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 717-783-2548.

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

2. **Reporting Period (start and end date of grant award period):** 1/1/2009-12/31/2012

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:** # 4100047645

6. **Project Number and Title of Research Project:** 13. Identification and Analysis of Arterial Blood Pressure Noise in Baroreceptor Denervated Rats.

7. **Start and End Date of Research Project:** 1/1/2009 – 6/30/2011

8. **Name of Principal Investigator for the Research Project:** Xiaorui Tang, Ph.D.

9. **Research Project Expenses**.

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

   $ $46,380 Total Costs

   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	Position Title	% of Effort on Project	Cost
Hu	Lab Tech	30 % - 2009-2010	$9,974.43
Snyder	Summer Intern	100% for 1 month	$2,457.87
Freet	Postdoc	55% - Yr 2.5	$5,919.68

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	Position Title	% of Effort on Project
Tang	Assistant Professor	10%
Norgren	Professor	5%

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
None		

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 to be awarded:
Identification and Analysis of Arterial Blood Pressure Noise in Baroreceptor Denervated Rats	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Nov. 18, 2008	$155,100	$Not funded
Identification and Analysis of Arterial Blood Pressure Noise in Baroreceptor Denervated Rats	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Mar. 16, 2010	$155,100	$Not funded

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

Yes_____ No___X_____

If yes, please describe your plans:

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

No further research will be conducted on this project.

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		1
Female				
Unknown				
Total	**1**	**1**		**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	1	1		1
Unknown				
Total	**1**	**1**		**1**

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

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.

This project involved using a unique animal model (the neuromuscularly blocked (NMB) rat model) which is only available at the Penn State Hershey Medical Center. This model allows us to conduct *in vivo* experiments that last for at least a week which enables us to study processes that require days or weeks to develop, such as the long lasting neural plasticity effect.

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 application's strategic plan). 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.

Project Overview

The arterial baroreceptor reflex rapidly stabilizes and accurately regulates blood pressure. A consistent and prominent feature, observed across many species is that obliterating the baroreceptor afferent input to the brain by sinoaortic denervation (SAD) more than doubles arterial pressure variability (APV). However, surprisingly few studies have investigated the neurophysiological sources, and/or regulatory function, if any, of the APV. Understanding APV is of clinical importance. Since the 1980's, despite numerous attempts to catalog APV changes in a range of diseases, the prognostic and diagnostic promise has yet to be realized. A possible reason for this is lack of fundamental understanding of the sources and causes of APV. This current study aims at identifying the potential key central noise sources that are responsible for the large post-SAD APV. Our long-term goal is to further develop APV analysis into a non-invasive and economical tool for the clinical prognosis and diagnosis of cardiovascular diseases.

Previously, using a unique chronically neuromuscular blocked (NMB) rat preparation, we found that SAD more than doubled APV, and that ganglionic block, using chlorisondamine, significantly reduced the large post-SAD APV. These results indicated (1) that the noise sources for APV are endogenous and central, and (2) that normally, APV is attenuated by the baroreflex. Studies have shown that the brain regions rostral to the collicular are not potential noise sources, which suggests that the caudal brain stem is likely to be the source of the noise. Using NMB rats, we will determine the obligate roles of the baroreflex key way stations in the brain stem, e.g., the rostral ventral lateral medulla (RVLM), in the large post-SAD APV. We hypothesize that the RVLM (The Specific Aim) has an important role in the post-SAD APV. The Aim consists of 4 phases: baseline (3 d) -> SAD (3 d) -> bilateral ibotenic lesions of the RVLM (3 d) -> ganglionically block the rat with intravenous chlorisondamine (2 d). A control, of bilateral lesions in a non-cardiovascular area of the brain stem, the cuneate nuclei, will parallel the Aim. The major outcome measurements include the APV, characterized as the standard deviation of the arterial pressure (AP) and the very low frequency (VLF: 0.01-0.2 Hz) power of the AP spectrum. For each variable, separately, a 2 x 3 mixed factorial ANOVA varying experimental conditions (control, lesion) x phases (baseline, SAD, lesion) will be used to determine the RVLM contribution to post-SAD APV.

Final Progress Report

Two major aims were achieved in this project. The first aim was to evaluate the hypothesis that the CNS is a major contributor to the observed large post-SAD APV. To this end, we evaluated arterial pressure variability (APV) after: (1) complete sino-aortic denervation (SAD); (2) ganglionic blockade; and (3) ganglionic blockade followed by infusion of pressors in SAD and baroreflex-intact rats. The second aim of this project was to examine

the relative contribution of the aortic and carotid sinus baroreflexes in the control of APV. Therefore, we measured APV following: (1) bilateral aortic denervation followed by SAD; (2) carotid sinus denervation followed by SAD; and (3) unilateral aortic denervation. Finally, to better understand detail characteristic features of the variability, APV was analyzed both in the time domain as the standard deviation (SD) of the hourly systolic arterial pressure and in the frequency domain with the Fast Fourier Transform power spectral analysis of the APV.

Experimental Methods:

Sprague-Dawley female (255-390 g) rats were used in the study. The experimental protocols were supervised and certified to be in compliance with National Institute of Health and American Physiology Society guidelines by the Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee (IACUC). On average, each preparation lasted for ~10 days, and multiple protocols were implemented in the same preparation. Each rat was monitored and attended to around the clock. The NMB rat was prepared as previously described in (*Tang X, and Dworkin BR. Baroreflexes of the rat. IV. ADN-evoked responses at the NTS. Am J Physiol Regul Integr Comp Physiol 293: R2243-2253, 2007*). Briefly, for maintaining the preparation, the following parenteral solutions were infused continuously: (1) An intra-arterial solution (0.6 ml/h) consisting of 50 ml H_2O, 50 ml 0.5 N lactated Ringer, 1.25 g oxacillin Na, 2.8 mg α-cobratoxin, 0.3 mg vitamin K (Synkavite) and 20 meq K^+ (as KCl); (2) an intra-venous solution (0.6 ml/h) consisting of 50 ml H_2O, 50 ml 0.5 N lactated Ringer and 1.25 g oxacillin Na for the initial 48 hours, then glucose, 8.5% Travasol amino acid and intralipid (fat emulsion) were added subsequently to provide necessary nutrients.

Surgery:

In brief, the general surgical procedures are as follows: the rat was deeply anaesthetized with >1.5% isoflurane and monitored to ensure that: (1) the electroencephalogram (EEG) was synchronized and dominated by high-voltage low frequency activity; (2) mean systolic arterial pressure was < 100 mmHg; (3) heart rate was < 420 beats/min; and (4) arterial pressure, heart rate and EEG did not respond to surgical manipulation. General surgery included implantation of a pair of subcutaneous precordial electrocardiogram (EKG) silver wire electrodes, a transurethral bladder cannula to allow drainage and measurements of the rate of urine production, a femoral artery cannula for arterial pressure measurement and a femoral vein cannula for recording venous pressure and administering parenteral solutions. General surgery also included implantations of EEG electrodes to assure maintenance of adequate levels of anesthesia during the surgery and the regularity of sleep and wakefulness cycles throughout the experiment. Core temperature was servo-regulated at 37 ºC. Neuromuscular block was induced with a 75 µg i.v. bolus of α-cobratoxin and maintained by continuous infusion at 250 µg/day. The rat was ventilated with positive pressure at a rate of 72 breaths/min and at a volume of 200 cc/min. The air mixture consisted of 48.5% O_2, 47% N_2, 3% CO_2 and 1.5% isoflurane. After the surgery, the rat was allowed to recover for two days during which the isoflurane level was gradually reduced to 0.5%. During the recovery, the adequate level of anesthesia was maintained by observing that the EEG, arterial pressure and heart rate signals were within the physiological range. If any of these signals indicated signs of distress during the reduction of anesthesia, the level of isoflurane was elevated to its

previous stage and maintained for a longer period of time before the next lowering attempt. All experimental data used for the final analysis in this study were acquired at 0.5% isoflurane level.

The aortic and carotid sinus denervation: Briefly, the aortic denervation was achieved by isolating and resecting the superior cervical ganglion, superior laryngeal nerve and aortic depressor nerve (ADN). The carotid sinus denervation was achieved by stripping off all connective tissue around the carotid sinus region and painting the region with a solution of 10% phenol in ethanol to remove any remaining baroreceptor afferent fibers. Complete SAD was achieved by bilateral aortic and carotid sinus denervation. This was confirmed by the absence of bradycardia in response to a phenylephrine challenge, which typically increased arterial pressure more than 30 mmHg.

Experimental Protocols

Protocols were arranged into two main categories corresponding to the two major aims of the study: (1) evaluating the CNS contribution to the post-SAD APV; and (2) evaluating the role of the aortic and carotid sinus baroreflexes in stabilizing cardiovascular variability.

(1) Protocols for evaluating the CNS contribution to the post-SAD cardiovascular variabilities.

In the baroreflex denervated rats: Baseline→ SAD→ chlorisondamine administration→ phenylephrine and epinephrine infusions: Following recovery from the general surgery, six hours of baseline data were acquired before SAD. Twenty-four hours following the SAD, a bolus of the ganglionic blocker, chlorisondamine (2.5 mg/kg, i.v.), was given. Six hours later, phenylephrine and epinephrine were infused continuously (i.v.) for six hours each in a random order at a dose that restored arterial pressure to or above baseline levels, as customized for each NMB rat (phenylephrine, 5 to 73 $\mu g \cdot kg^{-1} \cdot min^{-1}$; epinephrine, 2.6 to 36 $\mu g \cdot kg^{-1} \cdot min^{-1}$). To achieve the appropriate dose, the infusion rates of phenylephrine and epinephrine were adjusted from 5 to 33 µl/min and from 3 to 13 µl/min, respectively. The purpose of using two different pressors, i.e., phenylephrine (an alpha adrenergic agonist) and epinephrine (an alpha and beta adrenergic agonist), was to partially control for idiosyncratic effects of the particular agent on the APV. Saline infusion at a rate of 10 µl/min was used as a control.

In baroreflex-intact rats: Baseline→ Chlorisondamine administration→ phenylephrine and epinephrine infusions: Following recovery from the general surgery, a bolus of chlorisondamine (2.5 mg/kg, i.v.) was given. Six hours later, the pressors phenylephrine and epinephrine were continuously infused (i.v.) for six hours each in a random order as described above. Saline infusion at a rate of 10 µl/min was used as a control.

(2) Protocols for evaluating the role of the aortic and carotid sinus baroreflexes in stabilizing the APV

Bilateral aortic or carotid sinus denervation: Baseline → bilateral aortic (or carotid sinus) denervation→ bilateral carotid (or aortic) denervation, i.e., SAD: Following recovery from the general surgery, bilateral aortic (or carotid sinus) denervation was performed. This initial denervation was chosen randomly on either pair of the baroreceptors (i.e., half of the rats

began with the aortic then the carotid sinus denervation, and another half with the carotid sinus then the aortic denervation). Twenty four hours later, further denervation was conducted on the remaining pair of the baroreceptors.
Unilateral aortic denervation: Baseline → left or right aortic denervation: Following recovery from the general surgery, aortic denervation was performed on the left aortic baroreceptors.

Data acquisition and analysis:
All experimental signals were continuously acquired 24 hours a day and 7 days a week throughout the entire experiment using Spike2® and a Power 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK). Five hours of data were analyzed for each experimental condition in protocols evaluating the CNS contribution to the post-SAD cardiovascular variability. Ten hours were analyzed for each condition in protocols evaluating the role of the aortic and carotid sinus baroreflexes in stabilizing the APV. To eliminate potential transitional effects of switching from one experimental condition to another, signals were often acquired for more than five or ten hours in an experimental condition, and those from the last five or ten hours were analyzed for that condition. Data used for final analysis were always acquired at a 0.5% isoflurane level. Arterial pressure variability (APV) and heart rate variability (HRV) were calculated as follows: First, beat-to-beat systolic arterial pressure and heart rate were generated from the arterial pressure and EKG waveforms respectively using a peak detection algorithm in the Spike2® software. APV and HRV were calculated in the time domain as the standard deviations of systolic arterial pressure and heart rate in each hour; and the standard deviations across five or ten hours were averaged and reported for each experimental condition. In the frequency domain, Fast Fourier Transform (0.012 Hz resolution, Hanning window, no overlapping samples) power spectral analysis was conducted on systolic arterial pressure for each hour and the very low frequency (VLF: 0.01-0.15 Hz) and low frequency (LF: 0.15-0.6 Hz) powers were calculated by integration of the areas in the corresponding frequency ranges. The VLF and LF powers across five and ten hours were averaged and reported for each experimental condition.

Statistical Analysis: A separate analysis of variance (ANOVA) with repeated measures was used to determine the effect of each experimental intervention on APV, HRV and the VLF and LF powers of the systolic arterial pressure spectra; Newman-Keuls post-hoc comparisons were applied when appropriate. Differences were considered significant at $p<0.05$. A Student *t-test* was used to evaluate the effect of unilateral aortic denervation on the APV and differences were considered significant at $p<0.05$. All results are presented as the mean ± SE.

Results
Results are arranged according to the two major aims of this study: (1) evaluating the CNS contribution to the post-SAD cardiovascular variability; and (2) evaluating the role of the aortic and carotid sinus baroreflexes in stabilizing the APV.

(1) The CNS has a significant contribution to the post-SAD APV.

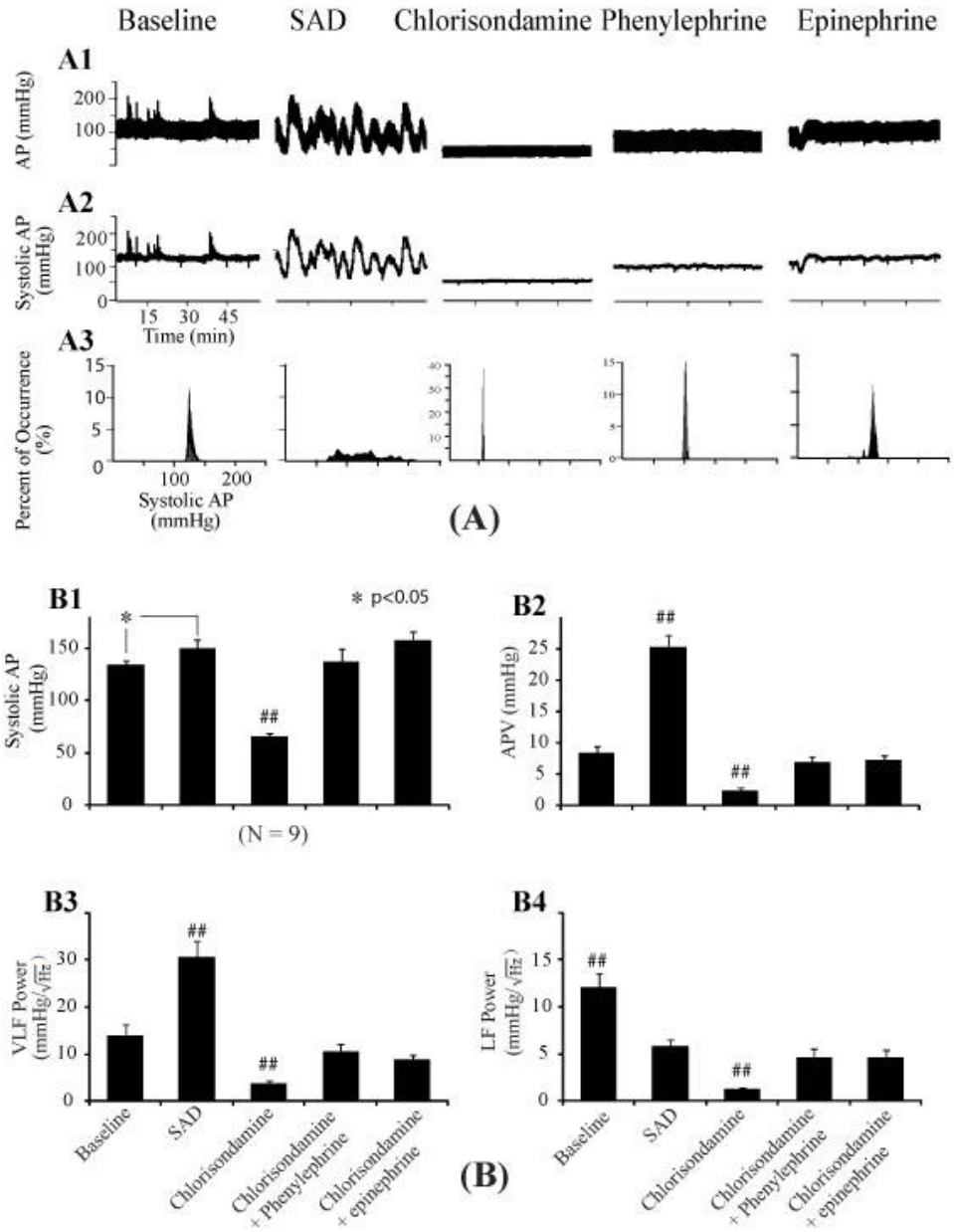

Fig. 1: Effects of sinoaortic denervation (SAD), chlorisondamine administration, intravenous infusions of phenylephrine, and epinephrine on the arterial pressure (AP) and arterial pressure variability (APV) in NMB rats. Panel A shows representative traces of AP (A1) and systolic AP (A2) and a frequency histogram of the systolic AP (A3) from each experimental procedure. Panel B summarizes the results of systolic AP (B1), APV (B2), very low frequency (VLF: 0.01-0.15 Hz, B3) and low frequency (LF: 0.15 – 0.6 Hz, B4) powers of the systolic AP spectra at baseline, following sinoaortic denervation, chlorisondamine administration and intravenous infusions of phenylephrine and epinephrine. While SAD significantly increased APV and VLF power, chlorisondamine decreased the values of both variables to below their corresponding baseline levels; subsequent phenylephrine and epinephrine infusions only slightly increased APV. APV was calculated as standard deviation of the hourly systolic AP. Data are mean ± SEM. Error bars = SEM. *p<0.05. ## Statistically significant difference from every other experimental stage.

SAD increased and chlorisondamine decreased APV in the NMB *rat* (Fig. 1, N=9)

Systolic AP following baseline, SAD, chlorisondamine, phenylephrine, and epinephrine infusion was analyzed using one-way analyses of variance (ANOVAs) with repeated measures across stage (Fig. 1B1, N=9). The main effect of stage was significant ($F(4, 38) = 50.18$, $p < 0.05$). Newman-Keuls post hoc test showed that, following SAD, systolic AP was significantly increased from the baseline (from 133 ± 4.9 to 151 ± 4.17 mmHg; $p<0.05$ vs. baseline); the subsequent application of chlorisondamine decreased systolic AP from 151 ± 4.17 to 67 ± 1.98 mmHg ($p<0.05$ vs. every other experimental stage). In the presence of chlorisondamine, phenylephrine and epinephrine infusion both returned systolic AP to the baseline control level, from 67 ± 1.98 mmHg to 138 ± 7.72 mmHg for phenylephrine ($p<0.05$ vs. the chlorisondamine stage); and from 67 ± 1.98 mmHg to 153 ± 5.26 mmHg for epinephrine ($p<0.05$ vs. the chlorisondamine stage).

APV was analyzed using repeated measures of ANOVAs across stage (Fig. 1B2, N=9). The main effect of stage was significant ($F(4, 38) = 84.31$, $p < 0.05$). Newman-Keuls post hoc test showed that, following SAD, APV was significantly increased from the baseline (from 8.3 ± 1.4 to 25 ± 1.98 mmHg; $p<0.05$ vs. baseline); the subsequent application of chlorisondamine decreased APV from 25 ± 1.98 to 2.44 ± 0.36 mmHg ($p<0.05$ vs. every other experimental stages). In presence of chlorisondamine, phenylephrine and epinephrine infusion both only slightly increased APV, from 2.44 ± 0.36 to 7.18 ± 0.47 mmHg for phenylephrine ($p<0.05$ vs. the chlorisondamine stage); and from 2.44 ± 0.36 to 7.46 ± 0.71 mmHg for epinephrine ($p<0.05$ vs. the chlorisondamine stage). These data suggest that the CNS has a significant contribution to the post-SAD APV. In corollary, these data also suggest that the baroreflex is frequently engaged to restrain moment-to-moment APV.

VLF power was analyzed using repeated measures of ANOVAs across stage (Fig. 1B3, N=9). The main effect of stage was significant ($F(4, 38) = 41.97$, $p<0.05$). Newman-Keuls post hoc test showed that, following SAD, VLF was significantly increased from the baseline (from 13.23 ± 2.07 to 30.89 ± 2.38 mmHg/√Hz; $p<0.05$ vs. baseline); the subsequent application of chlorisondamine decreased VLF power from 30.89 ± 2.38 to 3.77 ± 0.51 mmHg/√Hz ($p<0.05$ vs. every other experimental stages). In the presence of chlorisondamine, phenylephrine and epinephrine infusion both slightly increased VLF power, from 3.77 ± 0.51 to 11 ± 0.99 mmHg/√Hz for phenylephrine ($p<0.05$ vs. the chlorisondamine stage); and 3.77 ± 0.51 to 9.87 ± 0.85 mmHg/√Hz for epinephrine ($p<0.05$ vs. the chlorisondamine stage). VLF was identified as the principal frequency region of the baroreflex system. The VLF changes in response to each experimental intervention corresponded well with the APV changes, further suggesting that a substantial proportion of the APV likely arises from the CNS, and the baroreflex is frequently engaged to restrain moment-to-moment APV.

LF power was analyzed using repeated measures of ANOVAs across stage (Fig. 1B4, N=9). The main effect of stage was significant ($F(4, 38) = 20.63$, $p < 0.05$). Newman-Keuls post hoc test showed that, following SAD, LF power was decreased from the baseline (from 12.17 ± 1.68 to 6.34 ± 0.43 mmHg/√Hz; $p<0.05$ vs. baseline); the subsequent application of chlorisondamine further decreased LF power from 6.34 ± 0.43 to 1.48 ± 0.11 mmHg/√Hz

($p<0.05$ vs. every other experimental stages). In the presence of chlorisondamine, the phenylephrine and epinephrine infusion both slightly increased LF power, from 1.48 ± 0.11 to 4.98 ± 0.59 mmHg/√Hz for phenylephrine ($p<0.05$ vs. the chlorisondamine stage); and 1.48 ± 0.11 to 4.76 ± 0.45 mmHg/√Hz for epinephrine ($p<0.05$ vs. the chlorisondamine stage). These data suggest that LF is the resonant (i.e., oscillation) region of the baroreflexes. When denervation eliminated the negative feedback, it also eliminated the oscillation in this closed loop negative feedback system, which resulted in a power decrease in the LF band of the APV spectrum.

***Chlorisondamine, phenylephrine, and epinephrine infusion produced similar effects in baroreflex intact NMB rats as in SAD NMB rats* (Fig. 2)**

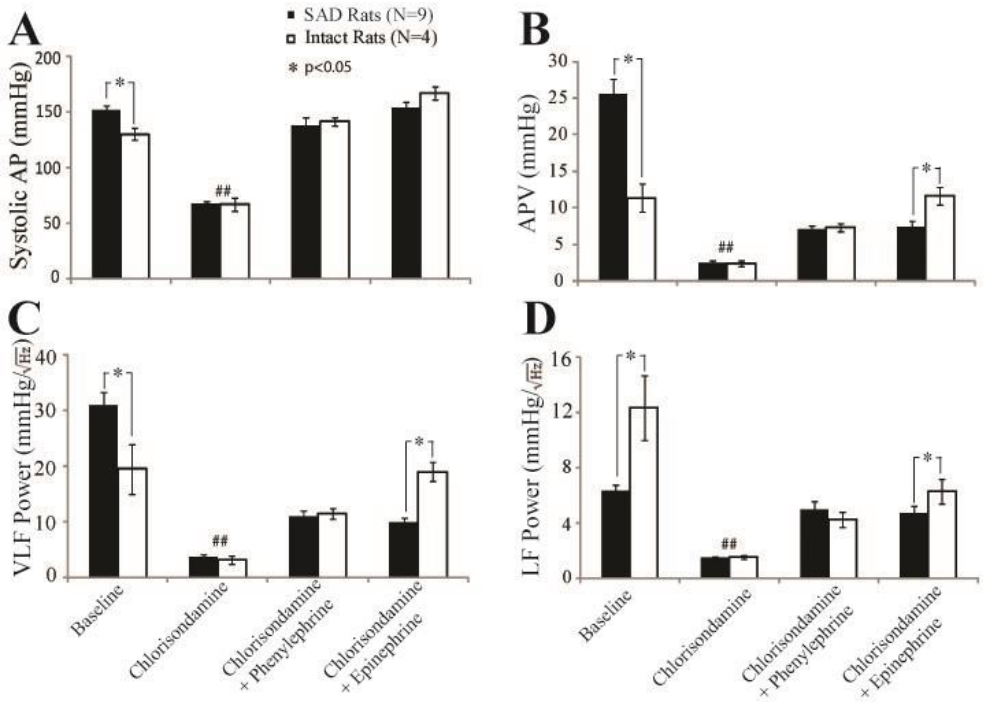

Fig. 2: Chlorisondamine administration, intravenous infusions of phenylephrine, and epinephrine produced similar effects on the systolic arterial pressure (AP), arterial pressure variability (APV), VLF and LF powers of the systolic AP spectra in both SAD and baroreflex-intact NMB rats. (A) The systolic AP. In both SAD and baroreflex intact rats, chlorisondamine significantly decreased systolic AP and subsequent infusions of phenylephrine and epinephrine both returned systolic AP to or above the corresponding baseline levels. (B) The APV. In both SAD and baroreflex intact rats, chlorisondamine significantly decreased the APV and subsequent infusions of phenylephrine and epinephrine both increased APV to the baroreflex intact baseline variability level. (C) VLF power of the systolic AP spectrum. In both SAD and baroreflex intact rats, chlorisondamine significantly decreased the VLF power and subsequent infusions of phenylephrine and epinephrine both increased the VLF power to the baroreflex intact baseline level. (D) LF power of the systolic AP spectrum. In both SAD and baroreflex intact rats, chlorisondamine significantly decreased LF power and subsequent infusions of phenylephrine and epinephrine both increased the power to the SAD level. Moreover, the SAD rat had a higher systolic AP, larger APV and VLF, but a lower LF power than

the baroreflex intact rat during the baseline stage. In the baseline stage, the baroreflexes were completely denervated for the 'SAD Rats' and un-manipulated for the 'Intact Rats'. APV was defined as standard deviation of the hourly systolic AP. Data are mean ± SEM. Error bras = SEM. *p<0.05. ## Statistically significant difference from every other experimental stage.

Systolic AP, APV, VLF and LF powers following baseline, chlorisondamine, phenylephrine, and epinephrine infusion in the baroreflex intact and SAD rat were analyzed separately using repeated measures of two-way ANOVAs varying group and stage. There was a significant 2-way interaction varying group and stage in systolic AP, APV, VLF, and LF (systolic AP, $F(3, 33) = 3.82$, $p < 0.05$, Fig. 2A; APV, $F(3, 33) = 32.93$, $p < 0.05$, Fig. 2B; VLF, $F(3, 33) = 11.69$, $p<0.05$, Fig. 2C; and LF, $F(3, 33) = 9.76$, $p < 0.05$, Fig. 2D). Newman-Keuls post hoc test showed that, similarly, in both SAD and baroreflex intact NMB rats, chlorisondamine significantly decreased systolic AP, APV, VLF and LF powers compared with the pre-chlorisondamine baseline condition ($p<0.05$ vs. every other experimental stages); the subsequent phenylephrine and epinephrine infusion (in presence of the chlorisondamine) both returned systolic AP to the baseline condition, and increased APV, VLF and LF power compared with the chlorisondamine condition ($p<0.05$ vs. the chlorisondamine stage). Between the SAD and the baroreflex intact rats, the SAD rats had a higher systolic AP (Fig. 2A), APV (Fig. 2B), and VLF power (Fig. 2C), but a lower LF power (Fig. 2D) than the baroreflex intact rats during the pre-chlorisondamine baseline stage ($p<0.05$, for all variables). Note that, the baroreflexes were intact for the 'Intact Rats,' and completely denervated for the 'SAD Rats' in the baseline stage in Fig. 2. Interestingly, epinephrine infusion produced a larger increase in APV, VLF power and LF power in the baroreflex intact rats than in the SAD rats, the significance of this increase, if any, has yet to be determined.

Independent of prior status of the baroreflex system (i.e., SAD or intact), a bolus application of chlorisondamine substantially decreased APV and VLF in both SAD and baroreflex intact NMB rats, suggesting that, by interrupting the baroreflex efferent pathway, chlorisondamine blocked the CNS contribution to the APV. Moreover, in presence of the chlorisondamine, subsequent phenylephrine and epinephrine infusion both returned systolic AP to or above the baseline levels in both SAD and intact rats (Fig. 2A, $p < 0.05$). This restoration of systolic AP significantly increased APV (Fig. 2B), VLF (Fig. 2C) and LF powers (Fig. 2D) in both SAD and intact NMB rats, suggesting that the APV is not only modulated by the CNS, but by the peripheral system as well.

(2) The aortic baroreflex has a more dominant role in the control of APV than the carotid sinus baroreflex

The aortic denervation produced a larger increase in APV than the carotid sinus denervation in the NMB *rat* (Fig. 3, N=6 in each group):

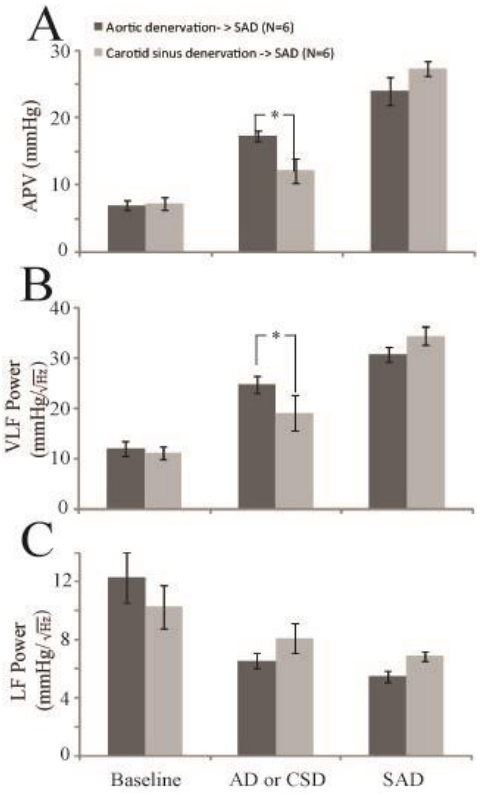

Fig. 3: While bilateral aortic and carotid sinus denervation significantly increased APV and VLF, bilateral aortic denervation produced a larger increase in APV than carotid sinus denervation. Panels A, B and C depicted the effects of different denervation (i.e., AD, CSD or SAD) on APV, VLF and LF, respectively. Bilateral aortic and carotid sinus denervation both significantly increased APV and VLF power compared to the baroreflex intact baseline ($p < 0.05$ vs. baseline, for the APV and the VLF power); the subsequent SAD further increased APV and VLF. ($p<0.05$ vs. baseline and AD or CSD denervation stages). Bilateral aortic denervation produced a larger increase in APV and VLF than carotid sinus denervation ($p < 0.05$, aortic vs. carotid sinus denervation). Data are mean ± SEM. Error bars = SEM. *$p<0.05$. APV was calculated as standard deviation of the hourly systolic arterial pressure. VLF and LF: the very low frequency (VLF: 0.01-0.15 Hz) and the low frequency (LF: 0.15 – 0.6 Hz) power of the systolic arterial pressure spectra, respectively. AD: aortic denervation; CSD: carotid sinus denervation; SAD: sinoaortic denervation.

APV, VLF power, and LF power following baseline, bilateral aortic or carotid sinus denervation and complete SAD were analyzed using separate two-way analyses of variance (ANOVAs) with repeated measures varying group and stage. There was a significant 2-way interaction varying group and stage in APV and VLF (APV, $F(2,20) = 5.09$, $p<0.05$, Fig. 3A; and VLF, $F(2, 20) = 3.63$, $p<0.05$, Fig. 3B). Newman-Keuls post hoc test showed that, for the APV (Fig. 3A), it was increased from the baseline 7.02 ± 0.66 to 17.27 ± 0.82 mmHg following bilateral aortic denervation ($p<0.05$ vs. baseline), and further increased to 24.02 ± 2.07 mmHg following the subsequent complete SAD ($p<.05$ vs. baseline and bilateral aortic denervation); the APV was increased from the baseline 7.31 ± 0.96 to 12.14 ± 1.83 mmHg following bilateral carotid sinus denervation ($p<0.05$ vs. baseline), and further increased to 27.29 ± 1.12 mmHg following the subsequent complete SAD ($p<0.05$ vs. baseline and bilateral carotid sinus denervation). For the VLF power (Fig. 3B), it was increased from the baseline12.01 ± 1.5 to 24.69 ± 1.73 mmHg/√Hz ($p<0.05$ vs. baseline) following bilateral aortic denervation, and further increased to 30.63 ± 1.53 mmHg/√Hz following the subsequent complete SAD ($p<.05$ vs. baseline and bilateral aortic denervation); the VLF power was increased from 11.21 ± 1.31 to 19.12 ± 3.49 mmHg/√Hz following bilateral carotid sinus denervation ($p<0.05$ vs. baseline), and further increased to 34.38 ± 1.85 mmHg/√Hz following the subsequent complete SAD($p<.05$ vs. baseline and bilateral aortic denervation). These data suggest that while both aortic and carotic sinus baroreflexes are

important in stabilizing APV, the aortic baroreflex has a more dominant role the carotid sinus baroreflex. These data also suggest that the effect of complete SAD on APV, VLF and LF variables is independent of the sequence of the denervation.

A two-way analysis of variance with repeated measures revealed a significant difference on the main effect of stage in LF ($F(2, 20) = 21.33$, $p<0.05$). Newman-Keuls post hoc test indicated that, when combined, baseline LF was higher in baseline than in the partial denervation (i.e., AD or CSD) stage ($p<.05$, Fig. 3C).

***Single side aortic denervation did not elicit a significant increase in APV in the NMB rat* (Fig. 4, N=6):**

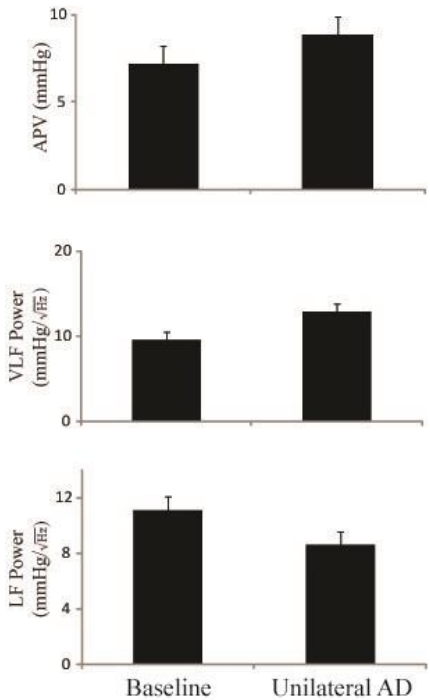

Fig. 4: Unilateral aortic denervation had no effect on the APV. From top to bottom, APV was characterized as the standard deviation of the hourly systolic arterial pressure. The VLF and LF were the very low frequency (VLF: 0.01-0.15 Hz) and the low frequency (LF: 0.15 – 0.6 Hz) power of the hourly systolic arterial pressure spectra, respectively. Data are mean ± SEM. Error bras = SEM. *$p<0.05$.

Following unilateral (left or right only) aortic denervation in the NMB rat, APV increased from 7.2 ± 1.03 to 8.87 ± 1.47 mmHg and VLF power from 9.54 ± 0.95 to 12.81 ± 1.89 mmHg/√Hz. LF power decreased from 11.06 ± 2.04 mmHg/√Hz to 8.58 ± 1.48 mmHg/√Hz (N=6 for all; Fig. 4). None of above variables were significantly altered by unilateral aortic denervation. Taken together with the results shown in Fig. 3, these data suggest that with the carotid sinuses intact, only a single aortic limb was required to stabilize the pressure.

***SAD had no effect on the HRV* (Fig. 5, N= 9)**

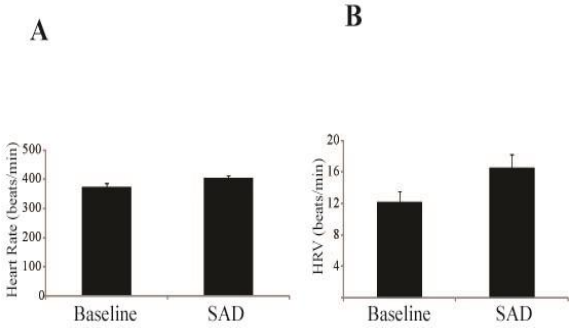

Fig.5: Sinoaortic denervation (SAD) had no effect on both the heart rate (A) and the heart rate variability (HRV) (B). Data are mean ± SEM. Error bras = SEM. *p<0.05.

In the NMB rat, baseline values of heart rate and HRV were 373 ± 13 and 12 ± 1 beats/min respectively. Following SAD, neither heart rate nor HRV were significantly altered (p>0.05; Fig. 5). The different outcomes of SAD on the APV and HRV suggest that different mechanisms are involved in producing and stabilizing heart rate and arterial pressure variability.

Published Abstracts:

1. **Tang, X.**, Browning, K. and Travagli, A., Gastrointestinal motility and vago-vagal reflexes in an animal model of the critically ill patient. *Digestive Disease Week, Chicago, IL, May 7-10, 2011*
2. Chen, L, Liu, Y, Chen, J., Dworkin, B. and **Tang, X.**, A simulation on baroreflex and long-term blood pressure regulation. *Neuroscience Conference, Washington DC, Nov.12-16, 2011.*
3. Doheny K., **Tang, X.** Browning K., Palmer C., and Travagli R., Correlation between decreased vagal activity and necrotizing enterocolitis (NEC), *Digestive Disease Week. San Diego, CA, May 2012.*

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?

_____Yes
__X___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?

_____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?

_____Number of subjects originally targeted to be included in the study
_____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:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____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.)

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, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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. Baroreflex and chemoreflex control of sympathetic activity following intermittent hypoxia	Christopher S. Freet, James F. Stoner, and Xiaorui Tang	Autonomic Neuroscience: Basic and Clinical	5/22/2012	☒Submitted ☐Accepted ☐Published

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

If yes, please describe your plans:

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.

None

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Xiaorui Tang, Ph.D. : (PI) Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Yunnan University, Kunming, Yunnan, P.R.China	B.S.	1989	Information & Electronic Science
New Jersey Institute of Technology, Newark, NJ	M.S.	1995	Electrical Engineering
New Jersey Institute of Technology, Newark, NJ with the doctoral dissertation conducted at Penn State College of Medicine, Hershey, PA	Ph.D.	2000	Electrical Engineering (Biomedical Engineering Focus)
The John Hopkins University, School of Medicine, Baltimore, MD	PostDoc.	2003	Biomedical Engineering, (Electrophysiology, & Autonomic Nervous Systems Focus)

Dr. Tang has extensive training in electrophysiology of the autonomic nervous system, cardiovascular sciences, and computer and electrical engineering. She is skilled in micro-surgery and has longstanding interests in baroreflex research. She has over 10 years experience with the chronically neuromuscular blocked (NMB) rat.

Ralph Norgren, Ph.D. Co-Investigator

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Pennsylvania, Philadelphia, PA	AB	1965	Psychology
University of Michigan, Ann Arbor, MI	MA	1968	Physiol. Psychology
University of Michigan, Ann Arbor, MI	PhD	1969	Physiol. Psychology
The Rockefeller University, New York, NY	Postdoc.	1969-1971	Neuroscience

Dr. Norgren has done research in the caudal brainstem of rodents for almost 40 years. Much of this activity involved neuroanatomical, neurophysiological, and behavioral studies of taste. Dr. Norgren also has extensive experience using electrophysiological guidance to make discrete lesions in both the nucleus of the solitary tract and the pontine parabrachial nucleus.