discharge. **(C)** Summary data. *P<0.05

not perform a similar set of electrophysiological patch-clamp experiments as illustrated in Figures 1 and 2 due to in vivo data that was generated in our laboratory at the same time. In these experiments, we used a behavioral paradigm to test whether deletion of the TRPV1 channel disrupted behavioral responses induced by acute changes in plasma sodium concentrations and osmolality. As illustrated in Figure 3, deletion of the TRPV1 or TRPV4 channel did not disrupt water intake produced by acute hypernatremia. We also found that thirst responses to chronic dehydration produced by drinking 2% NaCl for 48 hours were not different. Finally, analysis of Fos expression (a marker of neuronal activation) after acute hypernatremia was not different across strains of animals. These findings were published recently (Kinsman et al AJP Reg, 2014). In addition, deletion of TRPV1 does not disrupt osmotically-induced vasopressin secretion (Tucker & Stocker, 2015). Altogether, these findings suggest that TRPV1 channels do not play a pivotal role in osmoregulatory responses.

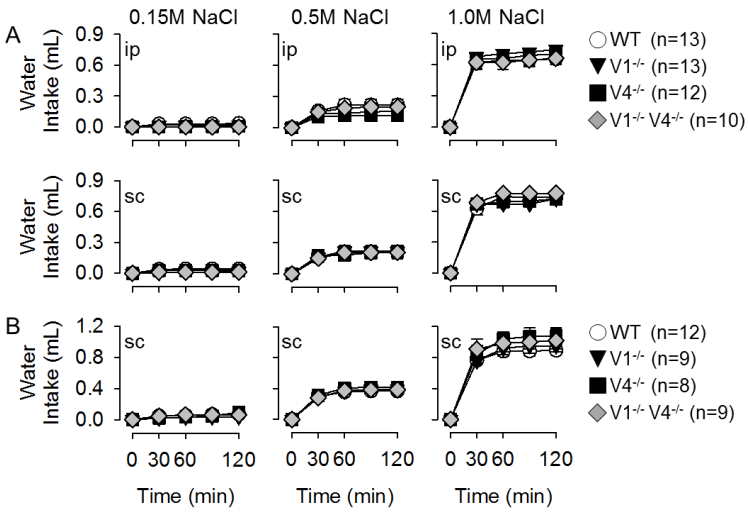

Figure 3. Water intake in wild-type, TRPV1-/-, TRPV4-/-, or TRPV1xTRPV4-/- mice after ip or sc injection of 0.15M, 0.5M, or 1.0M NaCl. (A) Water access was delayed, (B) water access was immediate.

Specific Aim 2 will determine whether excess dietary salt intake alters the excitability of catecholaminergic neurons in the rostral ventrolateral medulla (RVLM) through activation of angiotensin II type 1 receptors, mammalian target of rapamycin signaling, and oxidative stress. These experiments will use electrophysiology, RT-PCR, and Western blot analysis in rodents.

Methods – Electrophysiological Recording of RVLM Neurons. Male Sprague-Dawley rats were placed on a low (0.4%) or high (2%) NaCl for 2 weeks. Then, rats were anesthetized with 2-3% isoflurane, artificially ventilated, and instrumented with arterial and venous catheters to measure arterial blood pressure and sympathetic nerve activity. Animals were placed into a stereotaxic head frame with the skull level between bregma and lambda. After a limited craniotomy, the caudal pole of the facial nucleus was located by field potential recording during electrical stimulation of the facial nerve (100 µs, 1-2 mA, 1 Hz). The area of the medulla with the highest density of RVLM sympathetic neurons is approximately 0-300 µm behind the caudal pole of the facial nucleus. In addition, a concentric bipolar electrode was placed into the dorsolateral quadrant of the spinal cord at T1-T2 to antidromically activate RVLM neurons by applying square-wave current pulses (0.5 ms, <1mA, 1 Hz). Extracellular single-unit recordings were made using an Axoclamp 2B amplifier in bridge mode and glass microelectrodes filled with 3% Neurobiotin dissolved in 0.5M sodium acetate (pH 7.5). Signals were amplified, and action potentials were counted by a window discriminator. RVLM neurons were selected based on the following criteria: 1) spontaneous activity, 2) antidromic activation from the spinal cord, and 3) time-locked decrease in neuronal discharge by an increase in arterial blood pressure.

Experimental Protocol. RVLM neuronal discharge were recorded in response to the following stimuli: 1) chemoreflex activation produced by NaCN (1-200 µg/kg, iv), 2) chemoreflex activation produced by hypoxia (10% O_2, 30 s), 3) chemoreflex activation produced by hypercapnia (8% CO_2), 4) activation of somatic afferents (1 Hz, 1 s duration, 100-500µA), and 5) baroreflex activation produced by sodium nitroprusside (1-20 µg/kg, iv) or phenylephrine (1-20 µg/kg, iv).

Identification of Catecholaminergic Neurons. After the experimental protocol, the neurons were juxtacellularly labeled by applying current pulses (200 ms, 50% duty cycle) of increased amplitude (1-10 nA) through the recording electrode until the discharge is entrained to the current pulses for a minimum of 30 s. Then, the animal was perfused with 4% paraformaldehyde, brain harvested and sectioned at 50 µm on a vibratome and stored at -20ºC in a cryoprotectant solution. To identify whether the recorded cells are catecholaminergic, sections were incubated with a monoclonal anti-tyrosine hydroxylase antibody or polyclonal sheep anti-PNMT in 0.1 M PBS with 5% donkey serum followed by an overnight incubation with an Alexa Fluor 594 conjugated secondary donkey anti-mouse or sheep. Juxtacellular labeled cells were visualized with a streptavidin alexa-fluor 488. Sections were mounted on slides and visualized with appropriate filters on a Nikon FN-1 upright microscope using NIS Elements software.

Using the approaches described above, we performed two set of experiments. First, we wanted to identify whether dietary salt intake altered sympathetic and blood pressure responses evoked by various sympathetic reflexes dependent upon neurotransmission in the RVLM. Second, we wanted to determine the mechanisms by which NaCl worked centrally to alter RVLM neuronal activity and sympathetic reflexes.

Figure 4 illustrates that a high salt diet exaggerates sympathetic nerve activity (SNA) and blood pressure responses during activation of sciatic nerve afferents. Rats fed 4.0% NaCl chow displayed much greater changes in blood pressure and SNA during every frequency. Interestingly, lesion of the osmosensitive sites including the OVLT completely reversed the exaggerated responses. Although not illustrated here, we examined a variety of other sympathetic reflexes and found that a high salt diet exaggerated sympathetic and blood pressure responses to: 1) activation of aortic depressor nerve, 2) activation of vagal afferent, 3) volume expansion, and 4) central infusion of hypertonic NaCl. Interestingly, dietary salt intake did not alter sympathetic or blood pressure responses to chemoreceptor activation via hypoxia or hypercapnia. The ability of dietary salt intake to exaggerate a number of sympathetic reflexes and blood pressure would be expected to produce greater fluctuations in blood pressure throughout the day. Therefore, a final set of experiments tested whether dietary salt intake altered blood pressure variability in salt-resistant rats. Male Sprague-Dawley rats were implanted with Data Sciences Telemetry Units to chronically measure blood pressure and heart rate. After a 2 week recovery period, animals were fed 0.1% or 4.0% NaCl chow for an additional 2 weeks. Figure 5 illustrates that a high salt diet increases blood pressure variability at night (SD of systolic blood pressure) without a change in mean arterial blood pressure. Altogether, these data suggest that increased dietary salt intake alters sympathetic reflexes dependent upon RVLM neuronal transmission to increase blood pressure variability. These findings were published recently (Simmonds et al Hypertension 2014).

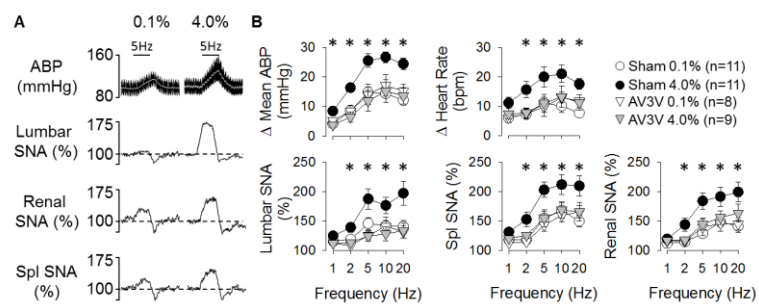

Figure 4. (A) Arterial blood pressure, lumbar sympathetic nerve activity (SNA), renal SNA, splanchnic SNA during stimulation of the sciatic afferents in animals fed 0.1% versus 4.0% NaCl chow. (B) Rats fed 4.0% NaCl chow had much greater responses versus rats fed 0.1% NaCl chow. Lesion of osmosensitive regions in the hypothalamus reversed these responses.

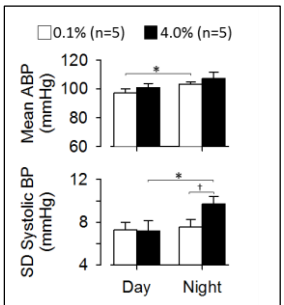

Figure 5. A high salt diet increased blood pressure variability without a changes in mean blood pressure.

A second major goal of Specific Aim 2 was to determine how dietary salt intake altered RVLM neuronal function. Recent findings in both animals and humans suggest dietary salt intake increases NaCl concentrations in plasma and cerebrospinal fluid. Therefore, we tested whether increases in cerebrospinal fluid sodium concentrations altered sympathetic function through change in RVLM neuronal activity. Figure 6 represents the typical responses observed when cerebrospinal fluid NaCl concentrations are acutely increased by infusion of 1.0M NaCl (5uL/10min) into the lateral ventricle. Infusion of 1.0M

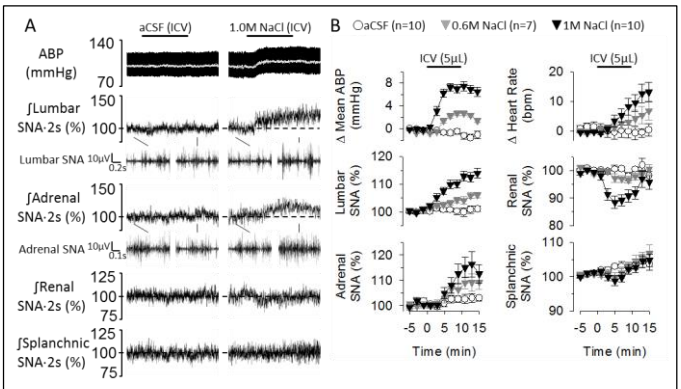

Figure 6. (A) Arterial blood pressure, lumbar SNA, adrenal SNA, renal SNA, and splanchnic SNA during acute infusion of 0.15M or 1.0M NaCl into the lateral ventricle. (B) Summary data illustrated as mean±SEM.

NaCl increased arterial blood pressure, lumbar SNA, and adrenal SNA but did not alter splanchnic SNA and even decreased renal SNA. Additional experiments demonstrate that ganglionic blockade with chlorisondamine (5mg/kg, IV) or lesion of osmosensitive regions in the hypothalamus abolish the changes in SNA and blood pressure (data not shown). These data suggest that increases in cerebrospinal fluid NaCl concentration differentially affect sympathetic outflow through osmosensitive neurons in the hypothalamus to increase blood pressure.

To determine the extent by which cerebrospinal fluid hypernatremia activates RVLM neurons to increase sympathetic outflow and blood pressure, different compounds were microinjected into the RVLM during central infusions of NaCl. Inhibition of the RVLM by bilateral injection of the $GABA_A$ agonist muscimol promptly reduced lumbar SNA (-76±6%, P<0.01), renal SNA (-53±10%, P<0.01), adrenal SNA (-62±6%, P<0.01), splanchnic SNA (-35±9%, P<0.01), mean ABP (88±6 to 57±7 mmHg, P<0.01), and heart rate (405±12 to 365±19 bpm, P<0.01). Importantly, bilateral injection of muscimol abolished the lumbar sympathoexcitatory and pressor response to central infusion of 1.0M NaCl (Figure 7). The adrenal sympathoexcitatory response was partially attenuated. Bilateral injection of aCSF into the RVLM did not significantly alter any baseline variable (data not shown) or alter the responses to ICV infusion of NaCl (Figure 7). To identify the neurotransmitter receptor in the RVLM that mediates the above responses, central infusions were performed after blockade of ionotropic glutamate or angiotensin II type 1 (AT1) receptors in the RVLM. Bilateral injection of kynurenic acid into the RVLM produced a small reduction in lumbar SNA (-6±1%, P>0.05) but did not alter renal SNA (-4±5%, P>0.01), adrenal SNA (-4±7%, P>0.05), splanchnic SNA (-1±1%, P>0.05), mean ABP (85±5 to 84±4 mmHg, P<0.05), and heart rate (350±18 to 346±13 bpm, P>0.05). Blockade of RVLM glutamate receptors with kynurenic acid significantly attenuated the increase in lumbar SNA, adrenal SNA, heart rate, and mean ABP to ICV infusion of 1.0M NaCl (Figure 8). In addition, KYN also reduced the renal sympathoinhibitory response. In contrast, bilateral injection of the AT1 receptor antagonist losartan did not alter lumbar SNA (10±7%, P>0.05), renal SNA (2±3%, P>0.05), adrenal SNA (-1±3%, P>0.05), splanchnic SNA (3±2%, P>0.05), mean ABP (90±4 to 92±5 mmHg, P>0.05), and heart rate (371±15 to 368±14 bpm, P>0.05). In addition, bilateral injection of losartan did not alter the sympathetic, tachycardic, and

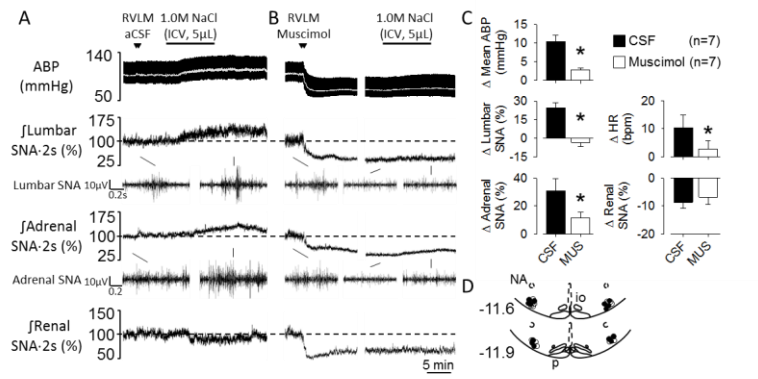

Figure 7. Bilateral inhibition of the RVLM with injection muscimol prevented the changes in blood pressure and SNA during central infusion of 1M NaCl. (A, B) Example of blood pressure and SNA, (C) summary data illustrated as peak changes (mean±SEM), *P<0.05. (D) injection site

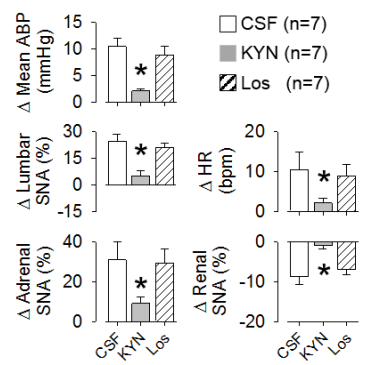

Figure 8. Peak changes in mean arterial blood pressure, SNA and heart rate in response to central infusion of NaCl after RVLM injection of vehicle, kynurenic acid, or losartan. *P<0.05 vs CSF

pressor responses to central infusion of 1.0M NaCl (Figure 8). Collectively, these data suggest that increases in cerebrospinal fluid NaCl concentration affect RVLM neurons through increased glutamatergic receptor activation.

To establish that cerebrospinal fluid hypernatremia alters the discharge of RVLM neurons, we recorded from 24 barosensitive, spinally-projecting RVLM sympathetic neurons. These cells were subsequently divided into 3 classes of neurons based on discharge responses to central infusion of 1.0M NaCl. Type I neurons (11/24, 46%) had a baseline discharge of 11±2 Hz and conduction velocity of 2.1±0.40 m/s. Central infusion of 1.0M NaCl increased cell discharge within 2 min after onset of the infusion, and the activity remained elevated throughout the infusion despite an increase in mean ABP (Figure 9). Type II neurons (9/24, 37%) had a baseline discharge and conduction velocity of 11±3 Hz and 2.6±0.3 m/s, respectively. However, infusion of 1M NaCl decreased the discharge rate of these neurons at 4 min after the onset of the central infusion (Figures 9 and 10). Type III neurons (4/24, 17%) displayed a baseline discharge and conduction velocity of 17±8 Hz and 2.3±0.5 m/s, respectively. Infusion of 1M NaCl did not alter discharge rate in these neurons (Figure 9). A previous study suggested that RVLM neurons may be distinguished between C1 and non-C1 cells based on the conduction velocity as slowly conducting neurons (<1 m/s) were consistently identified as C1 neurons (Schreihofer et al J Comp Neurol 1997). Interestingly, every slowly-conducting RVLM neuron defined as a conduction velocity ≤1m/s (mean conduction velocity: 0.8±0.1 m/s; n=4) displayed a lower discharge rate (7.5±3.0 Hz, P<0.05) and increased discharge response to central infusion of 1.0M NaCl. Therefore, every slowly-conducting RVLM neuron was deemed a Type I cell (Figure 9). Altogether, these findings suggest that increases in cerebrospinal fluid hypernatremia selectively increase the activity of a specific class of RVLM neurons.

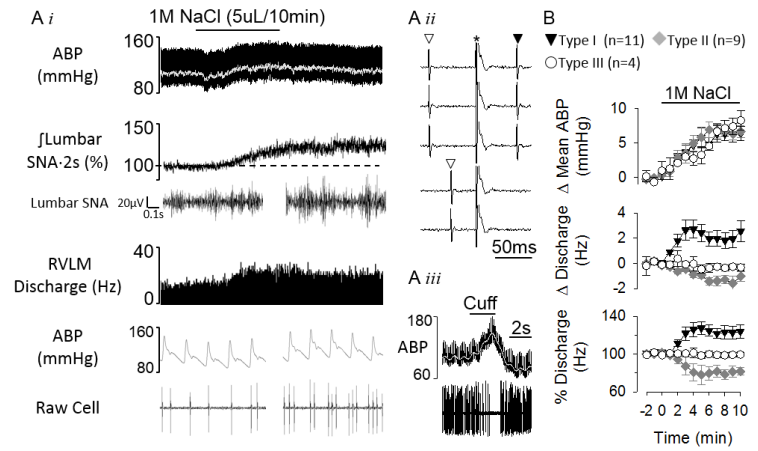

Figure 9. Type I Neuron. (A, i) Arterial blood pressure, SNA, and RVLM discharge during central infusion of NaCl. (ii) This neuron was antidromically activated from the spinal cord and (iii) barosensitive. (B) Summary data presented as mean±SEM indicate the present of Type I, Type II, and Type III neurons.

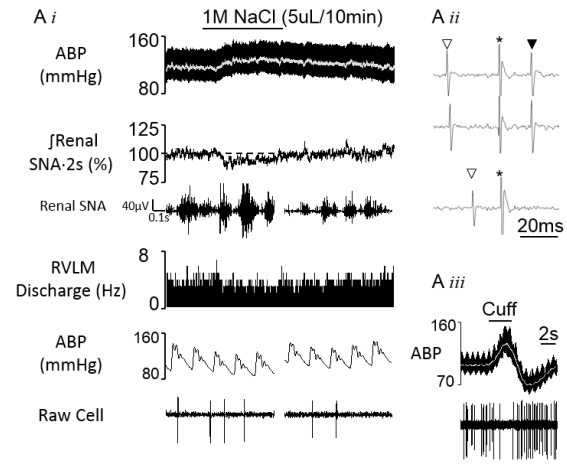

Figure 10. Type II Neuron. (A, i) Arterial blood pressure, SNA, and RVLM discharge during central infusion of NaCl. (ii) This neuron was antidromically activated from the spinal cord and (iii) barosensitive.

Specific Aim 3 will determine (A) the clinical populations (i.e. men versus women) that excess dietary salt intake affects sympathetic-cardiovascular regulation, and (B) determine whether these changes are correlated with circulating cytokines and oxidative stress. These experiments

will place humans on low and high salt diets for 2 weeks and then measure sympathetic nerve activity, blood pressure, and renal blood flow at rest and in response to a variety of physiological stressors.

Studies were conducted in the General Clinical Research Center (GCRC) at the Milton S. Hershey Medical Center. Studies were randomized, double-blinded, and cross-over (each subject received low and high salt condition). Subjects had an initial health screen at the GCRC followed by 2 additional visits (1 visit after each 2 week diet) to test sympathetic-cardiovascular responses to baroreflex, isometric handgrip, hypoxia, hypercapnia, and apnea. Subject populations and inclusion/exclusion criteria are described below.

Medium versus High NaCl Diet - Subjects were randomly assigned to a "low" or "high" NaCl diet for 2 wk and then cross-over to the other dietary condition. Both dietary conditions contain the identical base diet (Table 1) supplemented by placebo (low) or salt (high) capsules to produce a medium (~2.5 mg Na+) versus high salt (~12.5 g Na+). Subjects were instructed to supplement the diet with water and any fresh fruit or vegetables. This experimental design permits changes in salt content without gross changes in other dietary factors. Subjects were instructed to ingest capsules in divided doses (morning, lunch, and night). Efforts to enhance compliance were made by 1) discussions with subjects to emphasize the importance of the capsules and diet, 2) phone calls (2x/week), 3) return unused and extra capsules at the end of the study, and 4) subjects were asked to record meal and capsule use in a diary. Definitive physiological measurements were be used to validate effects of low versus high salt diets (see below). At the end of the 2 week dietary salt condition, subjects reported to the GCRC for the following procedures and physiological monitoring.

Table 1. Base diet for medium and high salt conditions

Meal	Sodium (mg)	Calories
Breakfast. Cheerios (1 serving) + 1 cup milk	300	250
Lunch / Dinner / Snack. Healthy Choice Entrees (3)	1500	900
Snack. Power Bars (2)	240	480
Snack. Carnation Instant Shake (3) *Boost shakes can be used to ensure adequate caloric intake	270	400
TOTAL	2310	2030

Biochemistry. Blood samples were obtained to measure plasma electrolytes, fasting glucose and insulin levels, cytokines, and renin activity. In addition, 24-h urine was analyzed for urine sodium excretion. Dietary salt loading has been repeatedly reported to raise plasma sodium concentration, lower plasma potassium concentration, increase sodium excretion, and reduce plasma renin activity. These measurements will provide a physiological assessment on the effectiveness of the low versus high NaCl diet.

ABP, Heart Rate and SNA. ABP variability was measured by automatic blood pressure cuff (SpaceLabs) worn by the subject at least 24 hr before a visit to the GCRC. Microneurography was used to obtain direct multifiber recordings of muscle sympathetic nerve activity (MSNA), and ABP was measured via Finapres as described previously (6-8).

Baroreflex Sensitivity (BRS). BRS was determined using the Modified Oxford technique. Sequential bolus infusions of nitroprusside (100 mcg) or phenylephrine (150 mcg) were performed to safely produce small changes in ABP (~15-25 mmHg). Sigmoidal curves plotting the change in R-R interval and MSNA as a function of blood pressure were constructed and the gain was compared within a subject between low versus high salt diet.

Isometric Handgrip Exercise. Isometric handgrip to fatigue was performed to mimic the somatic or exercise pressor reflex in laboratory animals (see Figure 1). The GCRC routinely performs this technique and details are described elsewhere.

Hypoxia, Hypercapnia, and Apnea. Subjects breathed room air through a facemask. The inspiratory port of the facemask was connected to a rubber bag containing 10% O_2, 90% N_2 or 5% CO_2, 20%O_2, 75% N_2 for 5-10 minutes. O_2 saturation will be measured by an oximeter clip placed on the finger. Respiration was monitored with a pneumobelt. Subjects were asked to hold their breath for short periods of time.

Blood Flow Measurement. Doppler ultrasound was used to determine regional blood flow to the kidney.

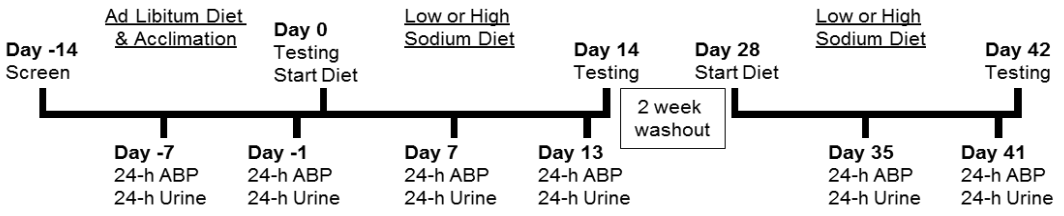

Figure 11. After a 2-week acclimation period, subjects will be randomly assigned to start on a low or high sodium diet and cross-over to the other condition after a 2 week washout. 24-h ABP, urine, and physiological tests are scheduled within each diet.

Figure 11 illustrates the typical experiments design for a subject which includes a 14 day control period, a 14 day test period, a 2 weeks washout, and then a 14 day test period. Subjects were randomly assigned to a "low" or "high" NaCl diet for 2 wk and then cross-over to the other dietary condition. Both dietary conditions contain the identical base diet (Table 1) supplemented by placebo (low) or salt (high) capsules to produce a medium (~2.5 mg Na+) versus high salt (~12.5 g Na+). +). As part of this protocol, we enrolled 12 subjects (10 males, 2 females). Only 8 subjects (7 males, 1 female) completed the protocol. The most common reason for subject drop-out was nausea reported during the high salt condition. Due to these factors, we submitted an amended IRB protocol to use "slow-release" salt tablets to avoid the nausea. In conjunction with this amendment, the IRB requested a complete review of all methodology and drugs. The re-review lasted several months. Consequently, the substantial delay severely impacted results from the study. During the IRB review, we were not permitted to perform any experiments. In addition, this project was submitted as a grant application to the American Heart Association (Grant-In-Aid: Monahan). The grant was funded. As a result, these experiments were performed under the American Heart Association Award. The CURE money was re-budgeted to enhance

the development of Experiments 1 and 2. Due to the 2 factors above, the data reported below represents findings from 3 subjects in which all measurements including microneurography and renal blood flow measurements were completed during the medium and high salt condition.

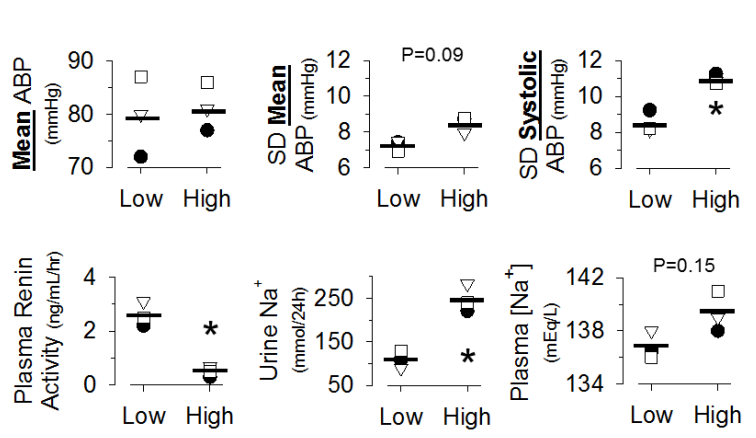

Figure 12. Characteristics of 3 subjects (▽,□,●) provided a low (2.5g) or high (6.5g) sodium diet for 2 weeks. **(TOP)** Mean ABP and standard deviation (SD) of mean or systolic ABP. **(BOTTOM)** Plasma renin activity, urine sodium excretion, and plasma sodium concentration. Mean, —; *P<0.05 versus low sodium (2-tailed)

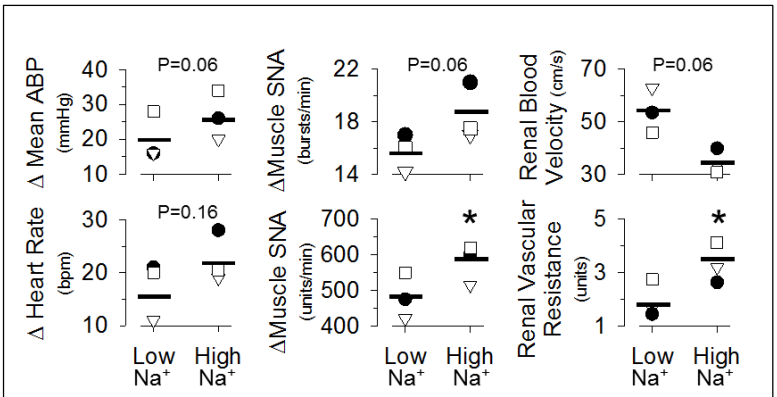

Figure 13. Sympathetic and cardiovascular responses to handgrip (30% max to fatigue) in 3 healthy subjects (▽,□,●) provided a low (2.5g) or high (6.5g) sodium diet for 2 weeks. **(LEFT)** Δmean ABP and heart rate, **(MIDDLE)** Δmuscle SNA, and **(RIGHT)** Renal blood velocity and vascular resistance. Mean, —; *P<0.05 vs low sodium (2-tailed)

Figure 12 illustrates baseline values for mean blood pressure, standard deviation of mean and systolic blood pressure, plasma renin activity, urine sodium excretion and plasma sodium concentration. A high salt diet did not change mean arterial blood pressure but increased blood pressure variability (SD of systolic blood pressure). As expected, a high salt diet decreased plasma renin activity, increased urine sodium excretion, and tended to increase plasma sodium levels.

Figure 13 illustrates the cardiovascular responses to handgrip exercise. Isometric handgrip exercise increased blood pressure, heart rate, muscle sympathetic nerve activity, and renal vascular resistance. Interestingly, the magnitude of these responses tended to be or were significantly greater in subjects fed high salt diets. Given the small number of subjects with completed measurements, there were no clear findings regarding dietary salt intake on sympathetic and cardiovascular responses to other stressors such as chemoreceptor stimulation. In light of the small subject number, blood samples were not analyzed for markers of oxidative stress.

The overall goal of this project is to build a basic science and clinical project to form the basis of a large research endeavor to study the impact of dietary salt intake in salt-resistant and salt-sensitive subjects. In this regard, we have expanded the clinical research study through collaboration with investigators at the University of Delaware. These investigators have a strong history of controlled-feeding studies in humans to examine similar endpoints. Although not directly funded by this grant mechanism, our collaborative team has presented several abstracts at national meetings to confirm and further demonstrate the impact of dietary salt in salt-resistant, normotensive subjects. This collaboration is ongoing and formed the basis of a multi-PI and multi-institutional grant submitted to NIH and the American Heart Association. Abstracts

are included below.

Published Abstracts / Conference Presentations

1. Stocker, SD. Acute hypernatermia increases the discharge of sympathetic-vasomotor neurons in the rostral ventrolateral medulla. 2011 American Heart Association Council for High Blood Pressure Research Meeting. *Hypertension 58:* e176, 2011.
2. Stocker, SD. Acute hypernatermia increases the discharge of sympathetic-vasomotor neurons in the rostral ventrolateral medulla. FASEB J, 26: 1091.62, 2012.
3. Stocker, SD. Assessment of chronic sympathetic nerve recording using Data Sciences International telemetry. FASEB J, 26: 1091.47, 2012
4. Wolfgang L & Stocker, SD. OVLT contributes to sodium-induced sympathoexcitation and hypertension. 2012 American Heart Association Council for High Blood Pressure Research Meeting
5. Browning BN & Stocker, SD. Osmosensitivity of neurons in the organum vasculosum of the lamina terminalis is mediated by benzamil-sensitive channels. 2012 American Heart Association Council for High Blood Pressure Research Meeting.
6. Stocker, SD. Dietary salt intake enhances sympathetic responses to central sodium and sciatic afferent nerve stimulation. FASEB J, 27: 695.6, 2013.
7. Frielle J & Stocker, SD. Celiac ganglionectomy attenuates central sodium-induced hypertension. FASEB J, 27: 695.8, 2013.
8. Lang SM & Stocker, SD. Glutamate receptor activation in the rostral ventrolateral medulla (RVLM) mediates the sympathetic response to increased cerebrospinal fluid (CSF) sodium concentration. FASEB J, 27: 695.4, 2013.
9. Simmonds SS & Stocker, SD. Lumbar denervation attenuates the sympathetic and cardiovascular response to acute increases in cerebrospinal fluid sodium concentration. FASEB J, 27: LB731, 2013.
10. Yamauchi, K. Tsuchimochi H, Stocker, SD**,** Kaufman MP. Increased dietary salt intake enhances the exercise pressor reflex. 2013 American Heart Association Council for High Blood Pressure Research Meeting.
11. Stocker, SD, Simmonds SS. Increased dietary salt intake enhances sympathetic and cardiovascular responses to various stressors. 2013 American Heart Association Council for High Blood Pressure Research Meeting.
12. Stocker, SD. Rostral ventrolateral medulla mediates sympathetic and cardiovascular responses to increased cerebrospinal fluid sodium concentration. 2013 American Heart Association Council for High Blood Pressure Research Meeting.
13. Simmonds SS & Stocker, SD. Dietary NaCl alters several sympathoexcitatory and sympathoinhibitory reflexes: dependence on the ventral lamina terminalis. FASEB J, 28: 683.4, 2014.
14. Brian M, Wenner M, Matthews E, Ramick M, Edwards D, Stocker, SD, Farquhar W. Dietary sodium loading increases systolic BP variability in salt resistant adults: a role for serum sodium. FASEB J, 28: LB701, 2014.
15. Kinsman B, Cowles J, Browning KN, & Stocker, SD. Influence of TRPV1 and/or TRPV4 receptors on osmoregulatory thirst in mice. FASEB J, 28: 683.2, 2014.
16. Simmonds SS & Stocker, SD. Blockade of transient receptor vanilloid channels prevents the sympathoexcitatory response to central NaCl infusion. FASEB J, 28: 683.3, 2014.

17. Matthews E, Stocker, SD, Brian M, Wenner M, Edwards D, Farquhar W. Blood pressure responses to skeletal muscle contraction relative to salt sensitivity in humans and rats. FASEB J, 28: 1165.11, 2014.
18. Stocker, SD and Simmonds SS. Transient Receptor Potential Vanilloid-1 (TRPV1) channels mediate the sympathoexcitatory and pressor responses to central NaCl stimulation. 20134 American Heart Association Council for High Blood Pressure Research Meeting, San Francisco, CA.
19. Tucker AB and Stocker, SD. Osmotically-induced vasopressin secretion is not altered in novel knock-out rats lacking the transient receptor potential vanilloid (TRPV1) channel. FASEB J, in press.
20. Stocker, SD, Simmonds SS, Wenner M, Farquhar WB. Chronic central infusion of hypertonic NaCl increases blood pressure variability and exaggerates sympathetic reflexes. FASEB J, in press
21. Kinsman B, Browning KN, Stocker, SD. OVLT Neurons Exhibit Exquisite Osmosensitivity to Elevated NaCl Concentrations. FASEB J, in press.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_X___Yes
_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

___3___Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

__40____Number of subjects originally targeted to be included in the study
__12____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
__10____Males
___2___Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
__12__Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
__12__White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

The treatment (medium and high dietary salt intake) was performed exclusively at Penn State College of Medicine. Due to the nature of the measurements involved in the study (microneurography, renal blood flow via Doppler Ultrasound), all of the subject recruitment and study coordination was performed at Penn State College of Medicine and Hershey Medical Center (Dauphin County).

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____ Yes

___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Chronic recording of sympathetic nerve activity in rats: surgical techniques, assessment of nerve activity, and quantification	Stocker, SD Muntzel, MS	*AJP Heart Circ Physiol* 305: H1407-1416	March 2013	☐Submitted ☐Accepted ■Published

2. Dietary salt intake exaggerates cardiovascular reflexes and increases blood pressure variability in normotensive rats	Simmonds SS Lay J Stocker SD	Hyp*ertension* 64(3): 583-589	January 2014	☐Submitted ☐Accepted ■Published
3. Osmoregulatory thirst in mice lacking the transient receptor potential vanilloid type 1 (TRPV1) and/or type 4 (TRPV4) receptor	Kinsman, B Cowles, JJ Simmonds, SS Lay, J Browning, BN Stocker, SD	*Am J Physiol Regul Integr Physiol* 307(9): R1092-R1000	March 2014	☐Submitted ☐Accepted ■Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

Stocker SD, Lang SM, and Simmonds SS. Central hypernatremia increases glutamatergic input to the rostral ventrolateral medulla. Hypertension, submitted.

Kinsman B, Browning KN, Stocker SD. Benzamil-sensitive channels underlying the brain osmoreceptor. Nat Neuroscience, In preparation.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The project has accumulated additional evidence in rodent models to establish that dietary salt intake may have adverse consequences in healthy, salt-resistant individuals. These adverse effects occur through the ability of dietary salt intake to alter the excitability of sympathetic networks to result in exaggerated changes in sympathetic outflow and blood pressure to a variety of different stressors or physiological challenges. In turn, these greater changes in sympathetic outflow and blood pressure would be expected to increase blood pressure variability. This has significant clinical ramifications as increased blood pressure variability is a strong prognostic indicator of future adverse cardiovascular events and disease. Consequently, subjects may ingest high level of dietary salt and have a normal mean arterial blood pressure but blood pressure variability has increased significantly thereby predisposing them to future adverse cardiovascular events. The hypothesis is based on the

above findings reported in rodents and we are currently pursuing these questions in human subjects in collaboration with human physiologists at the University of Delaware. Clinically, these findings may suggest that dietary salt intake can exert adverse effects on the cardiovascular system independent of mean arterial blood pressure.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Stocker, Sean D.	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) **Stocker**	Department of Cellular & Molecular Physiology and Neural & Behavioral Sciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Allegheny College, Meadville, PA	BS	05/97	Biology, Psychology
University of Pittsburgh, Pittsburgh, PA	PhD	01/02	Neuroscience
University of Texas HSC, San Antonio, TX	Postdoc	08/05	Physiology

A. Personal Statement

My research career has an established track record to investigate the neural circuits and cellular mechanisms that regulate sympathetic nerve activity and blood pressure regulation under physiological challenges and pathological disease states (hypertension). As a graduate student, I investigated how changes in blood pressure impacted homeostatic responses (thirst, vasopressin secretion) stimulated by angiotensin II and hypernatermia. As a postdoctoral fellow, I applied neurophysiological approaches to identify sites in the forebrain that integrate neural and humoral signals to regulate sympathetic nerve activity and blood pressure. As an independent

investigator, my laboratory investigates how the central nervous system senses circulating factors to regulate sympathetic function and blood pressure during a variety of conditions or diseases such as obesity and salt-sensitive hypertension.

My laboratory utilizes multiple approaches including simultaneous sympathetic nerve recordings, brain microinjections, in vivo single-unit recordings and juxtacellular labeling, functional neuroanatomy, chronic sympathetic nerve and blood pressure recordings using Data Sciences Telemetry system, and in vitro patch-clamp recordings. In the past 2 years at Penn State College of Medicine, we have initiated microneurography and renal blood flow measurements in humans through collaboration with Drs. Sinoway and Leuenberger in the Heart and Vascular Institute. This positions our research group to translate the current findings to humans. The current application builds logically upon my prior work and currently funded NIH R01. Dr. Browning will provide additional expertise for in vitro patch-clamp electrophysiology. In summary, I have demonstrated a track record of successful and productive research projects that will enhance the overall completion of the project.

B. Positions and Honors

Positions and Employment

2005-2009	**Assistant Professor**, Department of Physiology, University of Kentucky Gill Heart Institute Research Faculty, Cardiovascular Research Faculty Director, Rodent Telemetry Core Facility
2009-Present	**Associate Professor**, Department of Cellular & Molecular Physiology Pennsylvania State College of Medicine, Hershey, PA
2010-Present	Director, American Heart Association Summer Undergraduate Program
2013-Present	Director, Rodent Telemetry Core Facility
2014-Present	**Associate Professor**, Department of Neural & Behavioral Sciences Pennsylvania State College of Medicine, Hershey, PA

Other Experience / Professional Membership

1998-Present	American Physiological Society
2002-Present	American Heart Association – Council High Blood Pressure Research
2003-2006	American Physiological Society Water & Electrolyte Steering Committee
2003-2006	American Physiological Society Trainee Advisory Committee
2006-2007	American Physiological Society Strategic Planning Task Force
2007-2011	American Physiological Society Conference Committee
2008-2011	Secretary/Treasurer, American Physiology Society CNS Section
2006-Present	AHA Peer Review, Vascular Biology/Blood Pressure Regulation
2007-Present	American Physiological Society CNS Steering Committee
2011-2014	American Physiology Society Joint Program Committee
2011-Present	Program Committee, AHA Council for High Blood Pressure Research
2009-Present	Editorial Board Member, Hypertension
2013-Present	Editorial Board Member, Physiological Reports
2013-Present	Editorial Board Member, Am J Physiol Regul Integr Comp Physiol
2013-Present	Section Editor, Brain and Hypertension, Current Hypertension Reports
2014-Present	Associate Editor, Am J Physiol Regul Integr Comp Physiol
2014-Present	Associate Editor, Journal of Neurophysiology
2013-Present	Chair, Central Nervous System Section, American Physiological Society
2013-Present	Co-Chair, American Heart Vascular Biology/Integrative Blood Pressure
2014-Present	Vice Chair, American Heart Association Research Committee, Great Rivers Affiliate
2014-Present	Member, American Heart Association Strategically Focused Hypertension Research Center Committee

Honors

2000, 2004	Caroline tum Suden/Frances Hellebrandt Award
2001-2003	APS Water & Electrolyte Research Recognition Award
2005	APS Neural Control & Autonomic Regulation - Michael J. Brody Award
2007	AHA Council for High Blood Pressure Research - Harry Goldblatt Award
2008	APS Central Nervous System New Investigator Award
2009	Fellow of the American Heart Association (FAHA)
2012	Established Investigator of the American Heart Association
2012	Dean's Award for Excellence in Teaching

C. Selected Peer-Reviewed Publications (42 peer-review publications):

Most relevant to current application

1. **STOCKER SD** and Toney GM. Median preoptic neurons projecting to the hypothalamic paraventricular nucleus respond to osmotic, circulating AngII and baroreceptor input in the rat. J Physiol, 568: 599-615, 2005. PMCID: PMC1474729.

2. **STOCKER SD**, Simmons JR, Stornetta RL, Toney GM, and Guyenet PG. Water deprivation activates a glutamatergic projection from the hypothalamic paraventricular nucleus to the rostral ventrolateral medulla. J Physiol, 568: 599-615, 2005. PMCID: PMC2861548.

3. Adams JM, Madden CJ, Sved AF, **STOCKER SD**. Increased dietary salt enhances sympathoexcitatory and sympathoinhibitory responses from the rostral ventrolateral medulla. *Hypertension* 50: 354-359, 2007. PMID: 17592069.

4. Adams JM, McCarthy JJ, **STOCKER SD**. Excess dietary salt alters angiotensinergic regulation of neurons in the rostral ventrolateral medulla. *Hypertension* 52: 932-937, 2008. PMCID: PMC2635570

5. Adams JM, Bardgett ME, **STOCKER SD**. Ventral lamina terminalis mediates enhanced cardiovascular responses of rostral ventrolateral medulla neurons during increased dietary salt. *Hypertension* 54: 308-314, 2009. PMCID: PMC3024146.

6. Yamauchi K, Tsuchimochi H, Stone AJ, **STOCKER SD**, Kaufman MP. Increased dietary salt intake enhances the exercise pressor reflex. Am J Physiol Heart Circ Physiol 306: H450-H454, 2014. PMCID: PMC3920137

7. Simmonds SS, Lay J, **STOCKER SD**. Dietary salt intake exaggerates cardiovascular reflexes and increases blood pressure variability in normotensive rats. Hyp*ertension* 64:583-589, 2014. PMID: 24914195.

<u>Additional publications relevant to field</u>

8. STOCKER SD, Meador R, Adams JM. Neurons of the rostral ventrolateral medulla contribute to obesity-induced hypertension in rats. Hypertension 49: 640-646, 2007. PMID: 17190873.

9. Taylor AC, McCarthy JJ, **STOCKER SD**. Mice lacking the transient receptor vanilloid potential 1 channel display normal thirst responses and central Fos activation to hypernatremia. Am J Physiol Regul Integr Comp Physiol 294: R1285-R1293, 2008. PMID: 18272658

10. Bardgett ME, McCarthy JJ, **STOCKER SD**. Glutamatergic receptor activation in the rostral ventrolateral medulla mediates the sympathoexcitatory response to hyperinsulinemia. *Hypertension* 55: 284-290, 2010. PMCID: PMC2861553

11. **STOCKER SD**, Madden CJ, Sved AF. Excess dietary salt alters the excitability of central sympathetic networks. *Physiol Behav* 100: 519-524, 2010. PMCID: PMC3024145

12. Ward KR, Bardgett J, Wolfgang L, **STOCKER SD**. Sympathetic response to insulin is mediated by melanocortin 3/4 receptors in the hypothalamic paraventricular nucleus. *Hypertension* 57(3): 435-441, 2011. PMCID: PMC3580160

13. Luckett BS, Frielle J, Wolfgang L, **STOCKER SD**. Arcuate injection of the anti-insulin affibody prevents the sympathetic response to insulin. *AJP Heart Circ Physiol* 304: H1538-H1546, 2013. PMCID: PMC3680721

14. **STOCKER SD** and Muntzel MS. Chronic recording of sympathetic nerve activity in rats:

surgical techniques, assessment of nerve activity, and quantification. *AJP Heart Circ Physiol* 305: H1407-1416. PMCID: PMC3840263

15. Kinsman B, Cowles J, Simmonds SS, Lay J, Browning BN, **STOCKER SD**. Osmoregulatory thirst in mice lacking the transient receptor potential vanilloid type 1 (TRPV1) and/or type 4 (TRPV4) receptor. *Am J Physiol Regul Integr Physiol,* in press. PMID: 25100078

D. Research Support

Ongoing Research Support

12EIA8230000 **STOCKER, SD** (PI) 01/01/12 – 12/31/16
American Heart Association Established Investigator Award
"Neurogenic Actions of Dietary Sodium in Sympathetic-Cardiovascular Regulation"
The goal of this project is to develop a translational research program to examine how changes in dietary salt intake alter sympathetic cardiovascular regulation to cause salt-sensitive hypertension.

1R01HL113270-01A1 **STOCKER, SD** (PI) 02/01/13 – 01/31/17
NIH NHLBI
"Central Osmosensory Mechanisms in Salt-Sensitive Hypertension"
The goal of this project is to identify cellular elements involved in brain sodium-sensing and how changes in sodium concentration contribution to elevated sympathetic outflow in salt-sensitive hypertension.

14UFEL19160001 **STOCKER, SD** (PI) 06/01/14 – 05/30/16
American Heart Association
Penn State SURF Program in Cardiovascular Disease
The purpose of this training grant is to provide support for undergraduate students to spend 10-weeks in a cardiovascular research-intensive laboratory. The program also includes weekly career development and research seminars.

11PRE741007 Kinsman, B (PI) 07/01/14 – 06/30/16
American Heart Association
"Central Sodium Sensing in Salt-Sensitive Hypertension"
The goal of this fellowship is to provide training and identify how the central nervous system detects changes in plasma or cerebrospinal fluid sodium concentration to influence sympathetic nerve activity and blood pressure in salt-sensitive hypertension.
Role: Sponsor

Completed

10UFEL3900000 **STOCKER, SD** (PI) 06/01/10 – 05/30/12
American Heart Association SURF Grant
The goal of this project is to support training for promising undergraduate students in the field of cardiovascular disease. Students spend 10 weeks in a cardiovascular research laboratory at Penn State College of Medicine and Hershey Medical Center.

11PRE741007 Wolfgang, L (PI) 07/01/11 – 06/30/13
American Heart Association
"Hypothalamic Mechanisms Mediating Effects of Leptin in Diet-Induced Obesity"
The goal of this fellowship is to provide training and identify how leptin activates hypothalamic circuits to increase sympathetic nerve activity and blood pressure in diet-induced obesity.
Role: Sponsor

12UFEL10080000 **STOCKER, SD** (PI) 06/01/12 – 05/30/14
American Heart Association
Penn State SURF Program in Cardiovascular Disease
The purpose of this training grant is to provide support for undergraduate students to spend 10-weeks in a cardiovascular research-intensive laboratory. The program also includes weekly career development and research seminars.

1R01 HL090826-01 **STOCKER, SD** (PI) 02/15/08 – 01/31/14
NIH NHLBI
"Central Nervous System Mechanisms of Obesity Hypertension"
The goal of this grant is to identify the neural circuits and synaptic mechanisms by which hyperinsulinemia and hyperleptinemia increase sympathetic outflow and arterial blood pressure in obesity-induced hypertension.