RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Federal Award Date: 03/13/2020

Grant Number: 5R01HL128330-04 REVISED
FAIN: R01HL128330

Principal Investigator(s):
KATHRIN BANACH, PHD

Project Title: The Role of p21-activated Kinase (Pak1) in Atrial Fibrillation

Ms. Jennifer Garcia
Manager, Grant Proposals
Rush University Medical Center
1653 W. Congress Parkway
Chicago, IL 606120000

Award e-mailed to: Jane_Winger@rush.edu

Period Of Performance:
Budget Period: 05/01/2019 – 04/30/2020
Project Period: 07/01/2016 – 04/30/2020

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to RUSH UNIVERSITY MEDICAL CENTER in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Heart, Lung, And Blood Institute of the National Institutes of Health under Award Number R01HL128330. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

John Diggs
Grants Management Officer
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Additional information follows

SECTION I – AWARD DATA – 5R01HL128330-04 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$306,091
Fringe Benefits	$33,654
Personnel Costs (Subtotal)	$339,745
Materials & Supplies	$60,367
Travel	$3,000
Other	$17,179
Subawards/Consortium/Contractual Costs	$88,042
Publication Costs	$2,000
Federal Direct Costs	$510,333
Federal F&A Costs	$229,962
Approved Budget	$740,295
Total Amount of Federal Funds Obligated (Federal Share)	$740,295
Less Unobligated Balance	$328,064
TOTAL FEDERAL AWARD AMOUNT	$412,231
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
4	$412,231	$412,231

Fiscal Information:

CFDA Name:	Cardiovascular Diseases Research
CFDA Number:	93.837
EIN:	1362174823A1
Document Number:	RHL128330A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2019

IC	CAN	2019
HL	8475146	$412,231

NIH Administrative Data:
PCC: eRA Commons User Name / **OC**: 41025 / **Released**: eRA Commons 03/12/2020
Award Processed: 03/13/2020 12:02:41 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01HL128330-04 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01HL128330-04 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in

the final progress report when applicable.

f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01HL128330. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience.*

NIH strongly encourages electronic submission of the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final invention statement may be e-mailed as PDF attachments to: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – HL Special Terms and Conditions – 5R01HL128330-04 REVISED

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

REVISION#1- CARRYOVER AUTHORIZATION
This award includes authorization to utilize $328,064 Total Costs of the unobligated balance from the Yr 03 budget period. This adjustment is based on the carryover prior approval request submitted by Ms. Jennifer Garcia. No additional funds will be provided in any future year for this purpose.

NHLBI FUNDING GUIDELINES
This award is being issued in accordance with the NHLBI FY 2019 Operating Guidelines which can be found at: https://www.nhlbi.nih.gov/research/funding/general/current-operating-guidelines

CARRYOVER & SNAP REMOVED
Due to the large unobligated balance reported on the RPPR, carryover & SNAP designations have been removed. **Use of any unobligated funds requires prior approval from the NHLBI. Requests for carryover should only be submitted if there is an immediate need of funds in addition to the funds provided in this award and can be utilized in the fiscal year requested**.

CONSORTIUM/CONTRACTUAL COSTS
This award includes funds awarded for consortium activity with Northwestern University. The recipient, as the direct and primary recipient of NIH grant funds, is accountable to NIH for the performance project, the appropriate expenditures of grant funds by all parties, and all other obligations of the recipient, as specified in the NIH Grants Policy Statement. In general, the requirements that apply to the recipient, including the intellectual property requirements also apply to consortium participant (s).

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Shelia Ortiz
Email: ortizs@nhlbi.nih.gov **Phone**: (301) 827-8046 **Fax**: (301) 451-5462

Program Official: Yang Shi
Email: scarlet.shi@nih.gov **Phone**: 301-435-0455

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01HL128330-04 REVISED

INSTITUTION: RUSH UNIVERSITY MEDICAL CENTER

Budget	Year 4
Salaries and Wages	$306,091
Fringe Benefits	$33,654
Personnel Costs (Subtotal)	$339,745
Materials & Supplies	$60,367
Travel	$3,000
Other	$17,179
Subawards/Consortium/Contractual Costs	$88,042
Publication Costs	$2,000
TOTAL FEDERAL DC	$510,333
TOTAL FEDERAL F&A	$229,962
TOTAL COST	$412,231

Facilities and Administrative Costs	Year 4
F&A Cost Rate 1	55%
F&A Cost Base 1	$208,958
F&A Costs 1	$114,927
F&A Cost Rate 2	55%
F&A Cost Base 2	$209,154
F&A Costs 2	$115,035

A. COVER PAGE

Project Title: The Role of p21-activated Kinase (Pak1) in Atrial Fibrillation	
Grant Number: 5R01HL128330-04	**Project/Grant Period:** 07/01/2016 - 04/30/2020
Reporting Period: 05/01/2018 - 04/30/2019	**Requested Budget Period:** 05/01/2019 - 04/30/2020
Report Term Frequency: Annual	**Date Submitted:** 02/28/2019
Program Director/Principal Investigator Information: KATHRIN BANACH , PHD **Phone number:** 312-563-3553 **Email:** kathrin_banach@rush.edu	**Recipient Organization:** RUSH UNIVERSITY MEDICAL CENTER RUSH UNIVERSITY MEDICAL CENTER 1653 W CONGRESS PKWY CHICAGO, IL 606123833 **DUNS:** 068610245 **EIN:** 1362174823A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: JANE F WINGER 1653 W Congress Parkway CHICAGO, IL 60612 **Phone number:** 312 942-5000 **Email:** Jane_Winger@rush.edu	**Signing Official:** JENNIFER GARCIA 1653 W Congress Parkway Chicago, IL 60612 **Phone number:** 312-942-3554 **Email:** Jennifer_Garcia@rush.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The major goals of this applications are stated in the specific aims:
Aim #1: We will determine the role of Pak1 in AF by testing the hypothesis that i. Pak1 expression is decreased in the etiology of AF, ii. decreased Pak1 activity increases the AF inducibility.

Aim #2: We will determine the mechanism by which Pak1 regulates atrial ROS production and tissue remodeling by testing the hypothesis that i. Pak1 is a negative regulator of NOX2/ROS production; and ii. Pak1 regulates atrial ROS production through competitive binding of Rac1 while Pak1 kinase activity counteracts tissue remodeling.

Aim #3: We will determine the mechanism by which Pak1 influences atrial function during AF by testing the hypothesis that i. Pak1 regulates atrial ECC through NOX2/ROS and ii. stimulation of Pak1 in a RAP-AF model attenuates inducibility and duration of AF episodes.

The aims will not be modified.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Progress_Science_20190226.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4_TrainingPlan_2019.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The project will proceed as proposed in the original application. No changes to the Specific Aims or to the experimental plan are anticipated or planned.
Work planned in Aim 1 is still focused on the mechanism by which Pak1 is regulated in the atria and its role in atrial fibrillation. We already have considerable evidence that Pak1 is down-regulated under conditions of AF, but the focus on the RAP model in year 4 of the project will allow us to correlate changes in Pak1 expression with the severity of the pathophysiological phenotype. In addition our exciting new evidence that Pak1 is regulated in a gender specific manner will allow us to gain further insights into the mechanism that promote gender differences in the propensity for atrial fibrillation. Data of the large animal model will be supplemented with in vitro studies that help us detail the specific signaling molecules and transcription factors involved in the regulation of Pak1 expression.
In the work planned in Aim 2 we will further our effort to determine the mechanism by which Pak1 regulates atrial ROS production. While we have substantial experimental support that shows the Pak1 dependent regulation of NOX2 our new information also supports a Pak1 dependent regulation of IP3R. Further experiments will focus to separate the effects that Pak1 kinase activity, the role Pak1 plays as a component in signaling micro-domains, as well as its role in transcription factor activation.
A major focus of next years work will be the experiments proposed in Aim3. We have precluded experiments on the cellular level that determine the effects of Pak1 activator FTY720 on cellular ECC. Experiments in the large animal model focused on the acute effect of FTY720 as well as the dosing required for treatment in the canine model. The RAP model will provide tissue for the analysis of Pak1 expression as well its role in tissue remodeling during AF. It will provide in vivo data that detail the electrophysiological phenotype of the animals, the severity of tissue remodeling, and the burden of AF and therefore allow us to correlate this with Pak1 expression and signaling. Further the model allows us to understand how Pak1 stimulation interferes with the disease phenotype. The model will also provide isolated single cells that allow the characterization of cellular ECC and ROS production as well as the cells propensity for PACs. All these parameter will then be used as a read out to test the hypothesis that stimulation of Pak1 decreases the inducibility and duration of AF episodes in the etiology of AF.

B.2 What was accomplished under these goals?

Progress related to Aim 1: Determine the role of Pak1 in atrial fibrillation (AF) by testing the hypothesis that i. Pak1 expression is decreased in the etiology of AF, and ii. decreased Pak1 activity increases the AF inducibility. We previously demonstrated that Pak1 protein levels are down-regulated in a canine model of chronic AF. The tissue samples from canine left atrial appendage (LAA), were from control animals in sinus rhythm (SR) and a treatment group where AF was induced through rapid atrial pacing (RAP) that was maintained for a period of >6 month. One of our goals is to determine how changes in Pak1 expression are related to the severity and progression of AF. The animal model used in the current study is a RAP model however, ventricular rhythm is not maintained and animals are paced only for 4 weeks. So far, 4 animals have been generated of which 3 were RAP and 1 was a control animal. As previously reported, mRNA levels from LAA and posterior left atrium (PLA) were compared between Ctrl and RAP animals. The observed down-regulation of $Ca_v1.2$ and connexin 43 (Cx43) was consistent with the development of an AF phenotype. However, while there was a trend in PLA, no significant down-regulation of Pak1 was determined in this model. The focus in year 4 on the large animal model will increase tissue availability, provide critical control tissue, and allow us to correlate Pak1 expression to the severity electrophysiological phenotype.

The animals available for the large animal model of atrial fibrillation (AF) are predominantly female dogs whereas our cellular studies were conducted on male mice. To determine if there is a gender dimorphism in the expression and regulation of Pak1 in atrial tissue we compared Pak1 mRNA and protein levels in the atria of male and female WT mice (FVBN). Interestingly Pak1 mRNA as well as protein levels were significantly increased in female atria (Fig.1A-C). According to our hypothesis this would suggest an attenuated propensity for atrial arrhythmia in female mice. While this hypothesis remains to be tested further, it is in accordance with data published that indicate an increased protection from atrial arrhythmia in pre-menopausal women.

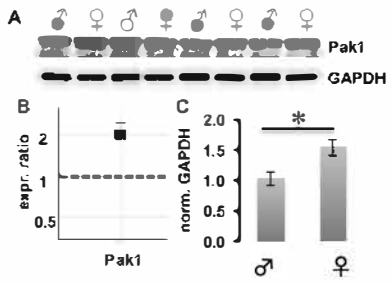

Fig. 1: Pak1 is up-regulated in female atria of mice. A. Western blot results of Pak1 protein levels in male an female mouse atria, normalized to the expression of GAPDH (C). B. Expression ratio of Pak1 mRNA in female atria determined by

To determine the impact that a difference in Pak1 expression has on the atrial phenotype of male and female mice we determined the mRNA levels of Ca handling proteins relevant for excitation-contraction coupling (ECC): sodium calcium exchanger (NCX), sarcoplasmic reticulum Calcium ATPase (SERCA), and the ryanodine receptor (RYR); and the expression levels of proteins relevant for the Pak1 signaling cascade: Rac1, Pak1, protein phosphatase 2A (PP2A), and NADPH oxidase 2 (NOX2). qPCR analysis revealed significantly increased levels of NCX mRNA in female atria and a trend for reduced mRNA levels of SERCA and $Ca_v1.2$. A shift from Ca reuptake to Ca extrusion via NCX is consistent with data on gender differences in the ventricular muscle, as well as our new data (see below) showing prolonged Ca transient decay constants in atrial myocytes from female mice. Of the proteins that were identified to be part of the Pak1 signaling axis, we determined that in addition to Pak1, Rac1 mRNA levels were increased. Levels of NOX2 ($gp91^{phox}$) and PP2A however, did not exhibit gender dependent differences.

To determine how these proteins are affected by the loss of Pak1 expression we compared mRNA levels in the atria of male and female $Pak1^{-/-}$ mice. Interestingly, loss of Pak1 eliminated gender differences in NCX and Rac1 mRNA levels determined between the atria of WT male and female mice. Overall the data support that Pak1 mRNA is expressed in a gender dependent manner and that loss of Pak1 itself contributes to an attenuation of gender differences. The mechanism of this regulation will be determined under Aim #2 in future experiments.

To determine if the difference in Pak1 expression in male and female atria has an impact on cardiac electrophysiological properties in vivo, we compared EKGs from male and female WT and $Pak1^{-/-}$ mice. During isoflurane anesthesia (2.5%) no differences were determined in spontaneous heart rate, P wave duration, and PR-interval between male and female mice in WT and $Pak1^{-/-}$ animals; also no significant differences were determined between WT and $Pak1^{-/-}$ animals of either sex. Block of the neurohumoral regulation of the heart rate by intraperitoneal injection of the β-blocker (propranolol) and muscarinergic receptor 2 blocker (Atropin), overall significantly reduced the heart rate in all animals; however, did not support sex specific differences or differences in neurohumoral regulation between WT and $Pak1^{-/-}$ animals. The same was true for the animal's response to β-adrenergic stimulation with isoprenaline. The data indicate that under physiological conditions, Pak1 expression has no significant impact on basal electrophysiological properties of the heart or its response to neurohumoral stimulation. Further analysis will be performed to determine and compare the occurrence of spontaneous premature atrial contractions (PACs) between male and female as well as WT and $Pak1^{-/-}$ animals.

Ca transients in atrial myocytes from male and female WT and Pak1$^{-/-}$ mice.

To determine if differences in Pak1 protein expression in male and female atria have consequences for cardiac ECC and the occurrence of spontaneous Ca release events we compared Ca transients in WT and Pak1$^{-/-}$ atrial myocytes isolated from male and female atria. Atrial myocytes (AMs) from female WT (WT$_{♀}$) mice exhibited a prolonged Ca transient decay constant (τ) and an attenuated SR load as determined by the caffeine transient amplitude. These data are consistent with a shift from Ca reuptake into the sarcoplasmic reticulutm (SR) by SERCA, to Ca extrusion via NCX. Such a shift is supported by the mRNA levels analyzed in male and female atria but western blotting results need to determine if the differences in mRNA translate into differences in protein level.

Ca transient amplitude and kinetic recorded in AMs from male and female Pak1$^{-/-}$ mice exhibited no gender differences. However, as previously reported (DeSantiago, 2018) compared to WT$_{♂}$ AMs the Ca decay constant was prolonged in Pak1$^{-/-}{}_{♂}$ and Pak1$^{-/-}{}_{♀}$ AMs. The result is consistent with the loss of gender disparity in mRNA levels between Pak1$^{-/-}{}_{♂}$ and Pak1$^{-/-}{}_{♀}$ atria, increased mRNA levels of NCX and attenuated mRNA levels of SERCA in Pak1$^{-/-}{}_{♂}$ vs. WT$_{♂}$ atria. No differences in mRNA expression for Ca handling proteins were determined between WT$_{♀}$ and Pak1$^{-/-}{}_{♀}$ atria. Overall the data reveal that Pak1 contributes to gender differences in atrial ECC and loss of Pak1 promotes a shift to a more female phenotype in male atria. Interestingly the number of spontaneous Ca release events was increased in Pak1$^{-/-}{}_{♂}$ as well as Pak1$^{-/-}{}_{♀}$ AMs compared to WT AMs of either sex. Based on our previous data (Desantiago, 2018), this increase in spontaneous Ca release events is a consequence of the increased NOX2 dependent ROS production in Pak1$^{-/-}$ AMs. Future experiments will aim to determine if this increase in NOX2/ROS is regulated in a gender dependent manner. An manuscript Unpublished is in preparation.

Progress related to Aim2:

In Aim 2 our overall aim is to determine if i. Pak1 is a negative regulator of NOX2/ROS production; and ii. Pak1 regulates atrial ROS production through competitive binding of Rac1 while Pak1 kinase activity counteracts tissue remodeling. Our previous experiments revealed that Pak1 is a negative regulator of NOX2 dependent ROS production under control conditions and during stimulation with AngII. We described (DeSantiago, 2018) that AngII stimulation resulted in an increase in ROS production as well as an increase in Ca transient amplitude. Activation of NOX2 is linked to a tyrosine kinase dependent activation process, whereas the rise in Ca has been linked to the activation of Phospholipase C (PLC) and subsequent IP_3 production. In recent experiments we aimed to determine if these two AngII signaling pathways are interrelated and both regulated by Pak1. Superfusion of AMs with AngII (1 µM) significantly increased diastolic $[Ca]_i$ ($\Delta F/F_0$, $Ctrl_{20'}$: 1.0±0.01, $AngII_{20'}$: 1.2±0.03; n=7; p<0.05), the field stimulation induced Ca transient amplitude ($\Delta F/F_0$, Ctrl: 2.0±0.17, AngII: 2.39±0.22, n=7; p<0.05), and spontaneous Ca release events that resulted in PACs (Ctrl: 0 s^{-1}, AngII: 0.15±0.05 s^{-1}; n=7; p<0.05). These effects, were suppressed by $InsP_3R2$ blocker 2-aminoethoxydiphenyl-borate (2APB; 1 µM)) and the PLC inhibitor, U73122 (10 µM), demonstrating their dependence on IP_3 induced Ca release events. At the same time, AngII induced an increase in ROS production that was sensitive to the NOX2 specific inhibitor gp91ds-tat (1 µM). In AMs deficient for NOX2 (gp91$^{phox-/-}$) however, AngII failed to increase $[Ca]_i$ and PACs indicating a sensitization of $InsP_3R$ and spontaneous Ca release by NOX2/ROS.

In saponin (0.005%) permeabilized AMs $InsP_3$ (5 µM) induced spatially defined Ca release events (sparks) that increased in frequency in the presence of exogenous ROS (tert-Butyl hydroperoxide: tBHP: 5 µM; $InsP_3$: 9.65±1.44 sparks*s^{-1}(100 µm)$^{-1}$; InsP3 + tBHP: 10.77±1.5 sparks*s^{-1}(100 µm)$^{-1}$, n=5, p<0.05). The combined effect of $InsP_3$ + tBHP was entirely suppressed by 2-APB suggesting a ROS dependent sensitization of the ryanodine receptor through IP_3 induced Ca release. The data overall support the hypothesis that while the AngII induced increase in $[Ca]_i$ depends on IP_3 production, the ROS dependent sensitization of IP_3R2 is required to induce significant changes in $[Ca]_i$.

Our prior experiments have demonstrated that attenuated Pak1 signaling significantly increases AngII induced changes in ROS production and spontaneous arrhythmic Ca release events in AMs isolated from mice or a dog model of atrial fibrillation (DeSantiago). Analysis of the $InsP_3R2$ mRNA levels between WT and Pak1$^{-/-}$ animals now revealed that $InsP_3R2$ is up-regulated in the atria of Pak1$^{-/-}$ mice. Up-regulation of $InsP_3R$ has been previously described under conditions of AF and increased triggered activity was observed in myocytes over-expressing IP_3R2. Future experiments will reveal if increased IICR in AF is a consequence of attenuated Pak1 activity. The data on the interplay of NOX2/ROS and IICR were presented at the annual meeting of the American Heart Association and a manuscript is in preparation.

Progress related to Aim3:

Determine the mechanism by which Pak1 influences atrial electrophysiological and contractile function by testing the hypothesis that Pak1 regulates Ca handling properties of atrial myocytes:

Our prior data demonstrated that attenuated Pak1 activity prolonged the Ca transient decay constant and increased the number of spontaneous Ca release events in male Pak1$^{-/-}$ atrial myocytes. The data supported the conclusion that loss of Pak1 increases NOX2/ROS production leading to increased NCX activity in combination with increased spontaneous Ca^{2+} transients (DeSantiago, 2018).

In our recent experiments we use the immunmodulating agent FTY720 (Fingolimod), a structural analog of sphingosine and a sphingosine-1-phosphate receptor modulator to stimulate Pak1 activity and to determine if Pak1 stimulation can attenuate the propensity for PACs and reduce AF burden. Atrial and ventricular myocytes from WT mice were superfused with FTY720 and Ca transient amplitude and kinetic analyzed. Under control conditions FTY720 had no acute effect on the Ca transient amplitude, or Ca transient kinetic in isolated atrial or ventricular myocytes. FTY720 also did not effect the load of the sarcoplasmic reticulum, however comparable to the effect of the NOX2 inhibitor gp91ds-tat, it attenuated the decay constant of caffeine induced Ca transients. The latter is indicative for a decreased activity of NCX. The data indicate that under physiological conditions, FTY720 mediated Pak1 stimulation has only limited impact on cardiac ECC.

Nevertheless, as previously reported, in isolated atrial myocytes from the canine model of RAP induced AF, we demonstrated that FTY720 suppressed an exaggerated response of the myocytes to AngII induced increase in $[Ca]_i$ and reduced the number of PACs in these cells under control conditions and in the presence of AngII. With this information we went on to determine in vivo in the canine RAP model of AF if FTY720 induced Pak1 stimulation can attenuate the burden of AF. Initial experiments were aimed to determine the acute effect of FTY720 in this model.

To determine the effect of FTY720 in vivo on atrial electrophysiological properties, dogs were implanted with pacemakers. A control animal was implanted but remained 'un-paced' whereas 3 dogs were paced at 600 bpm for an average of 3 weeks. The effective refractory period was determined by placing two rectangular, 21-electrode plaques epicardially on the posterior left atrium (PLA) and left atrial appendage (LAA). Using a single extra-stimulus at a cycle length of 400 ms the ERP was determined at each plaque on multiple electrodes. ERP and AF inducibility were determined under control conditions and after 40 min superfusion with FTY720 (intra venous injection at 0.5 mg/kg). In the control dog the basal ERP was 100 ± 8.1 ms, whereas in the RAP dogs ERPs were reduced to 56.7 and 26.7 ms, respectively. In the control and a RAP animal, FTY720 treatment at 0.5 mg/kg did not significantly alter the ERP after 0.5 and 1 h of injection. In the RAP dog treated with 1 mg/kg FTY720 AF was inducible from the beginning but transitioned into atrial flutter (AFl) after 38 min of FTY720 injection. AFl showed increasing cycle length and AF at this point became difficult to induce; however, reoccurring AFl prevented the analysis of ERP in the presence of FTY720. A complication was that at the higher concentration FTY720 induced chronic heart block after 40 min of treatment. Chronic heart block as well as bradycardia have been reported as a side effect of acute FTY720 administration. The effect is described to subside after 72 h of treatment. As a consequence we will adjust our experiments and in the futur treat dogs chronically (at 0.5 mg/kg/day for a week). The experiments will allow us to determine if Pak1 stimulation can attenuate AF inducibility, duration of AF episodes, and atrial remodeling in the dog model of RAP induced AF.

Significance

The significance of our experimental results is as follows:

1. We have determined that in atrial tissue Pak1 is expressed in a gender dependent manner and that loss of Pak1 expression attenuates gender differences in the expression of Ca handling proteins and proteins of the Pak1 signaling pathway. The higher expression of Pak1 could be a potential mechanism for attenuated propensity to atrial arrhythmia in pre-menopausal women. Further it could indicate an important regulation of Pak1 expression through sex hormones.

2. We have determined for the first time that the AngII induced increase in NOX2 dependent ROS production and the IP_3 induced Ca release (IICR) are interdependent and that an AngII induced increase in PACs depends on a ROS dependent sensitization of the IP_3R2. The data support that IP_3R2 is critically regulated by ROS and IICR can increase with increasing ROS alone during basal IP_3 production. Besides its role in the generation of PACs the data suggest a subtle ROS dependent regulation of IICR under physiological conditions such as stretch and a potential association of NOX2 and IP3R in signaling domains that allows this close regulation.

3. We demonstrate that stimulation of Pak1 with FTY720 does not have significant consequences for cellular ECC or for the atrial ERP in vivo. However, on the cellular level it can suppress PACs in a dog model of RAP induced AF as well as in mouse myocytes challenged with AngII. While initial preliminary experiments indicate that FTY720 can acutely attenuate AF inducibility, its propensity to induce chronic heart block during initial treatment requires us to use chronic treatment with FTY720 to determine the long-term benefit of Pak1 stimulation as a potential mechanism of treatment for AF.

Publications:

Manuscript: Desantiago, J., Bare, D. J., Varma, D., Solaro, R. J., Arora, R., & Banach, K. (2018). Loss of p21-activated kinase 1 (Pak1) promotes atrial arrhythmic activity. Heart Rhythm, 15(8), 1233–1241; *http://doi.org/10.1016/j.hrthm.2018.03.041*

Abstracts:

1. DeSantiago J, Varma D, Blatter LA & Banach K (2018): Relevance of $InsP_3$ receptor ROS regulation in atrial myocytes. *Circulation. 2018; 138: A16646*

2. DeSantiago J, Varma D, Blatter LA & Banach K (2018): Relevance of $InsP_3$ receptor ROS regulation in atrial myocytes. Rush University Medical Center, Research Symposium.

3. Ostro, R., Varma D, Bare DJ, DeSantiago J, & Banach K (2019). Pak1 contributes to gender differences in the propensity for atrial arrhythmia. Rush University Medical Center, Research Symposium

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

No specific training plan was provided by the project.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
In Process at NIHMS	The loss of p21-activated kinase (Pak1) promotes atrial arrhythmic activity. Heart rhythm.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	BANACH, KATHRIN	PHD	PD/PI	EFFORT					NA
eRA Commons User Name	N	Varma, Disha	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
eRA Commons User Name	Y	ARORA, RISHI	MD	Co-Investigator						NA
eRA Commons User Name	N	DeSantiago, Jaime	MD,MS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

E.IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

The biggest problem in the first two years was to find qualified post-doctoral candidates. In year 2 we could finally hire hire [Redacted by] and year 3 [Redacted by agreement] started working the budgeted [EFFO T] on the grant. We further hope to increase the efficiency by hiring of a technician [EFFORT] The technician [Redacted by] is currently a masters student in the laboratory and will start in the laboratory after finishing his thesis in April 2019. The progress in year 3 was swiftly and allowed us to finish the cellular experiments that form the basis for the large animal studies.
The large animal studies were now started in year 3 and will be the major focus of our work in year 4.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Rush University Medical Center	068610245	IL-007	Rush University Medical Center 1653 W Congress Parkway Chicago IL 606120000
Northwestern University	005436803	IL-007	750 North Lake Shore Drive Chicago IL 606114579

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

Yes

Estimated unobligated balance: 320000

G.10.b Provide an explanation for unobligated balance:
I do anticipate the appearance of a carryover greater than 25% of the current years approved spending. While spending was above 75% in year 2 and 3 of the project, the initial carryover from the first year was not entirely spent. The initial and continued carryover resulted from the significant difficulties in finding qualified post-doctoral fellows to work on the research project. However, this issue has been corrected with the addition of another technician working on the grant project. Below we provide a detailed budget plan for the use of the carryover in year 4 of the grant.

G.10.c If authorized to carryover the balance, provide a general description of how it is anticipated that the funds will be spent
The carryover balance will be utilized for salaries and increasing costs of supplies and animals in year 4. A. Salaries: $74,183: The carryover will be used for i. base-salary increase for [Redacted by] ($ 23,730) and [Redacted by] ($ 10,937) to comply with NIH standards and increase effort to [EFFORT] respectively; ii. recruitment of the Technician [Redacted by] starting May 1st 2019 [Institutional]. ii. Non-salary costs: Supplies ($40,649): i. increase in cost of supplies and consumables due to the increase in the number of experimenters. ii. cost-intensive large animal (dog) experiments previously budgeted continuously throughout all 4 years will be performed in year 4 (12 additional dog experiments); Animals ($ 8,000): renting of procedure rooms in animal facility for in vivo recordings. Travel ($6,000): Gordon Conference participation; Other: $29,000 repair and replacement of dysfunctional imaging set-up. B. Equipment: $ 36,000 for culture dish pacing system and telemetry system for exp. in Aim1; C. Subcontract: $ 40,000: the use of large animals that were scheduled to be used in the early grant years will be used in year 4 (12 dogs to be instrumented). The carryover is required to allow for purchase of animals that were originally budgeted.

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

RESEARCH & RELATED BUDGET - SECTION A & B

ORGANIZATIONAL DUNS*: 068610245

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: RUSH UNIVERSITY MEDICAL CENTER

Start Date*: 05-01-2019 **End Date*:** 04-30-2020

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Kathrin		Banach	PhD	Project Lead	Institutional Base Salary	EFFORT			67,000.00	18,405.00	85,405.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **85,405.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	EFFORT			56,800.00	15,603.00	72,403.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	**Total Number Other Personnel**					**Total Other Personnel**	**72,403.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**157,808.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 068610245
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: RUSH UNIVERSITY MEDICAL CENTER

Start Date*: 05-01-2019 **End Date*:** 04-30-2020

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		**0.00**
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 068610245
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: RUSH UNIVERSITY MEDICAL CENTER

Start Date*: 05-01-2019 **End Date*:** 04-30-2020

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	36,346.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	88,042.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animals	13,000.00
Total Other Direct Costs	**139,388.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**297,196.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Federal Direct Cost	55.0	209,154.00	115,035.00
		Total Indirect Costs	**115,035.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**412,231.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: BudgetJust_Banach_02262019.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Budget Justification:

A. Senior/Key Persons

Kathrin Banach, Ph.D., Principal Investigator (EFF month) is responsible for the administration and direction of the proposed project. She will also perform electrophysiological recordings on isolated atrial myocytes using the patch clamp technique as well as recordings from multicellular cardiac preparations using a multi-electrode array. (~$85,405)

B. Other Personnel

Redacted by agreement, (Post-Doctoral Student, EFF RT month effort): Redacted by agreement has extensive expertise in the analysis of intracellular Calcium signaling in cardiomyocyte using optical and electrophysiological techniques. He will be responsible to characterize the time dependent changes in ROS generation as well as the Ca handling aspects of excitation contraction coupling in atrial myocytes. He recorded the preliminary data shown in the proposal. In the first year of the application he will be trained to perform the in vivo electrophysiological recordings in the mice (~$25,494). *Should the carryover be approved,* Redacted by agreement *s effort will be increased to 6 month.*

Redacted by agreement (Post-Doctoral Student, EFF RT month effort): A research associate with a background in biochemistry and molecular biology is critically important of the success of Aim#1 and #2. He/she will be responsible for the characterization of protein expression in cardiac tissue, virus amplification and cell transfections. He/she will further assist with the breeding of the Pak1$^{-/-}$ mice and their genotyping (~$46,909). *Should the carryover be approved* Redacted by agreement *s effort will be increased to 12 month.*

With approval of the carryover we would like to hire Redacted by agreement *(Technician,* EFF RT *month effort): A research associate with a background in biochemistry and molecular biology is critically important of the success of Aim#1 and #2. He/she will be responsible for the characterization of protein expression in cardiac tissue, virus amplification and cell transfections. He/she will further assist with the breeding of the Pak1$^{-/-}$ mice and their genotyping. (~$38,241)*

Total: $ 157,808

C. Equipment: *not applicable*

D. Travel: *not applicable*

F. Other Direct Costs

1. Materials and Supplies

Chemicals and laboratory supplies, glassware and plastic ware: General chemicals include salts, buffers, intracellular organic molecules (ATP, enzymes and cofactors), drugs (agonists and inhibitors of enzymes, pumps, channels and receptors) to prepare experimental solutions; electrophysiology supplies include pipette glass, syringes, couplers and replacement parts for pullers; specific chemical compounds include various fluorescent probes (e.g. calcium indicators, in salt or membrane-permeant forms); general laboratory supplies include replacement pH electrodes, pipettors, glassware and plastic ware (cuvettes, pipettes, plastic dishes and tubes, glass and plastic flasks and cylinders, centrifugation tubes, cover slips, plastic tubing). For Antibodies, gels and activity assays for biochemical and molecular biological experiments. Enzyme solution for the cell isolation of mouse and dog atria ($ 36,346 per year).

2. Animals: Mice of 4 different phenotypes (WT, Pak1$^{-/-}$, gp91$^{phox-/-}$) will be used in the study as well as a dog model of rapid atrial pacing induced atrial fibrillation. The Pak1$^{-/-}$ mouse line is established and breeding is ongoing in the Rush animal facility. The WT and gp91$^{phox-/-}$ animals will be purchased from Jackson Labs. The mice will be housed and bred in the local animal facility. Funds are requested for purchase, shipping and housing as well as phenotyping of the animals. In addition extra charges are will be applied for the rent of procedure rooms in the animal facility and rent of anesthesia equipment. ($ 13,000).

3. Publication Costs

Funds are requested for submission charges and page charges. ($ 2,000).

RPPR

RESEARCH & RELATED BUDGET - SECTION A & B

FINAL

ORGANIZATIONAL DUNS*: 005436803

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: NORTHWESTERN UNIVERSITY AT CHICAGO

Start Date*: 05-01-2019 **End Date*:** 04-30-2020

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Prof	Redacted by agreement			MD	co-investigator	Institutional Base Salary	EFFORT			18,960.00	4,968.00	23,928.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **23,928.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			9,002.00	2,359.00	11,361.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	EFFORT			10,336.00	2,708.00	13,044.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**24,405.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,333.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 005436803
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: NORTHWESTERN UNIVERSITY AT CHICAGO

Start Date*: 05-01-2019 **End Date*:** 04-30-2020

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 005436803
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: NORTHWESTERN UNIVERSITY AT CHICAGO

Start Date*: 05-01-2019 **End Date*:** 04-30-2020

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animals	7,390.00
Total Other Direct Costs	**7,390.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**55,723.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Federal Indirect Cost	58.0	55,723.00	32,319.00
		Total Indirect Costs	**32,319.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**88,042.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: BudgetJust_NW_02262019.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Sub-contract for Northwestern University

For the successful completion of all 3 specific aims we will use tissue isolated from a dog model of atrial fibrillation. Our co-investigator Redacted by agreement has extensive experience in the use of this animal model and will perform these experiments at Northwestern. To cover the animal costs and the surgical intervention we request a consortium agreement for their efforts.

Redacted by agreement (Co-Investigator, EFFORT effort). Redacted by agreement is an expert in atrial fibrillation who has worked with small and large animal models of AF; his expertise is in-valuable throughout the duration of the grant. Redacted by agreement's expertise is needed specifically for the successful completion of Aims#1 and #3. He will train Redacted by agreement in the in vivo mouse studies proposed in Aim #1 where the inducibility of AF will be determined in WT and Pak1$^{-/-}$ mice. In years 2 and 3 in Redacted by agreement's laboratory a dog AF model will be established where AF is induced by rapid atrial pacing. Redacted by agreement will perform experiments that determine the inducibility of AF in these dogs in the presence and absence of pharmacologically increased Pak1 activity (Aim #3). (~$23,073 per year)

We further request support for a Post-Doctoral Student and a Research Technician that will support Redacted by agreement during the experiments as well as with the recordings, data analysis and cell isolation from the large animal model.

Redacted by agreement (Post-Doctoral Student, EFFORT effort) The post-doctoral student will assist Redacted by agreement to establish the dog model and perform the electrophysiological recordings. ($11,361 per year)

Redacted by agreement (Research Technician, EFFORT effort) In the 2nd and 3rd year of the application a research technician in Redacted by agreement's laboratory will isolate cells from the dog atria. ($ 10,336 per year)

Animals:

The dog model of atrial fibrillation is a well established disease model that has significant value for the understanding of atrial fibrillation in humans. The current budget allows us to use 3 animals which will be used in a model of rapid atrial pacing induced atrial fibrillation. Funds are requested for Redacted by agreement's part of the project and will include purchase, housing as well as surgical procedures for the dogs. ($ 7,390 fore year 4) *Should the carry over request be approved we can increase the number to 12 dogs which were originally scheduled to be used in the first two years of the grant application.*