RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE ON DRUG ABUSE

Issue Date: 04/15/2014

Grant Number: 5R01DA025011-05
FAIN: R01DA025011

Principal Investigator(s):
ANIL KUMAR, PHD

Project Title: Methamphetamine and AIDS in a Non-Human Primate Model

Manager, Pre Award
University of Missouri-Kansas City
5100 Rockhill Road
Kansas City, MO 64110

Award e-mailed to: ORS@umkc.edu

Budget Period: 04/01/2014 – 03/31/2015
Project Period: 09/30/2008 – 03/31/2015

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $351,733 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MISSOURI KANSAS CITY 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 Institute On Drug Abuse of the National Institutes of Health under Award Number R01DA025011. 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 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. 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,

Carol Alderson
Grants Management Officer
NATIONAL INSTITUTE ON DRUG ABUSE

Additional information follows

SECTION I – AWARD DATA – 5R01DA025011-05

Award Calculation (U.S. Dollars)

Federal Direct Costs	$256,731
Federal F&A Costs	$95,002
Approved Budget	$351,733
Federal Share	$351,733
TOTAL FEDERAL AWARD AMOUNT	$351,733
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$351,733

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$351,733	$351,733

Fiscal Information:

CFDA Number:	93.279
EIN:	1436003859A7
Document Number:	RDA025011A
PMS Account Type:	G (Pooled)
Fiscal Year:	2014

IC	CAN	2014
DA	8472629	$351,733

NIH Administrative Data:
PCC: PN/NPE / **OC**: 414E / **Released:** eRA Commons User Name 04/14/2014
Award Processed: 12/26/2013 10:57:56 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01DA025011-05

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 – 5R01DA025011-05

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 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. 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.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is excluded from Streamlined Noncompeting Award Procedures (SNAP).
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 Central Contractor Registration. 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) R01DA025011. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

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/#gps .

A final Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/#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) cash transaction data.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 90 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

Unless an application for competitive renewal is submitted, a final progress report must also be submitted within 90 days of the expiration date. Instructions for preparing a Final Progress Report are at: http://grants.nih.gov/grants/funding/finalprogressreport.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH **strongly encourages** electronic submission of the final progress report and 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 progress report and final invention statement may be e-mailed as PDF attachments to the NIH Central Closeout Center at: DeasCentralized@od.nih.gov.

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

NIH Division of Central Grants Processing, OER
6705 Rockledge Drive
Suite 5016, Room 5109
MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery 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 Progress Report is not required. However, a final 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.

Treatment of Program Income:
Additional Costs

SECTION IV – DA Special Terms and Conditions – 5R01DA025011-05

REMINDER: None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the current salary cap. Current salary cap levels can be found at the following URL: http://grants2.nih.gov/grants/policy/salcap_summary.htm.

All grantees must acknowledge funding received from the National Institute on Drug Abuse at the National Institutes of Health when issuing statements, press releases, requests for proposals, bid solicitations, and other documents describing projects or programs funded in whole or in part with NIDA money. (NIH Grants Policy Statement, October, 2013, http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch8.htm#_Toc271264948).

In conjunction with this requirement, in order to most effectively disseminate research results, advance notice should be given to NIDA that research finds are about to be published so that we may coordinate accurate and timely release to the media. This information will be embargoed until the publication date. Any press notification should be coordinated with the NIDA Press Officer who can be reached at (301) 443-6245.

NIDA has an interest in supporting HIV/AIDS and infectious disease research. The purpose of this support is to develop effective prevention, treatment, and service strategies for drug abusing youth and adults. To that end, awardees are encouraged to make every effort to incorporate scientific questions related to HIV/AIDS and other infectious diseases into research protocols. Principal Investigators will be required to provide information related to the development of research in this area in annual progress reports to allow NIDA to assess progress regarding HIV/AIDS research.

The National Institute on Drug Abuse (NIDA) encourages data harmonization to increase comparability, collaboration, and scientific yield of research on drug abuse. Towards that end, NIDA strongly encourages human-subject studies to incorporate a series of measures from the Substance Abuse and Addiction Core and Specialty collections, which are available in the PhenX Toolkit (www.phenxtoolkit.org). For more information about NIDA's data harmonization efforts, please see NOT-DA-12-008 at http://grants.nih.gov/grants/guide/notice-files/NOT-DA-12-008.html.

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: Carol Alderson
Email: aldersoc@nida.nih.gov **Phone**: 301-933-6196 **Fax**: (301) 594-6849

Program Official: Nancy S Pilotte
Email: np22f@nih.gov **Phone**: (30l) 435-1317 **Fax**: 301-594-6043

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01DA025011-05

INSTITUTION: UNIVERSITY OF MISSOURI KANSAS CITY

Facilities and Administrative Costs	Year 5
F&A Cost Rate 1	49%
F&A Cost Base 1	$193,882
F&A Costs 1	$95,002

Progress Report Scanning Cover Sheet

5R01DA025011-05

PI Name:	**KUMAR, ANIL**
Org:	**UNIVERSITY OF MISSOURI KANSAS CITY**
Start Date:	**04/01/2014**
Snap:	**N) (NEEDS TO BE BOOKMARKED)**
Appl ID:	**8652443**
Rec'd Date:	**03/14/2014**

Form Approved Through 08/31/2015 OMB No. 0925-0002

Department of Health and Human Services
Public Health Services

Grant Progress Report

Review Group	Type	Activity	Grant Number
5	5	R01	DA025011-05

Total Project Period
From: 09/30/2008 Through: 03/30/2015

Requested Budget Period
From: 04/1/2014 Through: 03/31/2015

1. TITLE OF PROJECT
Methamphetamine and AIDS in a Non-Human Primate Model

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR (Name and address, street, city, state, zip code)
Anil Kumar
Pharmacology and Toxicology
UMKC-School of Pharmacy
2464 Charlotte, HSB 3255
Kansas City, mO 64108

2b. E-MAIL ADDRESS
kumaran@umkc.edu

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Pharmacology and Toxicology

2d. MAJOR SUBDIVISION
School of Pharmacy

2e. Tel: 816-235-2415 Fax: 816-235-1776

3a. APPLICANT ORGANIZATION (Name and address, street, city, state, zip code)
University of Missouri Kansas City
5100 Rockhill Road
Kansas City, MO 64110

3b. Tel: 816-235-5389 Fax: 816-235-6532

3c. DUNS: 010989619

4. ENTITY IDENTIFICATION NUMBER
1436003859A7

6. HUMAN SUBJECTS ☒ No ☐ Yes

6a. Research Exempt	If Exempt ("Yes" in 6a): Exemption No.	If Not Exempt ("No" in 6a): IRB approval date
☐ No ☐ Yes		

6b. Federal Wide Assurance No.

6c. NIH-Defined Phase III Clinical Trial ☒ No ☐ Yes

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Lawrence A. Dreyfus, Ph.D .
Vice Chancellor for Res., University of Missouri-KC,
5100 Rockhill Road, Kansas City, Missouri 64110

Tel: 816-235-5839 Fax: 816-235-6532

E-MAIL: ors@umkc.edu

7. VERTEBRATE ANIMALS ☐ No ☒ Yes

7a. If "Yes," IACUC approval Date 07/13/2012

7b. Animal Welfare Assurance No. UMKC-A3397

10. PROJECT/PERFORMANCE SITE(S)

Organizational Name: University of Missouri-Kansas City

DUNS: 010989619

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT $247,539 8b. TOTAL $352,746

Street 1: 5100 Rockhill Road

Street 2:

9. INVENTIONS AND PATENTS ☒ No ☐ Yes

If "Yes," ☐ Previously Reported ☐ Not Previously Reported

City: Kansas City	County: Jackson
State: MO	Province:
Country: USA	Zip/Postal Code: 64110

Congressional Districts: MO-005

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 13)*
Dr. Lawrence Dreyfus

TEL: 816-235-5839	FAX: 816-235-6532	E-MAIL: ors@umkc.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 11. *(In ink)* Linda Dreyfus per Lawrence A. Dreyfus

DATE 3/11/14

Program Director/Principal Investigator (Last, First, Middle): Kumar, Anil

Use only if additional space is needed to list additional project/performance sites.

Additional Project/Performance Site Location

Organizational Name: University of Nebraska Medical Center

DUNS: 168559177

Street 1: 985880 Nebraska Medical Center | Street 2:

City: Omaha | County: | State: NE

Province: | Country: | Zip/Postal Code: 68198-7835

Project/Performance Site Congressional Districts: NE-002

Additional Project/Performance Site Location

Organizational Name:

DUNS:

Street 1: | Street 2:

City: | County: | State:

Province: | Country: | Zip/Postal Code:

Project/Performance Site Congressional Districts:

Additional Project/Performance Site Location

Organizational Name:

DUNS:

Street 1: | Street 2:

City: | County: | State:

Province: | Country: | Zip/Postal Code:

Project/Performance Site Congressional Districts:

Additional Project/Performance Site Location

Organizational Name:

DUNS:

Street 1: | Street 2:

City: | County: | State:

Province: | Country: | Zip/Postal Code:

Project/Performance Site Congressional Districts:

Additional Project/Performance Site Location

Organizational Name:

DUNS:

Street 1: | Street 2:

City: | County: | State:

Province: | Country: | Zip/Postal Code:

Project/Performance Site Congressional Districts:

Program Director/Principal Investigator (Last, First, Middle): Kumar, Anil

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM 04/01/2014	THROUGH 03/31/2015	GRANT NUMBER DA025011

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Anil Kumar	PD/PI	EFFORT			25,617	9,627	35,244
Peter Silverstein	Investigator				30,000	10,800	40,800
Ankit Shah	PDF				20,000	7,200	27,200
Austin Jackson	PDF				17,500	6,300	23,800
Luo Cao	Grad.Student				9,000	360	9,360
SUBTOTALS →					102,117	33,927	136,044

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* Tissue culture plastics and medium- $10,000, RNA Kit-10,000 Bioplex Kit- $10,000, Oxidative stress kits-$7,000 Real time RT-PCR Kit- $15,000 Antibodies-$6,000 Common Reagent-$4,000	62,000
TRAVEL For Drs. Kumar, Silverstein, Shah and Jackson to attend one meeting	8,000
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Tuition for one students (in proportinate to their involvement on project)-2010 CO2-$1000, Liquid Nitrogen-$1000	4,010
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	$ 210,414
CONSORTIUM/CONTRACTUAL COSTS – DIRECT COSTS	25,000
CONSORTIUM/CONTRACTUAL COSTS – FACILITIES AND ADMINISTRATIVE COSTS	12,125
TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Item 8a, Face Page)*	$ 247,539

BUDGET JUSTIFICATION	GRANT NUMBER DA025011

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

Personnel: Anil Kumar will spend EFFORT of his time on this project. He will be responsible for coordination between Drs. Shah, Jackson, Silverstein and Fox. Dr. Kumar will also be responsible for periodic teleconference (at least 4 times every year) between 2 sites. Dr. Kumar will also oversee overall progress and finalize manuscripts for publication, scientifc reports and presentation in different meetings.

Dr. Peter Silverstein will spend EFFORT of his time on this project. He will be responsible for daily supervision of part time graduate student and 2 post-doctoral fellows.

Dr. Ankit Shah will quantify viral loads in plasma, CSF, lymph nodes at different time points. He will also quantify viral loads in all different tissues collected at the time of necropsy. Dr. Shah has already optimized real time PCR for viral load and he is expected to start quantification soon. He will also identify presence of 3 virus in PBMC and CNS compartments over time as well in all tissues collected at the time of necropsy. He will also particpate in binding and neutralizing antibody analyses with Dr. Jackson.

Dr. Austin Jackson was hired two months ago and he will spend EFFORT of the time on this project. He will perform flowcytometry analyses of activation marker on lymphocytes, LN cells and cells from rectal biospy.He will also analyze type 1 and type 2 T cells in ELISPOT assay. He will also analyze binding and neutralizing antibody titer with Dr. Shah.

Ms. Lu Cao is a graduate student who will help Drs. Shah and Jackson.

We are requesting $62,000 for tissue culture supply, growth medium, antibodies, ELISA, ELISPOT, real time RT-PCR kit, primer/probe and other reagents. Dr. Fox will perfom histopathological analyses.

CURRENT BUDGET PERIOD	FROM 04/01/2013	THROUGH 03/31/2014

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.
N/A

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Ankit Shah	Post-doctoral Associate
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Year	FIELD OF STUDY
North Gujarat University, Gujarat, India	B. Pharm	2006	Pharmacy
University of Missouri-Kansas City	MS	2012	Cell and Molecular Biology
University of Missouri-Kansas City	PhD	2013	Pharmacology

A. Dissertation project: HIV-1 gp120 and methamphetamine-mediated induction of proinflammatory cytokines/chemokines and oxidative stress in astrocytes: Implications in neuroinflammation.

B. Personal Statement

Drug addiction is a major concern among the American population, majority of which consists of young adults. Almost all the abusive substances show negative impact on brain functionalities. Therefore, psychobiological analysis of this population is necessary to understand the cause of several chronic relapsing brain disorders. Additionally, studies indicate higher risk of STD acquisition among drug abusers. This further leads to spread of various blood-borne pathogens. I graduated with PhD in pharmacology and possess a training background in the field of HIV neuroinflammation and substance abuse, which enables me to explore the role of various substances of abuse such as cocaine, methamphetamine and alcohol. In our studies, HIV-1 gp120 was found to increase various pro-inflammatory cytokines such as IL-6, IL-8 and CCL5 via different mechanisms. Similarly, methamphetamine also showed increased expressions of IL-6 and IL-8 in NF-κB-dependent manner. In our studies concerning the role of methamphetamine in the pathogenesis of HIV associated neuroinflammation, we have already shown that methamphetamine interacts synergistically with gp120 and enhances expression of pro-inflammatory cytokines such as IL-6 and IL-8 in astrocytes. Furthermore, this leads to production of oxidative stress which worsens the condition among HIV patients who abuse methamphetamine. In future, I intend to continue our research focused in the field of HIV-infection and neurodegenerative disorders.

C. Honors

1. Awarded with Young Investigator Travel Award to partially cover the 20th SNIP meeting held at New Orleans, LA in April 2014
2. Awarded with Chancellor's Doctoral Fellowship for 2013-14.
3. Awarded with SGS travel grant to attend SNIP 2013 meeting in San Juan, PR
4. Awarded with IDSC Travel Grant to attend SNIP 2013 meeting in San Juan, PR
5. Awarded with Young Investigator Travel Award to partially cover the 19th SNIP meeting held at San Juan, PR in April 2013.
6. Awarded for best poster at Health Science Research Summit 2012 organized by UMKC.
7. Awarded with Young Investigator Travel Award to partially cover the 18th SNIP meeting held at Honolulu, HI in April 2012.
8. Awarded with the Dean's scholarship for 2011 in the Pharmacology & Toxicology Division at University of Missouri Kansas City.
9. Awarded with the NIDA AAPI travel fellowship to attend 2011 NIDA mini-convention on "Frontiers in Addiction Research" at Washington, D.C. in November 2011.
10. Awarded with Robert C. Lanman scholarship at UMKC for the academic year 2011-12
11. Awarded for best poster at Health Science Research Summit 2011 organized by UMKC
12. Awarded with a Young Investigator Travel Award to attend 17th SNIP meeting held at Clearwater Beach, Miami, FL in April 2011

13. Awarded as Best Student of the year 2010 in the Pharmacology & Toxicology Division at University of Missouri Kansas City.
14. Awarded with the Doctoral Scholarship award at University of Missouri-Kansas City for pursuing PhD.
15. Awarded with a scholarship at Fairleigh Dickinson University as a foreign International Graduate Student.
16. Awarded twice with Certificates by Shri Sarvajanik Pharmacy College for securing 1st position in Second Year B.Pharm (and 5th in North Gujarat University) and 2nd position in Third Year B.Pharm (and 13th in North Gujarat University).
17. Awarded with the Certificate by GENERAL HOSPITAL, MEHSANA P.P UNIT for whole heartedly participating in INTENSIFIED PULSE POLIO IMMUNISATION 2004-2005 for Eradication of Polio.

D. Professional Memberships

2003-2006 Indian Pharmaceutical Congress
2009-present SNIP
2009-present Science Advisory Board

E. Peer-reviewed Publications

1. Xun, L., **Shah, A.** et al. HIV-1Nef Induces CCL5 production in astrocytes through p38 MAPK and PI3K/Akt pathway and utilizes NF-κB, CEBP and AP-1 transcription factors." In Revision to Scientific Reports
2. Nookala, A., **Shah, A.** et al. HIV-1 Tat-mediated induction of CCL5 in Astrocytes involves NFκB, AP1, C/EBPα and C/EBPγ transcription factors and JAK, PI3K/Akt and p38MAPK signaling pathways." PLoS One, 8(11), e78855.
3. Gangwani, M., **Shah, A.** et al. "Human Immunodeficiency Virus Type 1 Type 1 Viral Protein R (Vpr) Induces CCL5 Expression in Astrocytes via PI-3K and MAPK Signaling Pathways." Journal of Neuroinflammation 2013, 10:136.
4. **Shah, A.** et al. "HIV-1 protein gp120 and methamphetamine co-operate synergistically to increase oxidative stress in astrocytes: Role of cytochrome P450" Cell death and Disease (2013) 4, e850.
5. **Shah, A.**, et al. "Synergistic cooperation between methamphetamine and HIV-1 gp120 through the PI3K/Akt pathway induces IL-6 but notIL-8 expression in astrocytes." PLoS One. 2012;7(12):e52060
6. Liu, X., **Shah, A.**, et al. "Methamphetamine increases LPS-mediated expression of IL-8, TNF-α and IL-1β in human macrophages through common signaling pathways". PLoS One 7 (2012) e33822.
7. Silverstein,P.S., **Shah, A.**, et al, HIV-1 gp120 and Drugs of Abuse: Interactions in the Central Nervous System, Curr HIV Res. 2012 Jul;10(5):369-83
8. **Shah, A.**, et al. Involvement of Metabotropic Glutamate Receptor-5 , AKT/PI3K Signaling and NF-kB Pathway in Methamphetamine-mediated increase in IL-6 and IL-8 Expression in Astrocytes. J Neuroinflammation 9 (2012) 52.
9. **Shah, A.**, et al., HIV-1 envelope protein gp120 up regulates CCL5 production in astrocytes which can be circumvented by gp120-specific siRNA and inhibitors of NF- kappa B pathway BBRC, 2011 Oct 14;414(1):112-7.
10. **Shah, A.**, et al., HIV-1 gp120 Induces Expression of IL-6 through a Nuclear Factor-Kappa B-Dependent Mechanism: Suppression by gp120 Specific Small Interfering RNA. PLoS One, 2011. 6(6): p. e21261.
11. Silverstein, P., **Shah, A.**, et al., Methamphetamine Toxicity and its Implications During HIV-1 Infection, J Neurovirol. 2011 Oct;17(5):401-15
12. Jin, M., **Shah, A.**, et al., A LC-MS/MS Method for Concurrent Determination of Nicotine Metabolites and Role of CYP2A6 in Nicotine Metabolism in U937 Macrophages: Implications in Oxidative Stress in HIV + Smokers. J Neuroimmune Pharmacol, 2011.
13. **Shah, A.** and A. Kumar., HIV-1 gp120-mediated increases in IL-8 production in astrocytes are mediated through the NF-kappaB pathway and can be silenced by gp120-specific siRNA. J Neuroinflammation, 2010. 7: p. 96.
14. **Shah, A.**, Shah, J.S., Sen, D.J., Comparison of pharmacological screening of CNS depressant action of fused ring heterocyclic adduct with fused ring aromatic with benzodiazepine as standard. Journal of International Drug Development and Research.2010: Vol.2 (1).

BIOGRAPHICAL SKETCH

NAME OF FELLOWSHIP APPLICANT Austin R. Jackson, Ph.D.	POSITION TITLE Post-Doctoral Fellow
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Missouri State University: Springfield, MO	B.S.	2008	Cell and Molecular Biology
University of South Carolina School of Medicine: Columbia, SC	Ph.D.	2008-2013	Biomedical Sciences
University of Missouri-Kansas City: Kansas City, MO	Post-Doc	2014-present	HIV: Neuroinflammation and Immunology

Please refer to the application instructions in order to complete sections A, B, C, and D of the Biographical Sketch.

A. Personal Statement

The goal I hold for my future is to significantly contribute to the knowledge base in the field of immunology while becoming a future educator and investigator. My research interest is finding the mechanism by which SIV/HIV causes neuroinflammation. My background in molecular and cell biology provides me with an excellent background and proves useful in studying the immunological parameters underlying this project. This training will be crucial in my future as an independent investigator in the HIV field.

B. Positions and Honors

ACTIVITY/OCCUPATION	BEGINNING DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/COMPANY	SUPERVISOR/ EMPLOYER
Undergraduate Research Assistant	08/07	05/08	Receptor Biology	Missouri State University	Richard Garrad
Graduate Research Assistant	08/08	11/13	Immunology	University of South Carolina	Mitzi Nagarkatti
Post-Doctoral researcher	01/14	Present	Viral Immunology	University of Missouri-Kansas City	Anil Kumar

Academic and Professional Honors

2004-2008: Board of Governor's Scholarship, Missouri State University
2004-2008: Dean's List, Missouri State University
2008: B.S. in Cell and Molecular Biology awarded, Summa Cum Laude, Missouri State University.
2010-2014: Alumni Association Board, University of South Carolina School of Medicine.
2010-2014: Graduate Advisory Committee, University of South Carolina School of Medicine
2011: Honorable mention in Newton's symposium, University of South Carolina
2011: 1st place, life sciences section, Graduate Student Day, University of South Carolina

2012: Dean Barnhardt Award Recipient, University of South Carolina
2012: Junior Editorial Internship, Laboratory Investigation.

C. Publications

1. Hegde VL, Tomar S, **Jackson A**, Rao R, Yang X, Singh UP, Singh NP, Nagarkatti PS, Nagarkatti M. 2013. Distinct microRNA expression profile and targeted biological pathways in functional myeloid-derived suppressor cells induced by Δ9-Tetrahydrocannabinol in vivo: Regulation of CCAAT/enhancer binding protein alpha by microRNA-690. J. Biol. Chem. 288(52): 36810-26.
2. **Jackson AR**, Hegde VL, Nagarkatti M, Nagarkatti PS. 2014. Characterization of Endocannabinoid-mediated Induction of Myeloid Derived Suppressor Cells Involving Mast Cells and MCP-1. J. Leukoc. Biol. PMID: 2431928
3. Unpublished

Unpublished

Abstracts:

National Meetings:

1. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Endogenous Cannabinoids regulate immune functions through induction of Myeloid Derived Suppressor Cells. Abstract for Poster Presentation: Society of Toxicology, Salt Lake City, UT, March 2010.
2. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Administration of Cannabinoids enhance growth and metastasis of 4T1 murine Breast Cancer through the induction of Myeloid Derived Suppressor Cells. Abstract for Poster Presentation: American Association of Immunologists, Baltimore, MD, May 2010.
3. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Anti-inflammatory properties of endogenous cannabinoids can be attributed to induction of a transient population of immunosuppressive Myeloid Derived Suppressor Cells that progress into immature macrophages. Abstract for Poster Presentation: Society of Toxicology, Washington, DC, March 2011
4. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Induction of Myeloid Derived Suppressor Cells by Endocannabinoids requires Mast Cells. Abstract for Poster Presentation: Society of Toxicology, San Francisco, CA, March 2012.

Regional:

1. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Jackson AR, Hedge VL, Nagarkatti M, Nagarkatti PS. Induction of Myeloid Derived Suppressor Cells by Endocannabinoids requires Mast Cells. Abstract for Poster Presentation: VA research Day, Columbia, SC, April 2012.
2. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Anti-inflammatory properties of endogenous cannabinoids can be attributed to induction of an immature population of immunosuppressive Myeloid Derived Suppressor Cells. Platform Talk, Newton's Symposium, University of South Carolina School of Medicine, Columbia, SC, February 2011.
3. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Endogenous cannabinoids induce regulatory myeloid-derived suppressor cells that have anti-inflammatory properties. Platform Talk, Newton's Symposium, University of South Carolina School of Medicine, Columbia, SC, February 2010.
4. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Administration of Cannabinoids enhance growth and metastasis of 4T1 Murine Breast Cancer. Abstract for Poster Presentation: Carolina Women's Health Research Forum, Columbia, SC, October 2010.
5. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Anti-inflammatory properties of endogenous cannabinoids can be attributed to induction of an immature population of immunosuppressive Myeloid Derived Suppressor Cells. Platform Talk, Newton's Symposium, University of South Carolina School of Medicine, Columbia, SC, February 2011.
6. Jackson AR, Hegde VL, Nagarkatti M, Nagarkatti PS. Induction of Myeloid Derived Suppressor Cells by Endocannabinoids requires Mast Cells. Abstract for Poster Presentation: VA research day, Columbia, SC, April 2012

Program Director/Principal Investigator (Last, First, Middle): Kumar, Anil

PROGRESS REPORT SUMMARY	GRANT NUMBER DA025011	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Anil Kumar	FROM 04/01/2013	THROUGH 03/31/2014

APPLICANT ORGANIZATION
University of Missouri-Kansas City

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Methamphetamine and AIDS in a Non-Human Primate Model

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

N/A

SCIENTIFIC PROGRESS

A: SPECIFIC AIM:

Specific Aim-1: To establish the SIV-SHIV/macaque model of chronic methamphetamine exposure and examine whether such exposure accelerates the onset of AIDS in macaques.
Specific Aim-2: To determine whether chronic methamphetamine exposure affects virus-specific cellular, humoral and mucosal immune responses in SIV-SHIV/macaque model of AIDS.
Specific Aim 3: To determine whether chronic methamphetamine exposure synergizes with SHIV/SIV infection to cause enhanced neuropathological effects in the brain and alterations in dopaminergic systems.

B: STUDIES AND RESULTS:

We reported last year that we acquired 18 rhesus macaques which were split into 4 groups (methamphetamine only=2; saline only=2; SIV Control=6 and SIV+methamphetamine=8). These animals were during early phase methamphetamine maintenance phase when last report was submitted. During last one year first two groups have completed 62-64 weeks of observation. Group 4 was infected with mixture of SHIV and SIV after 20 weeks of Methamphetamine maintenance phase while group 3 served as control for infection where animals received only saline. Periodic blood, CSF, LN and rectal biopsies have been collected. These animals have been followed for a total of 265 days after virus infection. Five of the 6 control animals have been euthanized out of which one animal was euthanized because of AIDS-unrelated reasons. In the methamphetamine group 6 of the 8 animals have been euthanized out of which 3 developed diseases within 100 days and they were defined as rapid progressors whereas other 3 were euthanized on days 139, 162 and 192, post-infection (termed as normal progressors). The 2 other animals are still alive. The survival curves for all these animals have been plotted using Kaplan-Meier survival analysis and results are shown in Figure-1. The results clearly show that the 3 animals defined as rapid progressors in methamphetamine group developed disease significantly earlier to control animals suggesting deleterious effect of methamphetamine in a subgroup of animals. A variety of tissues has been collected from all the animals that have been euthanized during last year. We plan to euthanize remaining animals within next 4 weeks.

B.1 Effect of methamphetamine and SIV infection on $CD4^+$ and $CD8^+$ T cell profile in blood of macaques:

All animals were periodically bled and effect of methamphetamine on CD4 and CD8 T cell profile was monitored over 62 week in 2 control (Figure 2A and B) and 2 methamphetamine-treated (Figure 2C and D) animals. The methamphetamine did not cause any significant change in these two cell populations as only normal fluctuation was observed in these 4 animals. The $CD4^+$ and $CD8^+$ T cell profiles were also assessed in 6 SIV controls (Figure 2E and F) and 8 SIV+Meth macaques (Figure 2G and H). All animals rapidly lost CD4 T cells but there was no significant difference between two groups. Likewise, the CD8 T cells profile also did not show any significant difference between two groups.

B.2 Effect of methamphetamine and SIV infection on $CD4^+$ and $CD8^+$ T cell profile in lymph nodes of macaques:

The animals were biopsied at 3 different time points and LN cells were obtained by teasing the tissue followed by collagenase treatment. The cell suspension was passed through Ficoll hypaque and interface was collected for lymphocyte population. These cells were stained with antibodies against CD3, CD4 and CD8. The CD4 and CD8 percentage was determined by acquisition using FACSCanto II. The results of these experiments are shown in Figure 3 and 4. The methamphetamine did not cause any effect on CD4 and CD8 T cells. However, methamphetamine caused significantly higher reduction in CD8 T cells ($p < 0.05$) after SIV infection suggesting that cellular response in these animals may be compromised. We will get better idea when we perform ELISPOT assays. The CD4 loss was massive after infection and there were practically no cells left 4 week after infection in either group.

B.3 Effect of methamphetamine and SIV infection on $CD4^+$ and $CD8^+$ T cell profile in mucosal compartment of macaques:
The rectal biopsies were obtained at different times and the cells were prepared as described above. These cells were stained with antibodies against CD3, CD4 and CD8. The CD4 and CD8 percentage was determined by acquisition using FACSCanto II. The results of these experiments are shown in Figure 5 and 6. Methamphetamine did not affect CD4 and CD8 populations. SIV infection cause massive CD4 loss but there was no significant difference between control and Meth groups in CD4 and CD8 T cell profile.

B.4 Methamphetamine significantly increased monocyte counts after SIV infection:
Methamphetamine did not affect monocyte number when given to two animals for 62 weeks or 8 animals that were administered for 20 weeks before infection. However, monocyte numbers were significantly higher in methamphetamine+SIV group ($p < 0.01$) suggesting possible higher MCP-1 and inflammation (Figure-7).

B.5 Effect of methamphetamine on expression of proinflammatory cytokines/chemokines:
Eight control and 10 methamphetamine macaques were bled at weeks 0, 4, 10 and 20 and the plasma was subjected to multi-cytokine assay using 8 cytokine/chemokine detection system (IL-1ra, IL-4, IL-6, IL-8, IL-12, IFN-γ, MCP-1 and sCD40L). Only 5 cytokines were detectable in plasma and results of this experiment are shown in Figure-8. There was no clear cut pattern observed in these animals suggesting that methamphetamine does not cause any significant change in systemic compartment. However, experiments are underway to determine cytokine/chemokine concentration in CSF.

B.6 Effect of methamphetamine and SIV infection on expression of proinflammatory cytokines/chemokines:
Six control and 8 methamphetamine macaques were infected with virus and plasma was collected at weeks 0, 4, 12 and 24. The plasma was used for detection of 8 cytokine/chemokines as described above. The results of this experiment are shown in Figure-9. SIV control animals showed gradual increase in IL8 in control SIV macaques whereas methamphetamine+SIV monkeys showed increased IL8 at week 12 followed by decline at week 24. In spite of the fact that total monocyte numbers were higher in methamphetamine+SIV group, the increase was more pronounced in control SIV group. Methamphetamine caused decrease in sCD40L level as evident by lower level at the time of infection but SIV infection caused rapid increase (Figure-9E)

B.7 Optimization of LC-MS method for methamphetamine and metabolites:

We have developed a novel method to quantify methamphetamine (MA) and its 6 metabolites using Liquid chromatography-Mass spectrometry (LC-MS). The normal monkey plasma was used to optimize these assays so that it can be used for determination in plasmas collected at different time point and determine whether there is any correlation between methamphetamine metabolism and disease progression.

Chemical and Reagents: MA, 4-OH MA, AM, 4-OH AM, norephedrine, MA-d5, and AM-d8 were purchased from Cerilliant Analytical Reference Standards (Sigma-Aldrich Company, Round Rock , TX). HPLC grade methanol, acetonitrile, ammonia solution, and formic acid were procured from Fisher Scientific (New Brunswick, NJ). All HPLC grade chemicals were utilized without further purification. An Xbridge HPLC reverse phase C18 column and HLB Oasis solid phase extraction cartridges (Waters Corporation, Milford, MA) were employed for the determinations of analytes.

Solutions and reference standards: MA, 4-OH MA, AM, 4-OH AM, norephedrine, MA-d5, and AM-d8 were dissolved in methanol at a concentration of 1 mg/mL (v/v). Stock concentrations were corrected as described earlier in our study (23122404). Standard curve for each analytes was generated using 20 µL working solution at different concentrations (800.00, 640.00, 486.40, 389.12, 214.02, 74.91, 14.98, 4.94, 1.09 ng/mL) in plasma obtained from a drug-free monkey. Similarly, the quality control (QC) samples were independently prepared at four concentrations (486.40, 214.02, 14.98 and 1.09 ng/mL) in drug-free monkey plasma.

System suitability and carry-over tests: The *system suitability test* for each analyte was performed independently by using six replicate injections of 800 ng/mL of the reference standard and internal standard (IS). The *system suitability test* for each analyte was further analyzed by calculating the coefficient of variation (CV) (standard deviation/mean concentration) multiplied by 100. The *carry-over test* was performed by injecting a blank plasma sample extract followed by immediate injection of an extract of the sample from the upper limit of quantitation (ULOQ) of the standard curve along with an IS.

Mass spectrometry optimization: The mass spectrometer (3200 QTRAP LC-MS/MS system, AB Sciex) was optimized for detection of MA and its four metabolites along with IS. Mass spectrometry data of each compound was first acquired in full scan mode from the range between 50–300 Da to identify their precursor ions. The most suitable proton adduct in an electrospray ionization $[M+H]^+$ precursor ions was determined for MA (150.5), 4-OH MA (166.3), AM (136.4), 4-OH AM (152.3), norephedrine (152.3), MA-d5 (155.5), and AM-d8 (144.5) (Figure 10). These precursor ions were optimized by setting the curtain gas, declustering potential, ion spray voltage, and source gas 1.

Tandem (MS/MS) mass spectrometry conditions: The proton adduct *m/z* $[M+H]^+$ precursor ions of MA and four of its metabolites along with IS were selected in positive mode for collision cell quadruople 2 (MS2). Precursor ions were fragmented by applying collisionally-activated dissociation gas and collision energy to obtain their most abundant and stable product ions. The product ions for MA (91.2), 4-OH MA (135.4), AM (91.3), 4-OH AM (135.2), norephedrine (134.4), MA-d5 (92.3), and AM-d8 (97.2) were optimized by adjusting collision energy, curtain gas, entrance potentials, and source gas 2 (GS2) (Figure 10). The multiple reactions monitoring (MRM) transitions (*m/z*) $[M+H]^+$, (Q_1/Q_3) selected for quantitative analyses were: 150.5/91.2 for MA, 166.3/135.4 for 4-OH MA, 136.4/91.3 for AM, 152.3/135.2 for 4-OH AM, 152.3/134.4 for norephedrine, 155.5/92.3 for MA-d5 and 144.5/97.2 for AM-d8 (Figure 1 and Supplemental Table 1). A dwell time of 200 ms and a source temperature of 450^{0}C were employed for all the analyte determinations.

LC-MS/MS chromatographic separation: An LC-MS/MS chromatographic separation was achieved by a reverse phase Xterra MS C 18 column (50 x 4.6 mm, i.d, 5µm) using UFLC Shimadzu LC-20AD HPLC (California, USA). An isocratic mobile phase composed of 55% acetonitrile in water containing 0.05% of formic acid at a flow rate of 0.4 mL/min was used. The samples were reconstituted in a 500 µL of acetonitrile-water-formic acid (70:30:0.05) solution. A 15 µL aliquot of each sample was injected into LC-MS/MS for quantitative analysis over 5 min. The LC-MS/MS acquired MRM data was processed uisng Analyst software (version 1.4.2, AB Sciex).

Sample preparation and extraction: A simple HLB SPE technique was used for sample extraction. Two hundred microliters of plasma from a drug-free monkey was aliquoted and to this 20 µL of 10 µg/mL IS (final concentration of ~1 µg/mL) was added. The mixture was vortex-mixed for 30 sec followed by the addition of 20 µL of an aqueous 10% formic acid solution, which was again vortex-mixed for 1.0 min prior to SPE. The SPE columns (HLB 30 mg, 1 mL cartridge) were preconditioned with 1 mL of methanol and equilibrated with 1mL of water. The plasma samples were loaded on the SPE cartridge and drained slowly by applying positive pressure at 15 psi with a 48 well plate Positive Vacuum Manifold. The SPE columns were washed with 1 mL of water. Next, the SPE columns were air dried by N_2 gas under positive vacuum at 20 psi for 2 minutes, and analytes were eluted with 1 mL

methanol. After elution, samples were evaporated using a speed vacuum at 35^{0}C for 60 min. Dry residue of each sample was re-dissolved in 200 µL of reconstitution solution.

The Specificity and Selectivity: The specificity and selectivity of the methods were tested by analyzing six monkey blank plasma samples. These blank matrices did not show measurable interference at analyte peak of interests for MA and its metabolites. Six samples were processed at the lower limit of quantitation (LLOQ, 1 ng/mL) in order to assess the blank plasma interference at the analyte peak of interest (Figure 11A). The percentage of interference determined in the blank was calculated by comparing the mean peak area of LLOQ of the analyte with the peak response obtained from the blank samples, which should be ≤ 20%.

Precision and Accuracy: Within-assay & between-assay, precision & accuracy experiments were performed by analyzing eight extracted calibration and four levels of QC standards. The pooled monkey blank plasma samples were used to prepare a calibration curve and QC standards. The standard samples were prepared based on the procedure described earlier (PMID 20172680). Precision was determined by analyzing six replicates at each of the four levels of QC standards using a previously described method from our group.

Recovery, matrix effect, and stability of analytes in monkey drug free plasma: Recovery of MA and its metabolites was estimated by analyses of two sets of six replicates of plasma extracted low, middle, and high QC standards and post-spiked (represent 100% recovery) sample along with IS. The three levels of recovered QC standards were prepared in extracted blank plasma. The aqueous dilutions were post-spiked in extracted blank plasma sample to get the same concentrations. An overall extraction recovery was determined by comparing the mean peak area ratios of the analytes with the IS obtained from the extracted QC (matrix samples from non-smokers versus the un-extracted standards).

Matrix effect of MA and its metabolites along with IS was evaluated by analyzing 2 sets of six replicates of each low, middle, and high QC standards from post-spiked (extracted blank plasma samples), and spiked standards in aqueous solutions (represent no matrix effect). Ninety blank matrix samples from a non-smoker and 18 samples of each analyte were processed and extracted as described above. The aqueous stock QC dilutions (low, middle, and high) were spiked in the extracted blank samples to obtain the QC standards (486.40, 214.02 and14.98 ng/mL). Similarly, these QC standards were prepared by spiking analytes in reconstitution solution to obtain the same concentrations. Matrix ion suppression was calculated by comparing the mean peak area ratios of each analyte and IS generated from the post-spiked QC standards from plasma samples from non-smokers with reconstituted spiked QC standards. A relative matrix effect was estimated by comparing the mean peak area ratios of the analytes to IS obtained from the post-spiked QC.

Six replicates of stability samples at concentrations of 486.40, 214.02, and14.98 ng/mL were prepared for each analyte in pooled monkey plasma. They were stored at -80^{0}C for several weeks to estimate the degradation of analyte in the matrix. Stability QC samples were extracted along with freshly prepared calibration standards in pooled monkey plasma. The stability samples were frozen and stored for 6 months at -80^{0}C. Stability QC samples were freeze-thawed for three cycles, and analyzed with freshly prepared calibration curve standards. These plasma samples were stored and stability was determined to anticipate the storage conditions of the actual test samples. The linearity was determined using freshly spiked calibration standards, which were analyzed in duplicate along with stability samples (*bench top*, *freeze* and *thaw* stability) processed as described previously (PMID 20172680). Based on our results we have developed a scheme for methamphetamine metabolism in rhesus macaques which is shown in Figure-13

Animal recruitment and plasma sample analysis: We have so far analyzed 10 monkey plasma samples from SIV+methamphetamine group and results from these experiments are shown in Figure-

14. Our results show different pattern of methamphetamine metabolism. A comprehensive analysis of plasma collected throughout study will provide definitive conclusion. However based on preliminary results the metabolic rate appears to be different in different macaques. Macaques RHd14 and RDu13 seem to be high metabolizer whereas macaques ROf14, RDy13 and Rue14 appear to be intermediate metabolizer. RMa14, Rtb14 and RQs13 seem to be slow metabolizer based on amphetamine production. In our future studies we will try to correlate this with SNP in CYP2D6 and CYP3A4 which are known to be involved in methamphetamine metabolism.

C: SIGNIFICANCE

1. We have developed a novel method for detection of methamphetamine and its metabolites in rhesus macaques.
2. The methamphetamine has been found to be associated with increased monocyte in blood after infection with pathogenic virus.
3. Decreased CD8 T cells in lymph nodes of macaques in methamphetamine group after viral infection suggest compromised CD8 response in these animals.
4. A subgroup of methamphetamine animals developed accelerated disease and succumbed to SHIV/SIV-induced AIDS in approximately 3 months.
5. There was no correlation between plasma cytokine/chemokine levels and disease progression.
6. Based on preliminary analysis we have found 3 groups of macaques with ability to metabolize methamphetamine differently but apparently there seems be to no correlation with disease progression.

D: PLANS:

1. We will determine viral loads in plasma, CSF and tissues.
2. We will perform antibody assay (both binding and neutralizing) and ELISPOT assay.
3. The methamphetamine metabolism analyses will be completed.
4. The cytokine/chemokine levels will be assessed in CSF.
5. Differential viral distribution in blood, CSF and tissue will be completed.

E: PUBLICATIONS:

1. A Nookala, A Shah, RJ Noel and A Kumar (2013). HIV-1 Tat-mediated induction of CCL5 in astrocytes involves NF-kB, AP-1, C/EBPα and C/EBPγ transcription factors and JAK, PI3K/Akt and MAPK signaling pathways. PLoS One , 8(11):e78855.
2. A Shah, S Kumar, SD Simon, DP Singh and A Kumar (2013). HIV gp120 and methamphetamine-mediated oxidative stress induces astrocyte apoptosis via cytochrome P450 2E1. Cell Death and Disease. 4:e850, PMID 24113184.
3. MR Gangwani, R Noel, A Shah, S Kumar, V Amill and A Kumar (2013). Human Immunodeficiency Virus Type 1 Viral Protein R (Vpr) Induces CCL5 Expression in Astrocytes via PI-3K and MAPK Signaling Pathways. Journal of Neuroinflammation. 10: 136.

F: PROJECT GENERATED RESOURCES:

None

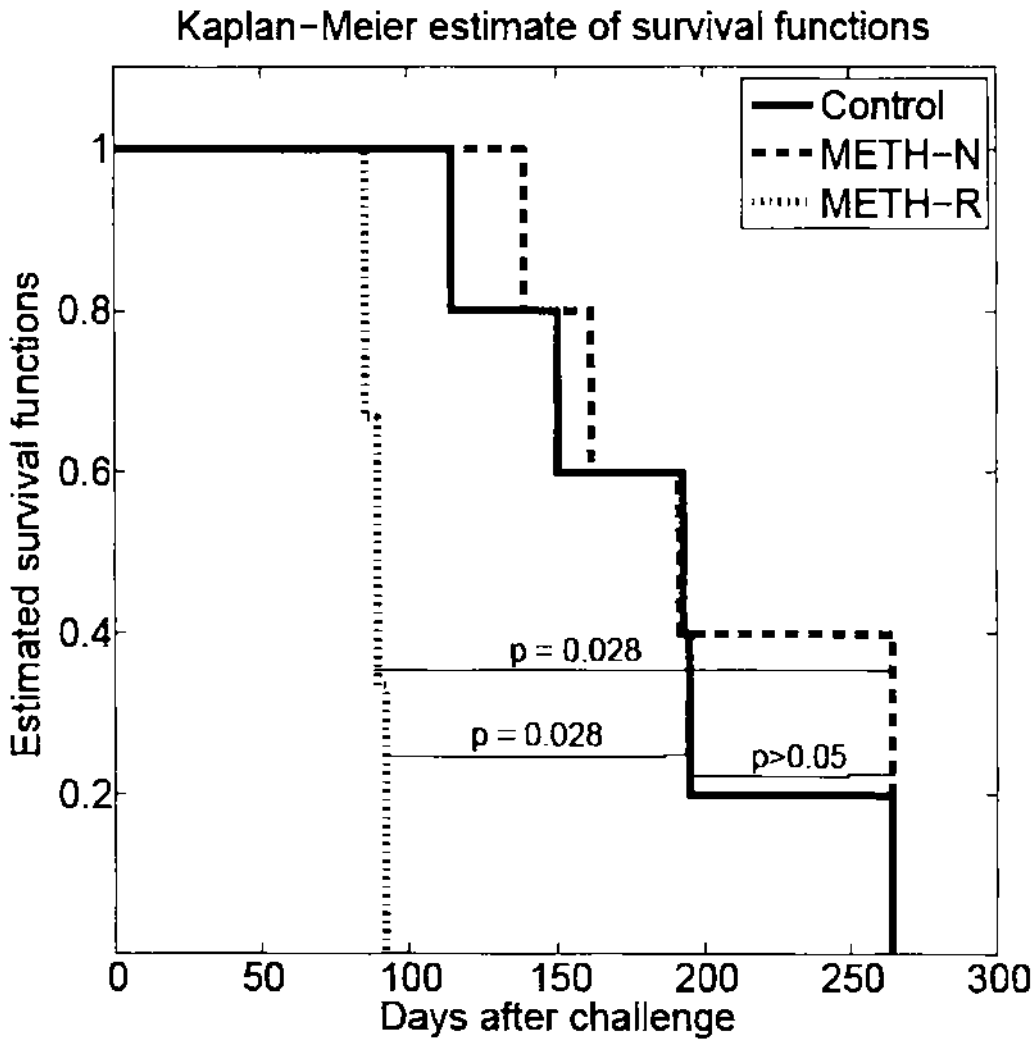


Figure-1: Kaplan-Meier curves of survival time in control and normal as well as rapid progressors in methamphetamine group.

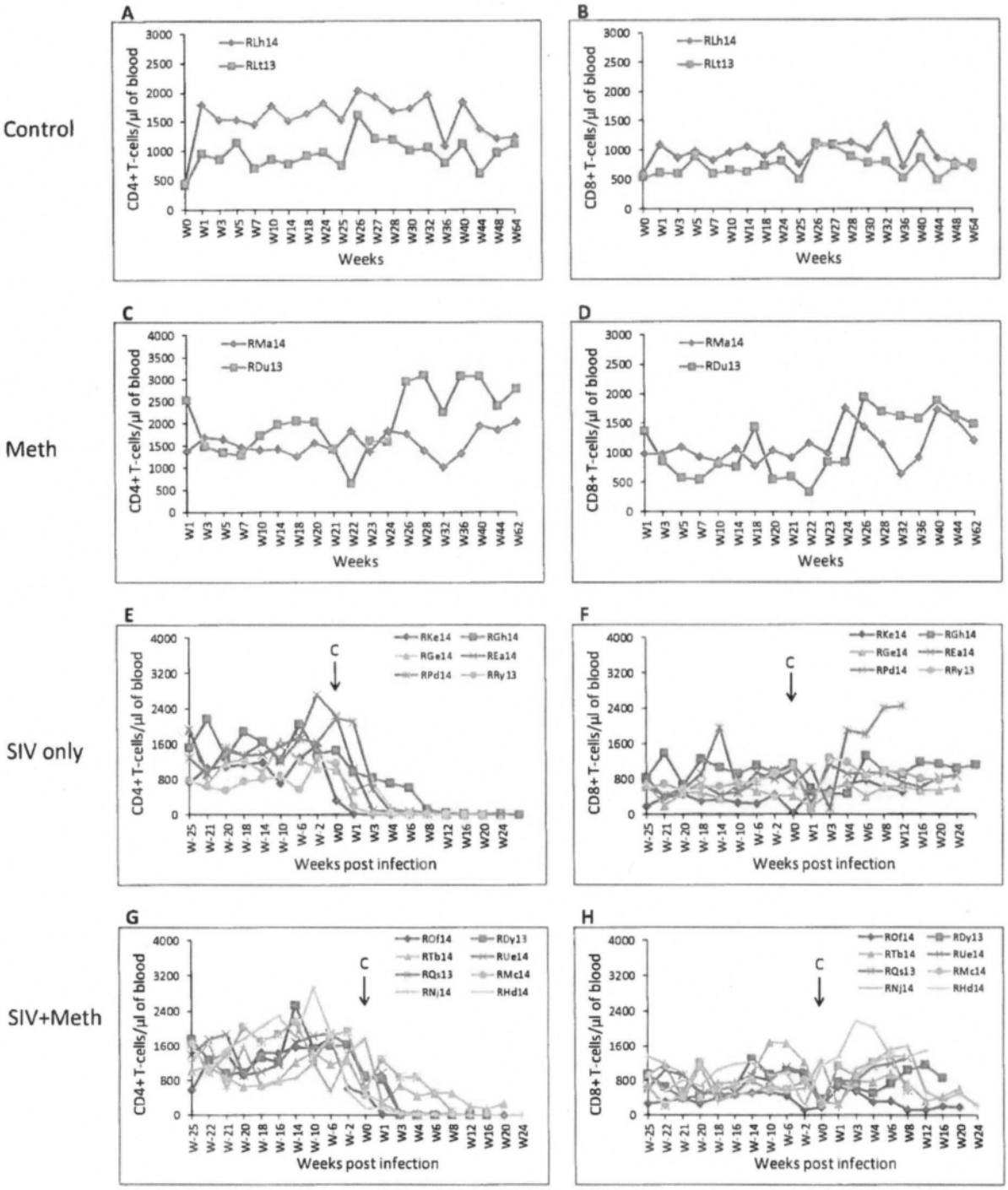


Figure. 2: $CD4^+$ and $CD8^+$ T cell profile in saline only (A, B); Methamphetamine only (C, D), SIV+saline (E, F) and SIV+Methamphetamine macaques (G, H). The methamphetamine was administered in escalating dose starting from 0.1 mg/KG twice every day, 5 days a week to 0.750.1 mg/KG twice every day, 5 days a week through weeks 1-4. Methamphetamine was then maintained at this for remained of observation period. The controls were given equal volume of the saline at same time. The animals in E,F, G and H were inoculated with mixture of $SHIV_{KU-1B}$, $SHIV_{89.6}P$ and SIV/17E-Fr. The CD4 and CD8 profile was determined by staining with a mixture of antibodies against CD3, CD4 and CD8. The absolute numbers of $CD4^+$ and $CD8^+$ T cells were calculated by multiplying the percentage of lymphocyte subset with lymphocyte number and divided by 100.

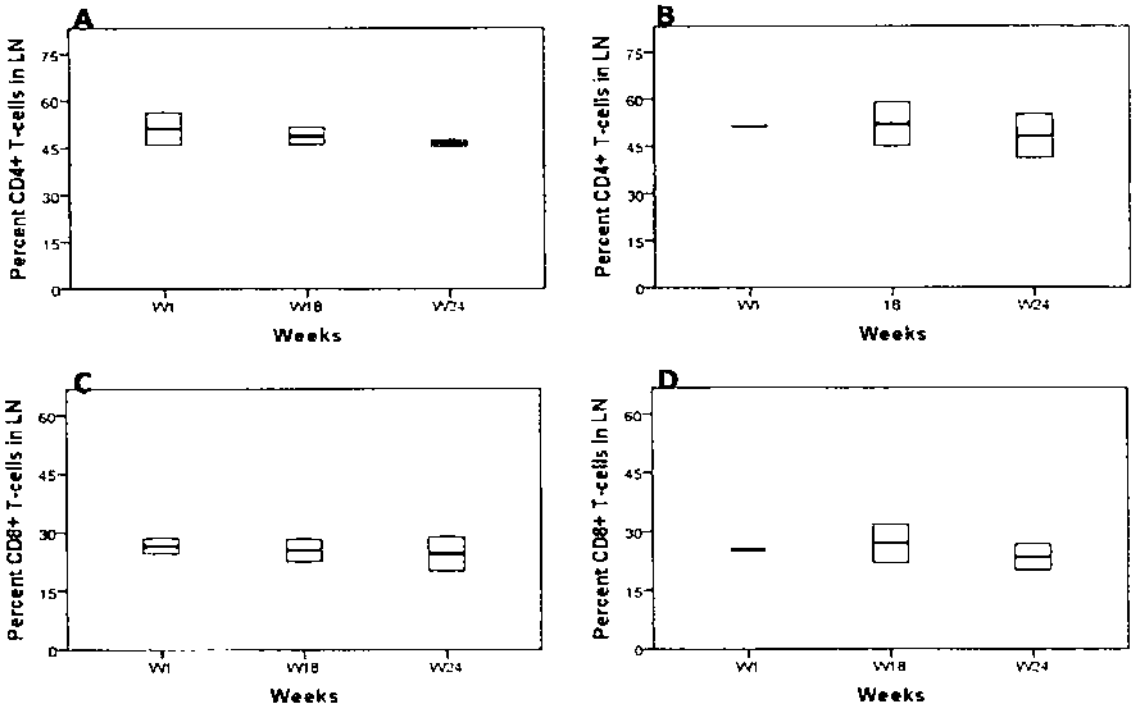


Figure-3: Effect of Methamphetamine on LN T cell population: Four rhesus macaques (2 Saline-A, C; and, 2 Methamphetamine-B, D) were administered Meth as described in Figure-1 for a period of 24 weeks. LN biopsy was performed at weeks 1, 18 and 24. The cells were separated by digesting LN tissue with collagenase and running a gradient through Ficoll-hypaque. The CD4 and CD8 percentage was determined by staining with a mixture of antibodies against CD3, CD4 and CD8 and acquisition using FACSCanto II.

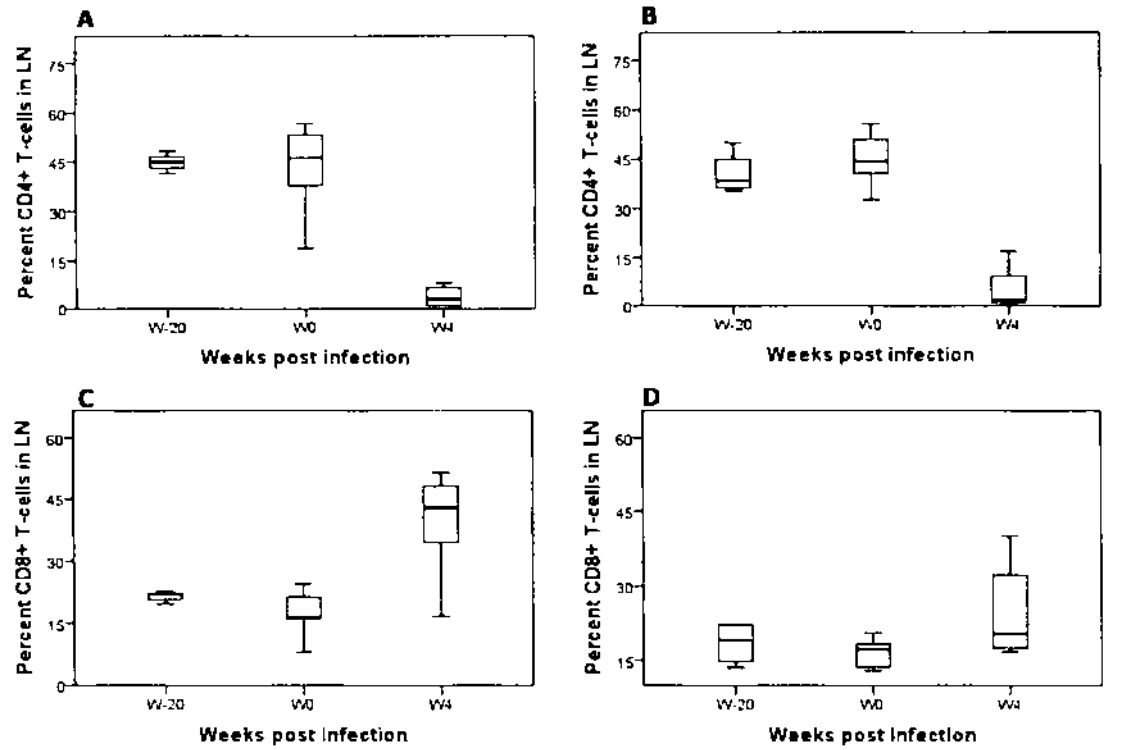


Figure-4: Effect of SIV infection on LN T cell population in control and Meth treated macaques: Lymph nodes from six saline (A, C) and, 6 Methamphetamine (B, D) treated rhesus macaques were collected at weeks -20, 0 and 4 (considering week 0 as time of infection). The cells were separated by digesting LN tissue with collagenase and running a gradient through Ficoll-hypaque. The CD4 and CD8 percentage was determined by staining with a mixture of antibodies against CD3, CD4 and CD8 and acquisition using FACSCanto II.

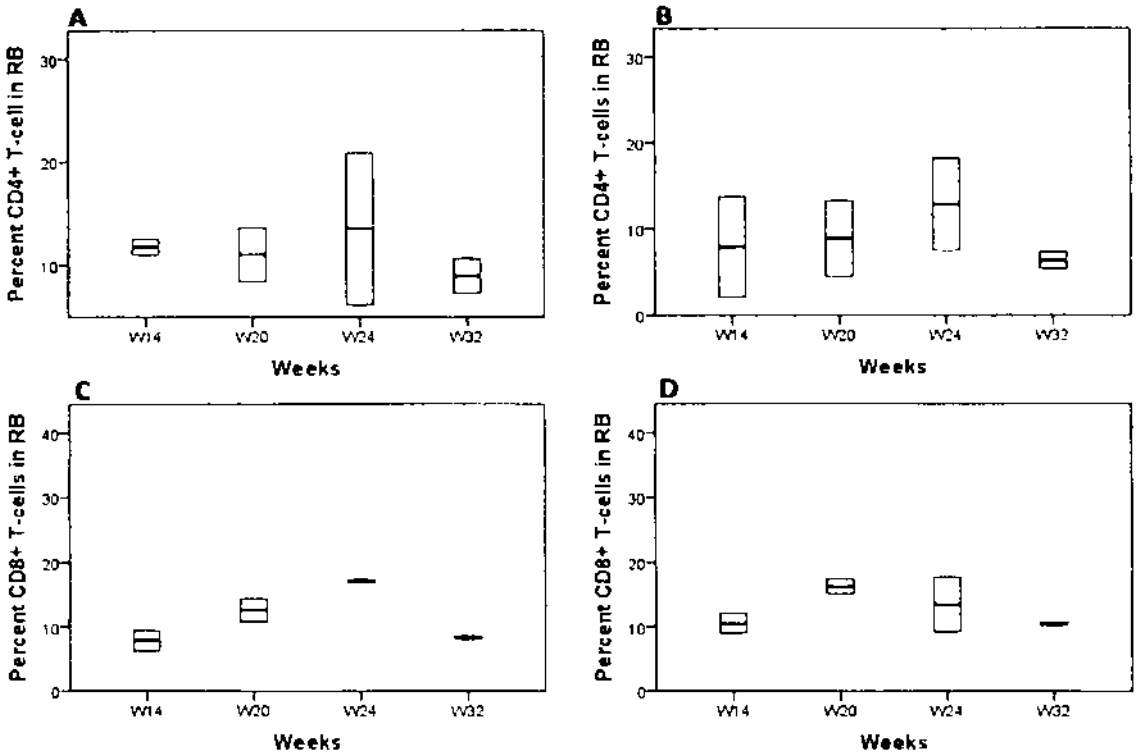


Figure-5: Effect of Methamphetamine on mucosal T cell population: Four rhesus macaques (2 Saline-A, C; and, 2 Methamphetamine- B, D) were administered Meth as described in Figure-1 for a period of 32 weeks. Rectal biopsies were performed at weeks 14, 20, 24 and 32. The cells were separated by digesting rectal biopsies with collagenase and running a gradient through Ficoll-hypaque. The CD4 and CD8 percentage was determined by staining with a mixture of antibodies against CD3, CD4 and CD8 and acquisition using FACSCanto II.

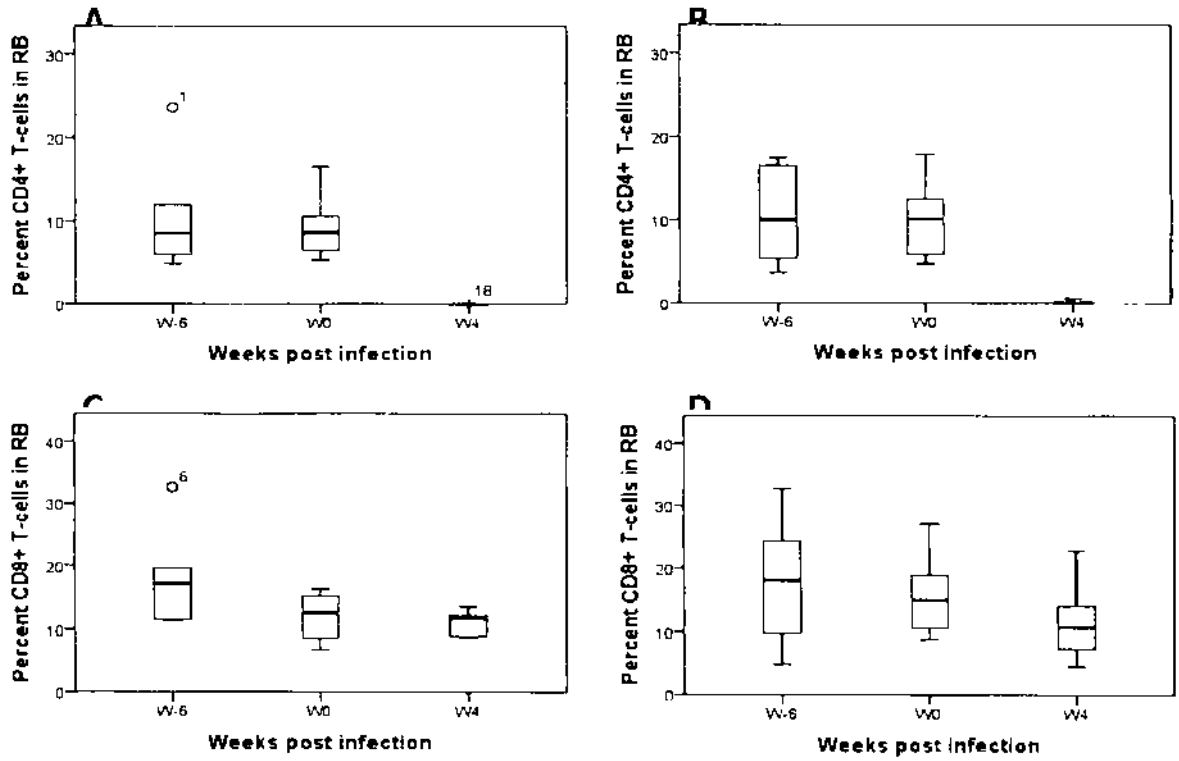


Figure-6: Effect of SIV infection on mucosal T cell population in control and Meth treated macaques: Rectal biopsies from six saline (A, C) and, 8 Methamphetamine (B, D) treated rhesus macaques were collected at weeks -6, 0 and 4 (considering week 0 as time of infection). The cells were separated by digesting rectal biopsies with collagenase and running a gradient through Ficoll-hypaque. The CD4 and CD8 percentage was determined by staining with a mixture of antibodies against CD3, CD4 and CD8 and acquisition using FACSCanto II.

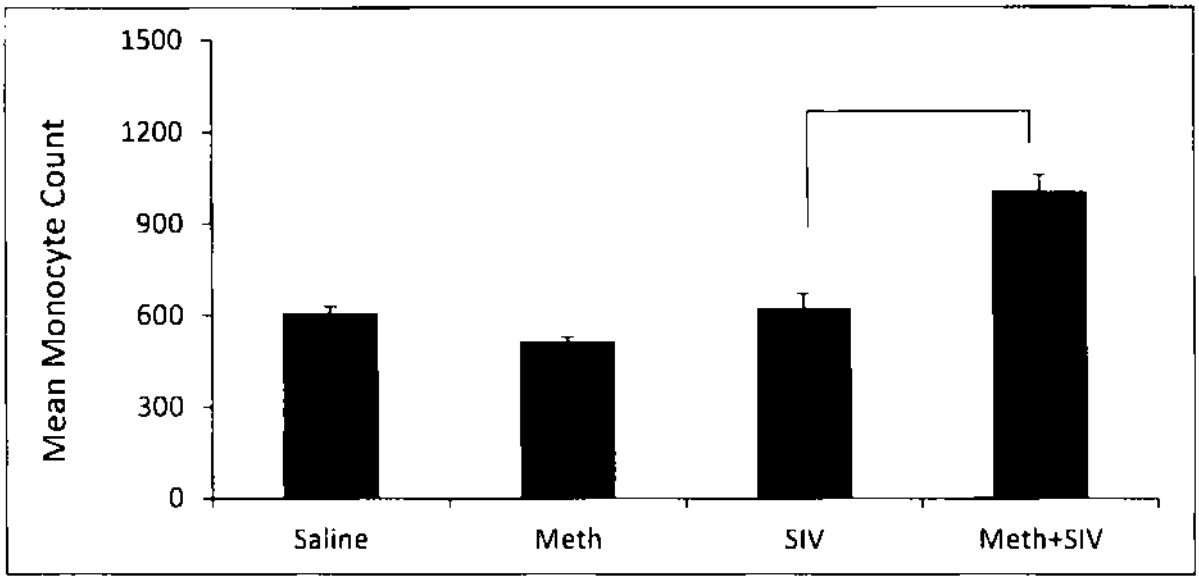


Figure-7: Effect of Meth and SIV infection on monocyte population in rhesus macaques. Average monocyte count in saline only, Methamphetamine only, SIV+saline and SIV+Methamphetamine macaques. The monocyte count were taken from complete blood count for each monkey in different group throughout observation period. The sample numbers varied depending on survival of the animal. The average count for each animal was obtained by adding all monocyte number divided by number of samples. The data shown are mean of 2, 2, 6 and 8 animals respectively.

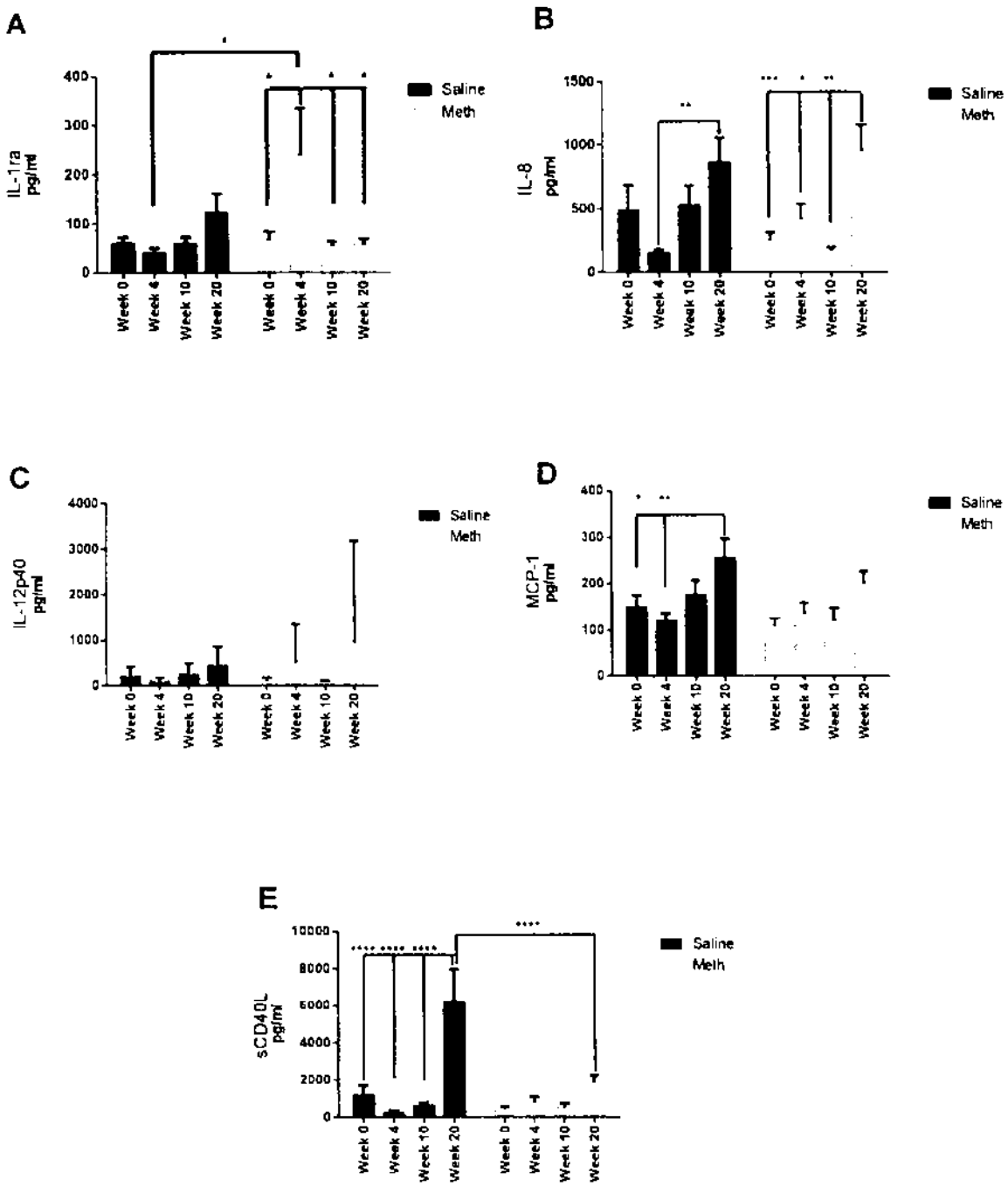


Figure-8: Effect of Methamphetamine on IL-1ra, IL-8, IL-12, MCP-1 and sCD40L. The blood samples were collected at weeks 0, 4, 10 and 20 from 8 controls and 10 Meth treated macaques. The plasma was subjected to 8-plex multi cytokine assay. The results are shown as mean± for each group. * p= <0.05; ** p= <0.01; *** p= <0.005, **** p= <0.001.

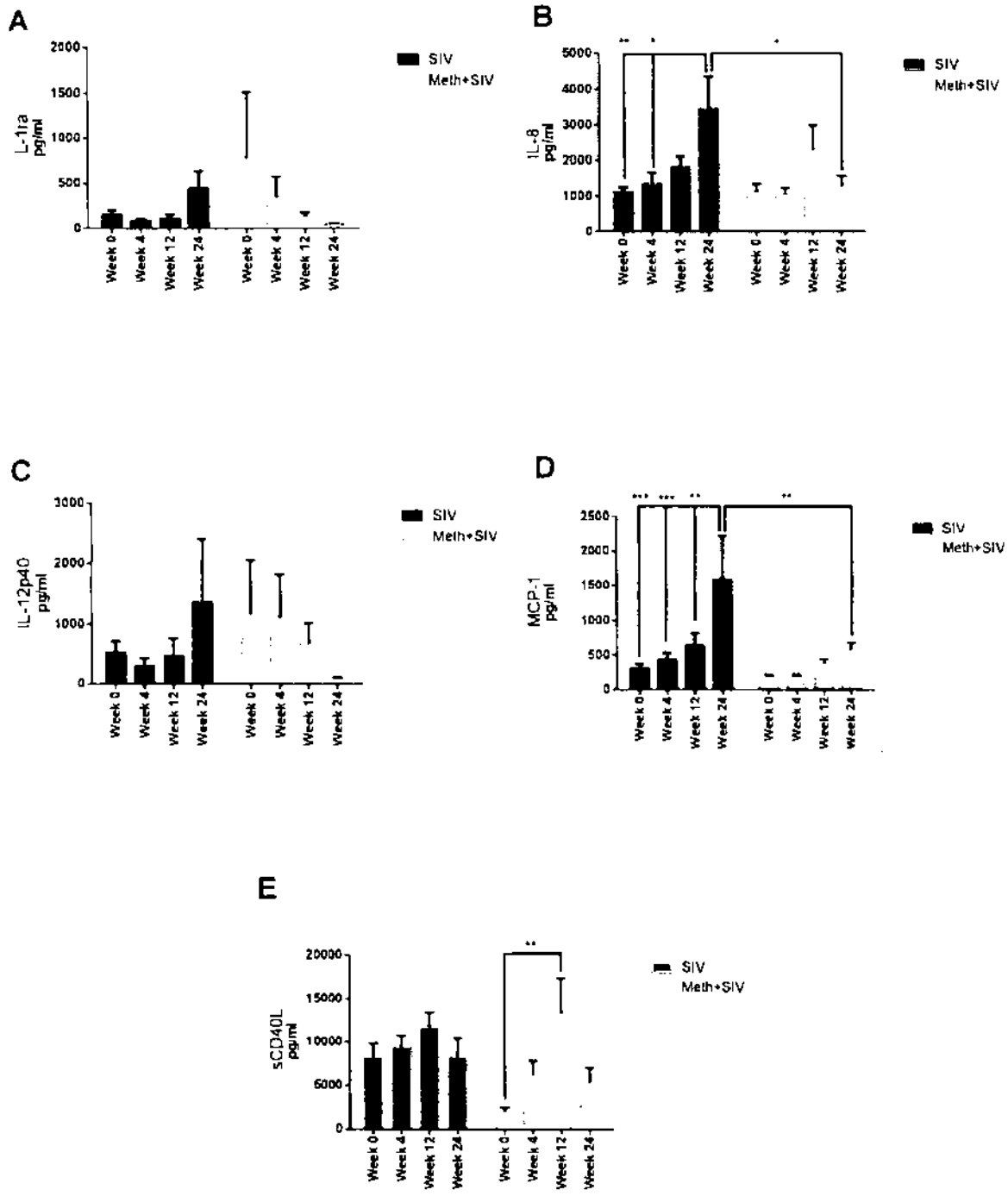


Figure-9: Effect of SIV infection on IL-1ra, IL-8, IL-12, MCP-1 and sCD40L levels in control and Meth treated macaques. The blood samples were collected at weeks 0, 4, 12 and 24 from 6 SIV controls and 8 Meth treated/SIV-infected macaques. The plasma was subjected to 8-plex multi cytokine assay. The results are shown as mean± for each group. * p= <0.05; ** p= <0.01; *** p= <0.005.

Figure 10

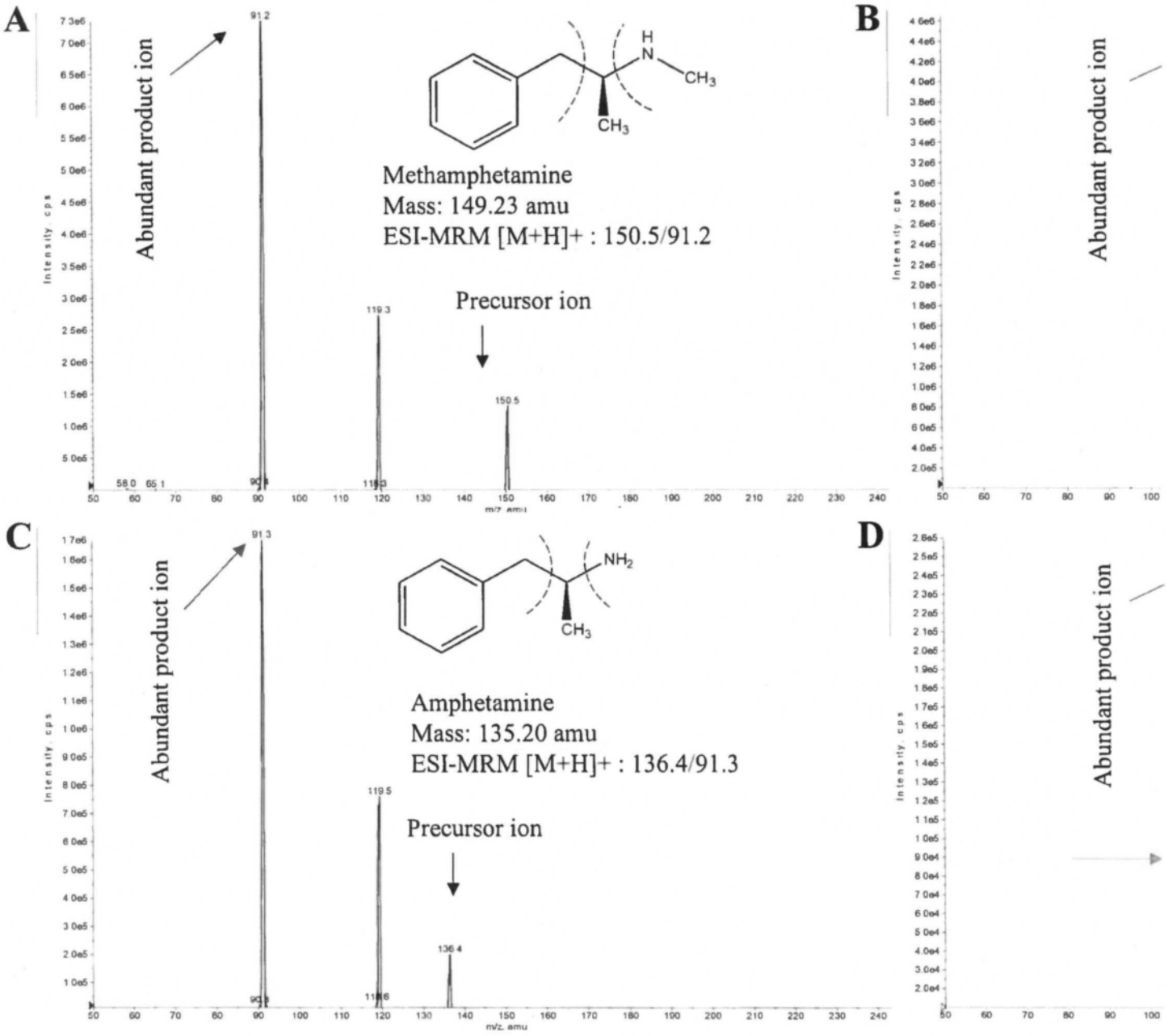


Figure 10 Contd.

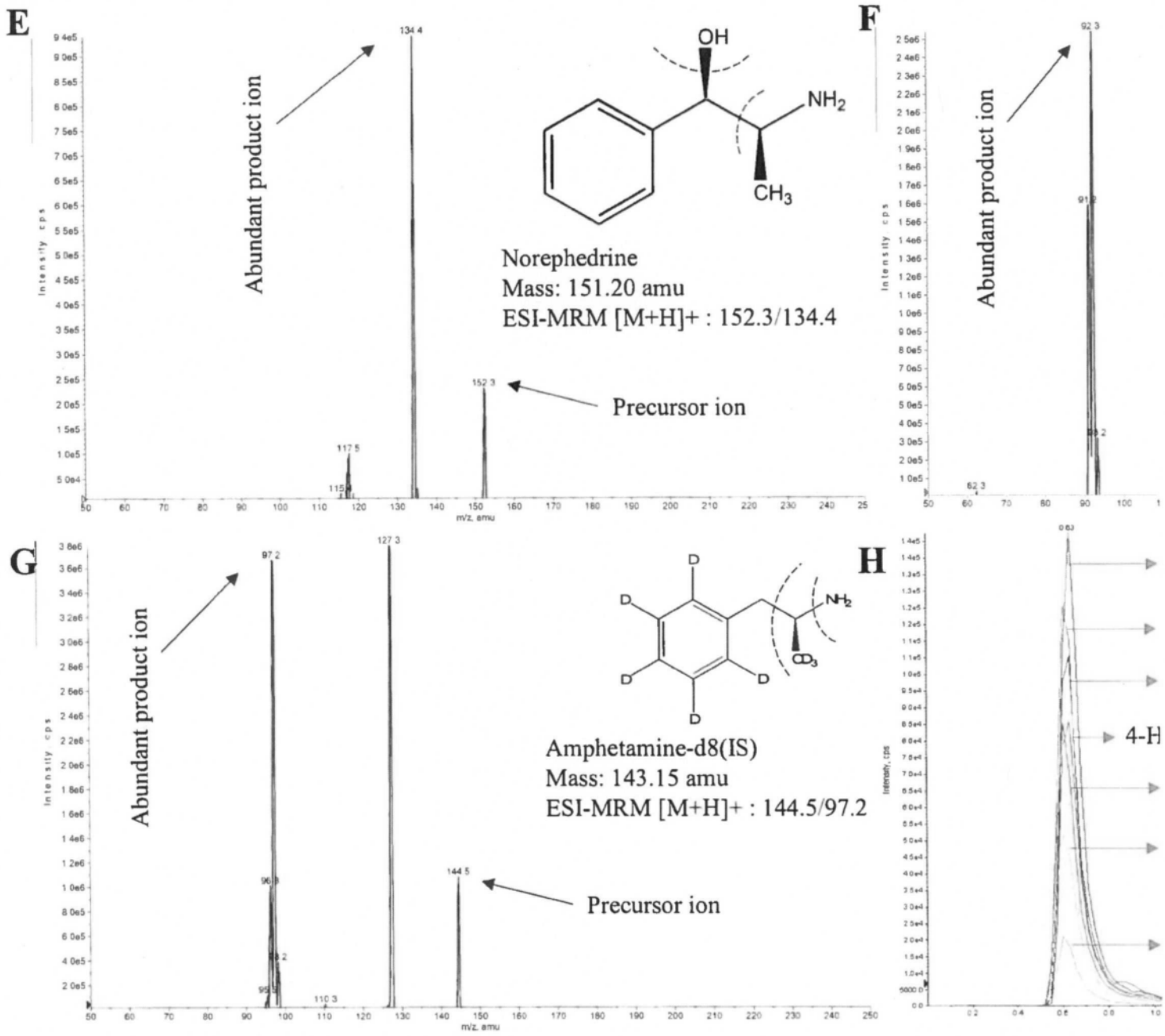


Figure 11

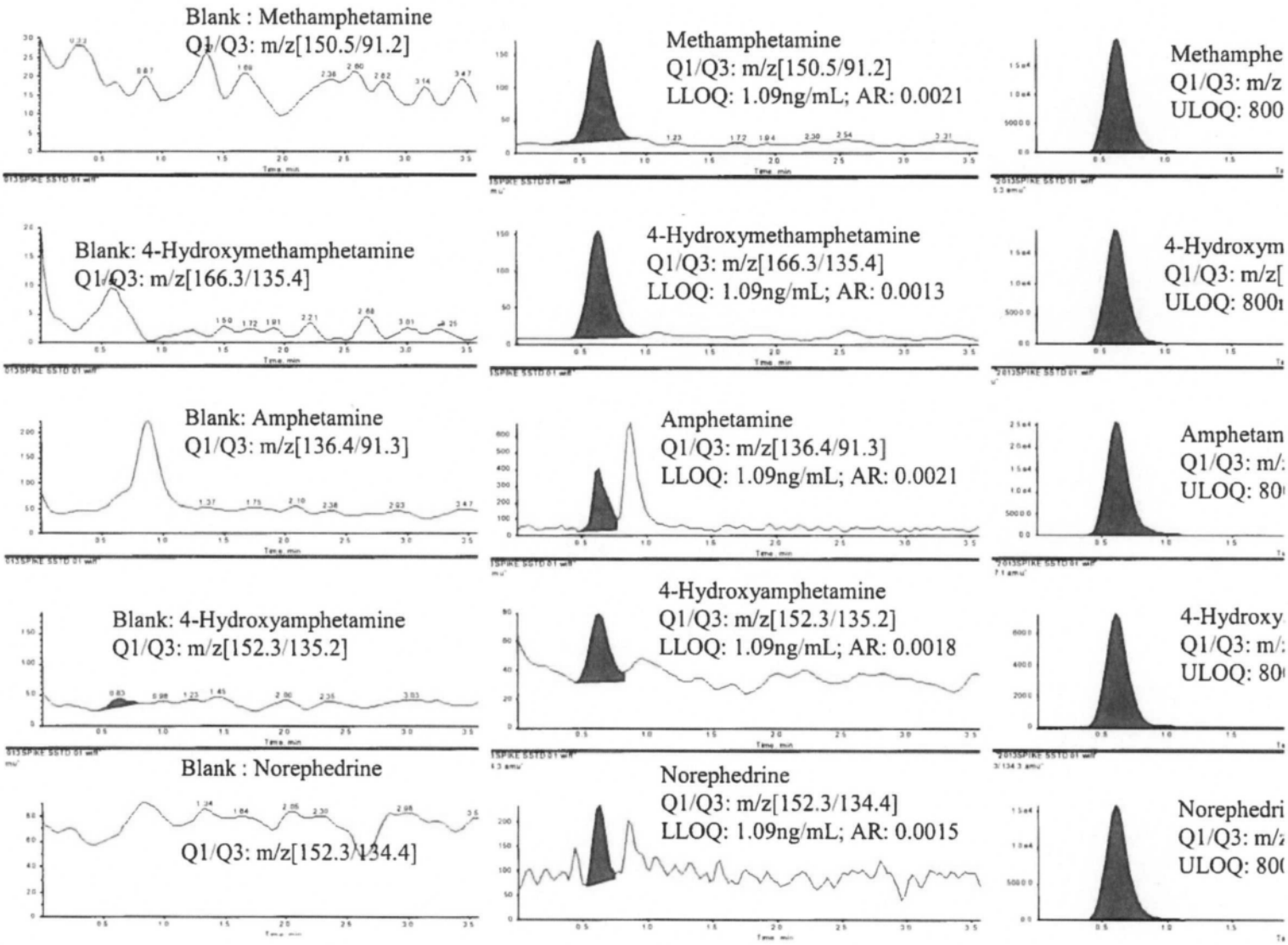
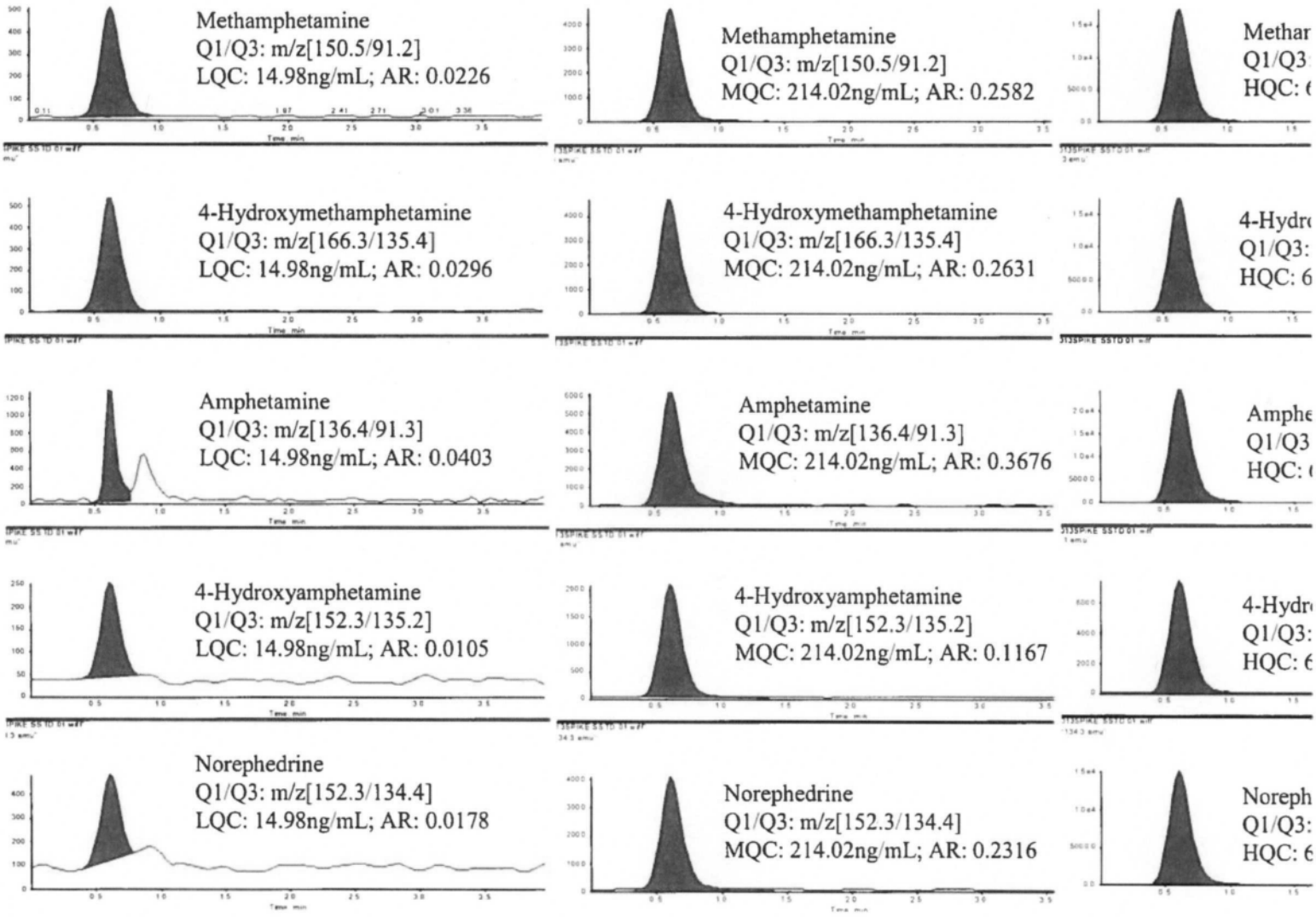


Figure 12



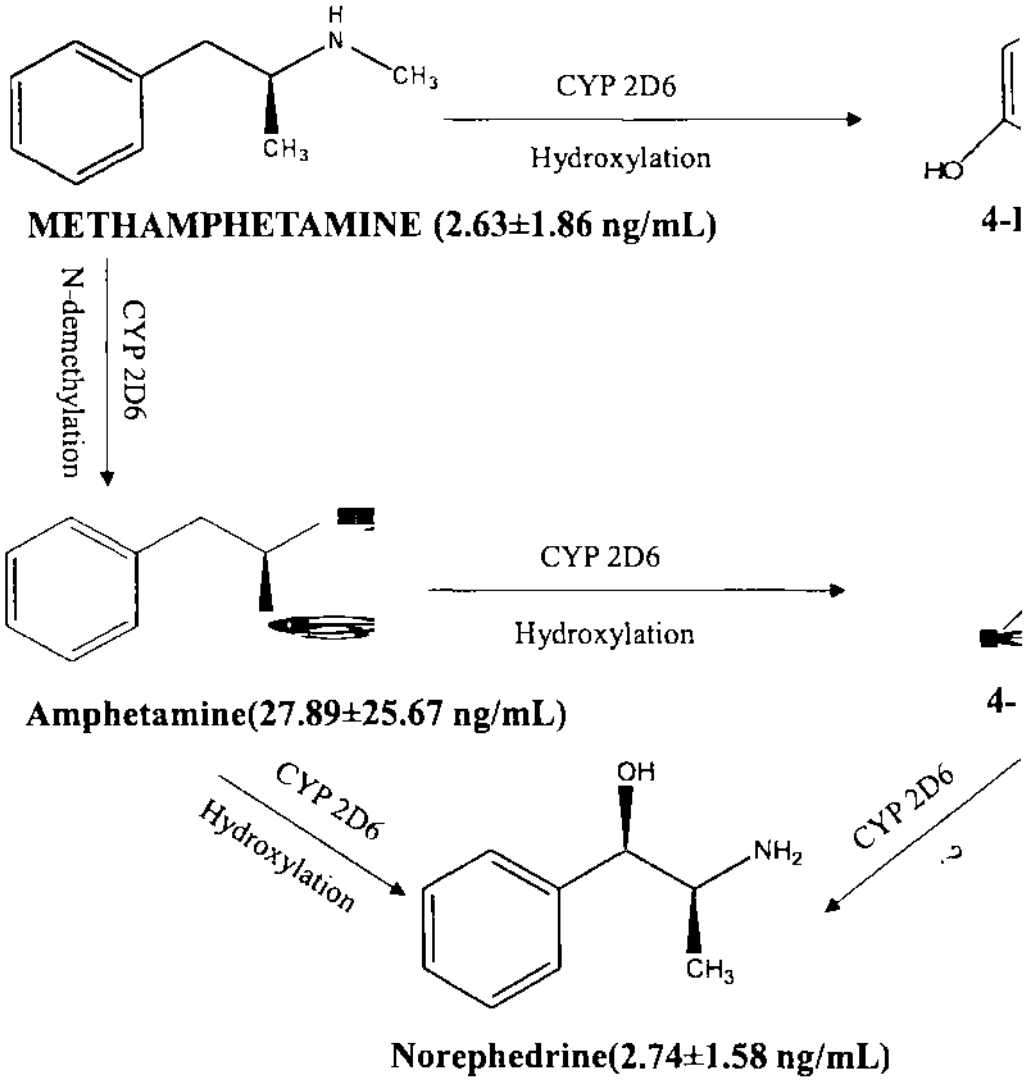


Figure-13. The mechanism of methamphetamine metabolic pathways in rhesus m

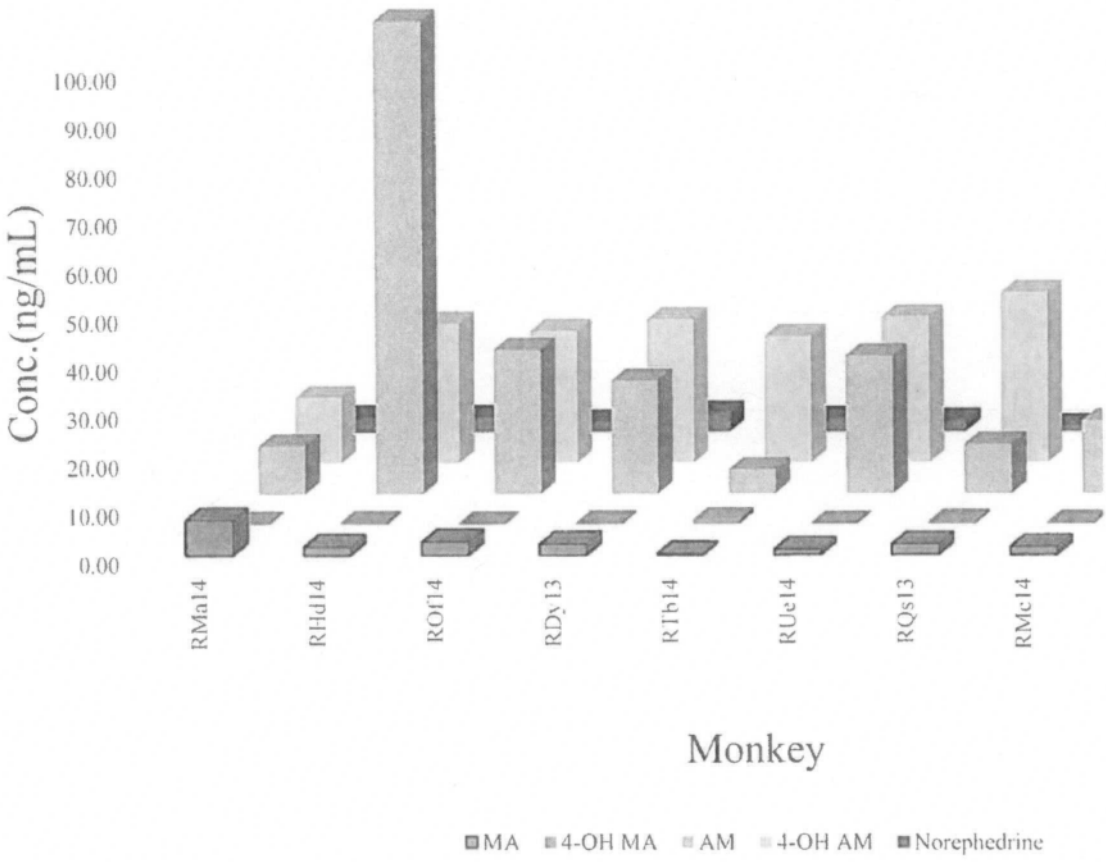


Figure -14. Methamphetamine (MA) and its metabolite concentrations were measured in r
mg/kg o.i.d. - 0.75 mg/kg b.i.d.) over a period of 20 weeks. The monkey plasma samples v
balance solid phase extraction method. The LC-MS/MS method was linear over concentra
quantitation is 1ng/mL and upper limit of quantitation is 800ng/mL for MA and its metabc

Program Director/Principal Investigator (Last, first, middle): Kumar, Anil

GRANT NUMBER
DA025011

CHECKLIST

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
04/01/2014- 03/31/2015	$0	

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III of the PHS 398, and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after the Progress Report (Form Page 5).

3. FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office.

F&A costs will ***not*** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications.

☒ DHHS Agreement dated: 07/23/2012 ☐ No Facilities and Administrative Costs Requested.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION*

Entire proposed budget period: Amount of base $ 210,414 x Rate applied 0.50 % = F&A costs $ 105,207

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

Program Director/Principal Investigator (Last, First, Middle): Kumar, Anil

ALL PERSONNEL REPORT

Place this form at the end of the signed original copy of the application. Do not duplicate.

GRANT NUMBER
DA025011

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI
- Co-Investigator
- Faculty
- Postdoctoral (scholar, fellow, or other postdoctoral position)
- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

If personnel are supported by a Reentry or Diversity Supplement please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
eRA Commons User Name	Anil Kumar	Ph.D.	Personal Info	PI		EFFORT		
	Peter S Silverstein	Ph.D.		Faculty				
	A, Nookala	B.S.		Graduate Student				
	Ankit Shah	Ph.D.		PDF				
	Austin Jackson	Ph.D.		PDF				
	V Trivedi			undergrad. student				