

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

		3. DATE RECEIVED BY STATE	State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier DA056410	
<input type="radio"/> Pre-application <input checked="" type="radio"/> Application <input type="radio"/> Changed/Corrected Application		b. Agency Routing Number	
2. DATE SUBMITTED	Application Identifier	c. Previous Grants.gov Tracking Number	
5. APPLICANT INFORMATION			UEI* : YH86RTW2YVJ4
Legal Name*: TRUSTEES OF INDIANA UNIVERSITY			
Department:			
Division:			
Street1*:	509 E 3RD ST		
Street2:			
City*:	BLOOMINGTON		
County:	MONROE		
State*:	IN: Indiana		
Province:			
Country*:	USA: UNITED STATES		
ZIP / Postal Code*:	474013654		
Person to be contacted on matters involving this application			
Prefix: Mr.	First Name*: STEVEN	Middle Name: ALLEN	Last Name*: MARTIN
Suffix:			
Position/Title:	ASSOCIATE VP FOR RESEARCH ADMINISTRATION		
Street1*:	509 E 3RD ST		
Street2:			
City*:	BLOOMINGTON		
County:	MONROE		
State*:	IN: Indiana		
Province:			
Country*:	USA: UNITED STATES		
ZIP / Postal Code*:	474013654		
Phone Number*:	317-278-3473	Fax Number:	Email: IUAWARD@IU.EDU
6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		1-356001673-A1	
7. TYPE OF APPLICANT*		H: Public/State Controlled Institution of Higher Education	
Other (Specify):			
Small Business Organization Type		<input type="radio"/> Women Owned	<input type="radio"/> Socially and Economically Disadvantaged
8. TYPE OF APPLICATION*		If Revision, mark appropriate box(es).	
<input type="radio"/> New	<input checked="" type="radio"/> Resubmission	<input type="radio"/> A. Increase Award	<input type="radio"/> B. Decrease Award
<input type="radio"/> Renewal	<input type="radio"/> Continuation	<input type="radio"/> C. Increase Duration	<input type="radio"/> D. Decrease Duration
	<input type="radio"/> Revision	<input type="radio"/> E. Other (specify):	
Is this application being submitted to other agencies?*		<input type="radio"/> Yes	<input checked="" type="radio"/> No
What other Agencies?			
9. NAME OF FEDERAL AGENCY*		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER	
National Institutes of Health		TITLE:	
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*			
Indiana University Bloomington (IUB) Center for Cannabis, Cannabinoids, and Addiction (C3A)			
12. PROPOSED PROJECT		13. CONGRESSIONAL DISTRICTS OF APPLICANT	
Start Date*	Ending Date*	IN-009	
07/01/2023	06/30/2028		

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE**14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

Prefix: First Name*: Kenneth Middle Name: P. Last Name*: Mackie Suffix: M.D.
 Position/Title: Professor
 Organization Name*: TRUSTEES OF INDIANA UNIVERSITY
 Department: PSYCHOLOGICAL & BRAIN SCIENCES
 Division: COLLEGE OF ARTS + SCIENCES
 Street1*: 702 N WALNUT GROVE AVE
 Street2:
 City*: BLOOMINGTON
 County: MONROE
 State*: IN: Indiana
 Province:
 Country*: USA: UNITED STATES
 ZIP / Postal Code*: 474052204
 Phone Number*: 812-855-2042 Fax Number: Email*: kmackie@indiana.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	\$8,106,448.00
b. Total Non-Federal Funds*	\$0.00
c. Total Federal & Non-Federal Funds*	\$8,106,448.00
d. Estimated Program Income*	\$0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

- a. YES THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
 DATE:
- b. NO PROGRAM IS NOT COVERED BY E.O. 12372; OR
 PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

I agree*

* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION

File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Mr. First Name*: STEVEN Middle Name: ALLEN Last Name*: MARTIN Suffix:
 Position/Title*: ASSOCIATE VP FOR RESEARCH ADMINISTRATION
 Organization Name*: TRUSTEES OF INDIANA UNIVERSITY
 Department:
 Division:
 Street1*: 509 E 3RD ST
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 City*: BLOOMINGTON
 County: MONROE
 State*: IN: Indiana
 Province:
 Country*: USA: UNITED STATES
 ZIP / Postal Code*: 474013654
 Phone Number*: 317-278-3473 Fax Number: Email*: IUAWARD@IU.EDU

Signature of Authorized Representative*

Mary Beth Brozo

Date Signed*

10/03/2022

20. PRE-APPLICATION File Name:**21. COVER LETTER ATTACHMENT** File Name:

424 R&R and PHS-398 Specific

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**Component
Summary**

Components	Component Project Title	Organization Name	Contact PD/PI Name or Project Lead Name
Overall	Indiana University Bloomington (IUB) Center for Cannabis, Cannabinoids, and Addiction (C3A)	TRUSTEES OF INDIANA UNIVERSITY	Mackie, Kenneth P.
Admin-Core-001 (087)	IUB C3A Administrative Core	TRUSTEES OF INDIANA UNIVERSITY	Mackie, Kenneth P.
Core-001 (086)	Multi-Scale Imaging Core (MSIC)	TRUSTEES OF INDIANA UNIVERSITY	LU, HUI-CHEN
Core-002 (106)	Bioactive Lipid Mediators Core (BLMC)	TRUSTEES OF INDIANA UNIVERSITY	Bradshaw, Heather Bryte
Core-003 (147)	IUB C3A Pilot Project Core	TRUSTEES OF INDIANA UNIVERSITY	Hajos, Norbert

**Project/Performance
Site Location(s) Summary**

Applicant Organization	City	State/Province	Country
TRUSTEES OF INDIANA UNIVERSITY	BLOOMINGTON	IN	UNITED STATES

Organization Name	City	State/Province	Country	Component
TRUSTEES OF INDIANA UNIVERSITY	BLOOMINGTON	IN	UNITED STATES	Admin-Core-001 (087)
TRUSTEES OF INDIANA UNIVERSITY	BLOOMINGTON	IN	UNITED STATES	Core-001 (086)
TRUSTEES OF INDIANA UNIVERSITY	BLOOMINGTON	IN	UNITED STATES	Core-002 (106)
TRUSTEES OF INDIANA UNIVERSITY	BLOOMINGTON	IN	UNITED STATES	Core-003 (147)
TRUSTEES OF INDIANA UNIVERSITY	BLOOMINGTON	IN	UNITED STATES	Overall

**Human Subjects
Clinical Trials
Vertebrate Animals
HESC
Human Fetal Tissue
Summary**

Component	Human Subjects	Clinical Trial / Anticipated Clinical Trial	Vertebrate Animals	HESC	Human Fetal Tissue
Overall	N	N	Y	N	N
Admin-Core-001 (087)	N	N	Y	N	N
Core-001 (086)	N	N	Y	N	N
Core-002 (106)	N	N	Y	N	N
Core-003 (147)	N	N	Y	N	N

Study Summary

Component	Study	Delayed Onset	Clinical Trial/Anticipated Clinical Trial	NIH Defined Phase III Clinical Trial

Composite Application Budget Summary

Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Salary, Wages and Fringe Benefits	621,983	634,421	646,886	659,822	673,019	3,236,131
Equipment	289,384	0	0	0	0	289,384
Travel	0	0	0	0	0	0
Participant/Trainee Support Costs	41,500	41,500	41,500	41,500	41,500	207,500
Data Management and Sharing (DMS) Costs*						0
Human Fetal Tissue (HFT) Costs*						0
Other Direct Costs (excluding Consortium)	327,941	307,241	308,591	309,941	311,141	1,564,855
Consortium Costs	0	0	0	0	0	0
Direct Costs	1,280,808	983,162	996,977	1,011,263	1,025,660	5,297,870
Indirect Costs	555,705	550,873	558,954	567,312	575,734	2,808,578
Total Direct and Indirect Costs	1,836,513	1,534,035	1,555,931	1,578,575	1,601,394	8,106,448

*HFT/DMS Costs, if present, are included in the budget summary for information only; the values have not been subtracted from "Other Direct Costs (excluding Consortium)"

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	1,280,808	983,162	996,977	1,011,263	1,025,660	5,297,870

Component Budget Summary

Components	Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Admin-Core-001 (087)	Salary, Wages and Fringe Benefits	217,038	221,379	225,583	230,095	234,696	1,128,791
	Equipment	0	0	0	0	0	0
	Travel	0	0	0	0	0	0
	Participant/Trainee Support Costs	41,500	41,500	41,500	41,500	41,500	207,500
	Data Management and Sharing Costs*						0
	Human Fetal Tissue Costs*						0
	Other Direct Costs (excluding Consortium)	61,000	61,000	61,000	61,000	61,000	305,000
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	319,538	323,879	328,083	332,595	337,196	1,641,291
	Indirect Costs	162,652	165,192	167,651	170,291	172,982	838,768
TOTALS	Total Direct and Indirect Costs	482,190	489,071	495,734	502,886	510,178	2,480,059
Core-001 (086)	Salary, Wages and Fringe Benefits	219,158	223,540	228,010	232,570	237,221	1,140,499
	Equipment	107,690	0	0	0	0	107,690
	Travel	0	0	0	0	0	0
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Data Management and Sharing Costs*						0
	Human Fetal Tissue Costs*						0
	Other Direct Costs (excluding Consortium)	115,241	115,241	115,241	115,241	115,241	576,205

	Consortium Costs	0	0	0	0	0	0
	Direct Costs	442,089	338,781	343,251	347,811	352,462	1,824,394
	Indirect Costs	195,623	198,187	200,802	203,469	206,190	1,004,271
TOTALS	Total Direct and Indirect Costs	637,712	536,968	544,053	551,280	558,652	2,828,665
Core-002 (106)	Salary, Wages and Fringe Benefits	185,787	189,502	193,293	197,157	201,102	966,841
	Equipment	181,694	0	0	0	0	181,694
	Travel	0	0	0	0	0	0
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Data Management and Sharing Costs*						0
	Human Fetal Tissue Costs*						0
	Other Direct Costs (excluding Consortium)	41,000	41,000	41,000	41,000	41,000	205,000
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	408,481	230,502	234,293	238,157	242,102	1,353,535
	Indirect Costs	132,670	134,844	137,061	139,322	141,630	685,527
TOTALS	Total Direct and Indirect Costs	541,151	365,346	371,354	377,479	383,732	2,039,062
Core-003 (147)	Salary, Wages and Fringe Benefits	0	0	0	0	0	0
	Equipment	0	0	0	0	0	0
	Travel	0	0	0	0	0	0
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Data Management and Sharing Costs*						0
	Human Fetal Tissue Costs*						0
	Other Direct Costs (excluding	110,700	90,000	91,350	92,700	93,900	478,650

	Consortium)						
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	110,700	90,000	91,350	92,700	93,900	478,650
	Indirect Costs	64,760	52,650	53,440	54,230	54,932	280,012
TOTALS	Total Direct and Indirect Costs	175,460	142,650	144,790	146,930	148,832	758,662
TOTALS		1,836,513	1,534,035	1,555,931	1,578,575	1,601,394	8,106,448

Categories Budget Summary

Categories	Components	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
R&R Budget - Senior/Key Person Funds Requested	Admin-Core-001 (087)	27,793	28,349	28,916	29,494	30,084	144,636
	Core-001 (086)	183,123	186,785	190,520	194,330	198,216	952,974
	Core-002 (106)	123,215	125,679	128,194	130,756	133,372	641,216
	Core-003 (147)	0	0	0	0	0	0
TOTALS		334,131	340,813	347,630	354,580	361,672	1,738,826
R&R Budget - Other Personnel Funds Requested	Admin-Core-001 (087)	189,245	193,030	196,667	200,601	204,612	984,155
	Core-001 (086)	36,035	36,755	37,490	38,240	39,005	187,525
	Core-002 (106)	62,572	63,823	65,099	66,401	67,730	325,625
	Core-003 (147)	0	0	0	0	0	0
TOTALS		287,852	293,608	299,256	305,242	311,347	1,497,305
R&R Budget - Section A & B. Total Salary, Wages and Fringe Benefits (A+B)	Admin-Core-001 (087)	217,038	221,379	225,583	230,095	234,696	1,128,791
	Core-001 (086)	219,158	223,540	228,010	232,570	237,221	1,140,499
	Core-002 (106)	185,787	189,502	193,293	197,157	201,102	966,841
	Core-003 (147)	0	0	0	0	0	0
TOTALS		621,983	634,421	646,886	659,822	673,019	3,236,131
R&R Budget - Section C. Total Equipment	Admin-Core-001 (087)	0	0	0	0	0	0
	Core-001 (086)	107,690	0	0	0	0	107,690
	Core-002 (106)	181,694	0	0	0	0	181,694

	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		289,384	0	0	0	0	0	289,384
R&R Budget - Domestic Travel	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Foreign Travel	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Section D. Total Travel	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Tuition/Fees/Health Insurance	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0

TOTALS		0	0	0	0	0	0	0
R&R Budget - Stipends	Admin-Core-001 (087)	36,000	36,000	36,000	36,000	36,000	36,000	180,000
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		36,000	36,000	36,000	36,000	36,000	36,000	180,000
R&R Budget - Trainee Travel	Admin-Core-001 (087)	5,500	5,500	5,500	5,500	5,500	5,500	27,500
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		5,500	5,500	5,500	5,500	5,500	5,500	27,500
R&R Budget - Subsistence	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Other Participants/Trainee Support Costs	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0

R&R Budget - Section E. Total Participants/Trainee Support Costs	Admin-Core-001 (087)	41,500	41,500	41,500	41,500	41,500	207,500
	Core-001 (086)	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0
TOTALS		41,500	41,500	41,500	41,500	41,500	207,500
R&R Budget - Materials and Supplies	Admin-Core-001 (087)	0	0	0	0	0	0
	Core-001 (086)	50,000	50,000	50,000	50,000	50,000	250,000
	Core-002 (106)	40,000	40,000	40,000	40,000	40,000	200,000
	Core-003 (147)	0	0	0	0	0	0
TOTALS		90,000	90,000	90,000	90,000	90,000	450,000
R&R Budget - Publication Costs	Admin-Core-001 (087)	5,000	5,000	5,000	5,000	5,000	25,000
	Core-001 (086)	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0
TOTALS		5,000	5,000	5,000	5,000	5,000	25,000
R&R Budget - Consultant Services	Admin-Core-001 (087)	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0
R&R Budget - ADP/Computer Services	Admin-Core-001 (087)	26,000	26,000	26,000	26,000	26,000	130,000

	Core-001 (086)	7,500	7,500	7,500	7,500	7,500	37,500
	Core-002 (106)	1,000	1,000	1,000	1,000	1,000	5,000
	Core-003 (147)	0	0	0	0	0	0
TOTALS		34,500	34,500	34,500	34,500	34,500	172,500
R&R Budget - Subawards/Consortium/Contractual Costs	Admin-Core-001 (087)	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0
R&R Budget - Equipment or Facility Rental User Fees	Admin-Core-001 (087)	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0
R&R Budget - Alterations and Renovations	Admin-Core-001 (087)	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0
R&R Budget - Other Direct Cost 1	Admin-Core-001 (087)	30,000	30,000	30,000	30,000	30,000	150,000
	Core-001 (086)	57,741	57,741	57,741	57,741	57,741	288,705

	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	36,900	30,000	30,450	30,900	31,300	159,550	
TOTALS		124,641	117,741	118,191	118,641	119,041		598,255
R&R Budget - Other Direct Cost 2	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	36,900	30,000	30,450	30,900	31,300	159,550	
TOTALS		36,900	30,000	30,450	30,900	31,300		159,550
R&R Budget - Other Direct Cost 3	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	36,900	30,000	30,450	30,900	31,300	159,550	
TOTALS		36,900	30,000	30,450	30,900	31,300		159,550
R&R Budget - Other Direct Cost 4	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Other Direct Cost 5	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0

	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Other Direct Cost 6	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Other Direct Cost 7	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Other Direct Cost 8	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Other Direct Cost 9	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0

TOTALS		0	0	0	0	0	0	0
R&R Budget - Other Direct Cost 10	Admin-Core-001 (087)	0	0	0	0	0	0	0
	Core-001 (086)	0	0	0	0	0	0	0
	Core-002 (106)	0	0	0	0	0	0	0
	Core-003 (147)	0	0	0	0	0	0	0
TOTALS		0	0	0	0	0	0	0
R&R Budget - Section F. Total Other Direct Cost	Admin-Core-001 (087)	61,000	61,000	61,000	61,000	61,000	61,000	305,000
	Core-001 (086)	115,241	115,241	115,241	115,241	115,241	115,241	576,205
	Core-002 (106)	41,000	41,000	41,000	41,000	41,000	41,000	205,000
	Core-003 (147)	110,700	90,000	91,350	92,700	93,900	93,900	478,650
TOTALS		327,941	307,241	308,591	309,941	311,141	311,141	1,564,855
R&R Budget - Section G. Total Direct Cost (A thru F)	Admin-Core-001 (087)	319,538	323,879	328,083	332,595	337,196	337,196	1,641,291
	Core-001 (086)	442,089	338,781	343,251	347,811	352,462	352,462	1,824,394
	Core-002 (106)	408,481	230,502	234,293	238,157	242,102	242,102	1,353,535
	Core-003 (147)	110,700	90,000	91,350	92,700	93,900	93,900	478,650
TOTALS		1,280,808	983,162	996,977	1,011,263	1,025,660	1,025,660	5,297,870
R&R Budget - Section H. Indirect Costs	Admin-Core-001 (087)	162,652	165,192	167,651	170,291	172,982	172,982	838,768
	Core-001 (086)	195,623	198,187	200,802	203,469	206,190	206,190	1,004,271
	Core-002 (106)	132,670	134,844	137,061	139,322	141,630	141,630	685,527
	Core-003 (147)	64,760	52,650	53,440	54,230	54,932	54,932	280,012
TOTALS		555,705	550,873	558,954	567,312	575,734	575,734	2,808,578

R&R Budget - Section I. Total Direct and Indirect Costs (G +H)	Admin-Core-001 (087)	482,190	489,071	495,734	502,886	510,178	2,480,059
	Core-001 (086)	637,712	536,968	544,053	551,280	558,652	2,828,665
	Core-002 (106)	541,151	365,346	371,354	377,479	383,732	2,039,062
	Core-003 (147)	175,460	142,650	144,790	146,930	148,832	758,662
TOTALS		1,836,513	1,534,035	1,555,931	1,578,575	1,601,394	8,106,448

**Senior/Key Personnel
Summary**

Name	Organization	Role on Project	Components
Mackie, Kenneth P.	TRUSTEES OF INDIANA UNIVERSITY	PD/PI(Contact)	Overall
Anakk, Sayeepriyadarshini	University of Illinois at Urbana-Champaign	Other: External Consultant - Key	Overall
Atwood, Brady	TRUSTEES OF INDIANA UNIVERSITY	Other: Project Lead	Core-003 (147)
Atwood, Brady	TRUSTEES OF INDIANA UNIVERSITY	Other: External Consultant - Key	Overall
Babalonis, Shanna	University of Kentucky	Other: External Consultant - Key	Overall
Barna, Laszlo	Trustees of Indiana University	Other: Core Technical Staff	Core-001 (086)
Barna, Laszlo	Trustees of Indiana University	Other: Core Technical Staff	Overall
Bradshaw, Heather Bryte	TRUSTEES OF INDIANA UNIVERSITY	Other: Core Lead	Core-002 (106)
Bradshaw, Heather Bryte	TRUSTEES OF INDIANA UNIVERSITY	Other: Project Lead	Core-003 (147)
Bradshaw, Heather Bryte	TRUSTEES OF INDIANA UNIVERSITY	Other: Core Lead	Overall
Bruchas, Michael R	UNIVERSITY OF WASHINGTON	Other: External Advisory Board	Overall
Cheer, Joseph Francois	University of Maryland	Other: External Consultant - Key	Overall
Dalkilic, Mehmet	Trustees of Indiana University	Other: Core Technical Staff	Core-002 (106)
Dalkilic, Mehmet	Trustees of Indiana University	Other: Core Technical Staff	Overall
Dani, John A.	University of Pennsylvania	Other: External Consultant - Key	Overall
Das, Aditi	University of Illinois at Urbana-Champaign	Other: External Consultant - Key	Overall
Dey, Sudhansu K	Cincinnati Children's Hospital	Other: External Consultant - Key	Overall
Dunaevsky, Anna	University of Nebraska Medical Center	Other: External Consultant - Key	Overall
DUNN, KENNETH W	INDIANA UNIVERSITY MEDICAL CENTER	Other: External Consultant - Key	Overall
Hajos, Norbert	TRUSTEES OF INDIANA UNIVERSITY	Other: Core Lead	Core-003 (147)
Hajos, Norbert	Trustees of Indiana University	Other: Core Lead	Overall
Hillard, Cecilia J	Medical College of Wisconsin	Other: External Consultant - Key	Overall
Hohmann, Andrea Grace	TRUSTEES OF INDIANA UNIVERSITY	Other: Significant Contributor - Key	Overall
Huang, Jui-Yen	Trustees of Indiana University	Other: Core Technical Staff	Core-001 (086)

<u>Huang, Jui-Yen</u>	Trustees of Indiana University	Other: Core Technical Staff	Overall
<u>HURD, YASMIN L.</u>	Icahn School of Medicine at Mount Sinai	Other: External Consultant - Key	Overall
<u>Johnson, Clare Therese</u>	Trustees of Indiana University	Other: Core Technical Staff	Core-002 (106)
<u>Johnson, Clare Therese</u>	Trustees of Indiana University	Other: Core Technical Staff	Overall
<u>Karhson, Debra Shamala</u>	University of New Orleans	Other: External Consultant - Key	Overall
<u>Katona, Istvan</u>	TRUSTEES OF INDIANA UNIVERSITY	Other: Core Co-Lead	Core-001 (086)
<u>Katona, Istvan</u>	TRUSTEES OF INDIANA UNIVERSITY	Other: Core Co-Lead	Overall
<u>Kawata, Keisuke</u>	TRUSTEES OF INDIANA UNIVERSITY	Other: Significant Contributor - Key	Overall
<u>Kepecs, Adam</u>	Washington University	Other: Significant Contributor - Key	Overall
<u>Kimbrough, Adam J</u>	Purdue University	Other: External Consultant - Key	Overall
<u>LU, HUI-CHEN</u>	Trustees of Indiana University	Other: Core Co-Lead	Core-001 (086)
<u>LU, HUI-CHEN</u>	Trustees of Indiana University	Other: Core Co-Lead	Overall
<u>Mackie, Kenneth P.</u>	TRUSTEES OF INDIANA UNIVERSITY	Other: Center Director	Admin-Core-001 (087)
<u>MCCARTHY, MARGARET M.</u>	University of Maryland	Other: External Advisory Board	Overall
<u>Mirnics, Karoly</u>	University of Nebraska Medical Center	Other: External Consultant - Key	Overall
<u>MORGAN, DANIEL J</u>	Marshall University	Other: External Consultant - Key	Overall
<u>Nah, Gabriel</u>	Trustees of Indiana University	Graduate Student	Overall
<u>Patel, Sachin</u>	NORTHWESTERN UNIVERSITY AT CHICAGO	Other: External advisory board	Overall
<u>Porreca, Frank</u>	UNIVERSITY OF ARIZONA	Other: External Consultant - Key	Overall
<u>SALVEMINI, DANIELA</u>	St. Louis University	Other: External Consultant - Key	Overall
<u>SOLTESZ, IVAN</u>	Stanford University	Other: External Advisory Board	Overall
<u>Sun, Xiaofei</u>	Cincinnati Children's Hospital	Other: External Consultant - Key	Overall
<u>VANDERAH, TODD W</u>	UNIVERSITY OF ARIZONA	Other: External Consultant - Key	Overall
<u>Wolf, Marina Elizabeth</u>	Oregon Health and Science University	Other: External Advisory Board	Overall
<u>Yasuda, Ryohei</u>	MAX PLANCK FLORIDA CORPORATION	Other: External Consultant - Key	Overall

BIOGRAPHICAL SKETCH

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

NAME: Ken Mackie

eRA COMMONS USER NAME (credential, e.g., agency login): kmackie

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Brown University, Providence, RI	Sc.B.	1976–1980	Engineering
Yale University	M.D.	1980–1984	Medicine
Rockefeller University (with Paul Greengard)	Postdoctoral	1984–1986	Molecular/Cell Biology
Yale University	Internship	1986–1987	Internal Medicine
University of Washington	Residency	1987–1990	Anesthesiology
University of Washington (with Bertil Hille)	Postdoctoral	1990–1992	Neuroscience

A. Personal Statement

Initially trained as an engineer, then as a clinician-scientist, the primary focus of my research for the past 25 years has been on identifying cannabinoid receptor signaling pathways, investigating the regulation of these pathways, understanding cannabinoid receptor function in health and disease, and identifying ways to manipulate cannabinoid receptors and endogenous cannabinoids for therapeutic benefit. Through these studies we have delineated the signaling pathways activated by CB1 and CB2 receptors, uncovered multiple roles for endogenous cannabinoids in neurodevelopment, dissected the cellular mechanisms involved in CB1 signaling in neurons during synaptic plasticity, explored the relevance of CB2 receptor functional selectivity from cell cultures to animal models of pain, defined two major pathways leading to tolerance at CB1 receptors, and characterized signaling and explored the function of atypical cannabinoid receptors such as GPR55 and GPR119. Our studies are highly collaborative and interdisciplinary (including many collaborations with Drs. Bradshaw, Hajos, Katona, and Lu, the other PIs on this P30 proposal), and we particularly enjoy working together with scientists from different institutions, laboratories, and perspectives. We employ imaging, molecular, biochemical, anatomical, and behavioral techniques, as most appropriate for the problem being studied.

Major funded projects in the lab include investigations into the effects of phytocannabinoids on the developing adolescent (in collaboration with Dr. Lu's group at IU Bloomington) and perinatal brains (in collaboration with Dr. Manzoni's group in Marseille). This work is funded by **R01 DA053746** and **R01 DA043982**, respectively. A second major project is examining the potential of CB2 agonists as therapeutics for neuropathic pain, particularly as an approach to decrease opioid use. This work involves a longstanding collaboration with Andrea Hohmann's group, also at Indiana University Bloomington and is funded by **R01 DA047858**. We also have a collaborative project with Dr. Penner's group in Honolulu looking at the biology of "minor" cannabinoids, those phytocannabinoids from cannabis, other than THC and CBD, which have some quite interesting analgesic and anti-inflammatory properties. This work is supported by **R01 AT011162**.

As I've progressed in my career, I have increasingly sought out (or they have found me) administrative roles, including Director of the Gill Center at IU Bloomington from 2008 to 2017, as we grew from two to five endowed Gill Chairs. I have enjoyed this administrative experience, seeing it as a way that I can positively influence Science both at IU Bloomington as well as across the US. Part of this satisfaction comes from the observation that while I can only do so much in my own lab, I can accomplish much more for the scientific community by recruiting accomplished neuroscientists with interests in substance use disorders to IU

Bloomington. One measure of my effectiveness in this strategy is that I have run five successful endowed chaired searches for the Gill Center and the IU Psychology Department over the last ten years. Two of these endowed chairs, Istvan Katona and Hui-Chen Lu are PIs with me on this P30 and another, Andrea Hohmann is an affiliate and a member of our internal advisory board. Other administrative work that is relevant to my qualifications to be PI on this Center proposal include: Being project PI on components of two different program project grants (**P01DA015916** and **P01DA009158**), being communicating PI on IUB's long-standing NIDA Training Grant (**T32DA024628**), serving as a member of NIDA's advisory council, and overseeing an IU Bloomington neuroscience core lab (including responsibility for its finances, upkeep, and equipment replacement). In addition, I have chaired or served on innumerable IU committees involving research, including *per diem* rate setting for our vivarium, animal user's group, Institutional Biosafety Committee, steering committee for Addictions Grand Challenge, advisor to the IU Vice President for Research, search committee for IU Bloomington Vice Provost for Research, etc. These latter duties have enabled me to establish excellent working relationships with research administration across the campus and throughout Indiana. These connections were invaluable in negotiating a 75% cost share on equipment for this P30, financial and logistical support for summer URM courses, etc. Together, I feel these activities and my experiences position me well to be PI of the P30 Center, *IU Bloomington Center for Cannabis, Cannabinoids, and Addiction*.

Returning to my academics, below are four of our more influential reviews in the cannabinoid field.

1. Howlett, A. C., F. Barth, T. I. Bonner, G. Cabral, P. Casellas, W. A. Devane, C. C. Felder, M. Herkenham, **K. Mackie**, B. R. Martin, R. Mechoulam and R. G. Pertwee (2002). *International union of Pharmacology. XXVII. Classification of cannabinoid receptors*. Pharmacological Reviews **54**(2): 161-202.
2. **Mackie, K.** (2006). Cannabinoid receptors as therapeutic targets. *Annual Review of Pharmacology and Toxicology*. Palo Alto, Annual Reviews. **46**: 101-122.
3. Lu, H.C. and **K. Mackie** (2016). *An introduction to the endogenous cannabinoid system*. Biol. Psychiatry **79**(7): 516-525. PMCID: PMC4789136.
4. Atwood, B. K. and **K. Mackie** (2010). *CB(2): A cannabinoid receptor with an identity crisis*. British Journal of Pharmacology **160**(3): 467-479. PMCID: PMC2931549.

B. Positions, Scientific Appointments, Honors, and Employment:

- 2018-present Distinguished Professor and Linda and Jack Chair of Neuroscience, Dept. of Psychological and Brain Sciences, Indiana Univ., Bloomington, IN.
- 2007–present Adjunct Professor of Anesthesiology, Indiana University School of Medicine
- 2007–2018 Professor and Linda and Jack Chair of Neuroscience, Dept. of Psychological and Brain Sciences, Indiana Univ., Bloomington, IN.
- 1992–present Adjunct Assistant (1992-1998), Associate (tenured) (1998-2001), Professor (tenured) (2001-2007), and Affiliate (2007-present) Dept of Phys. and Biophysics, Univ. WA, Seattle, WA
- 1992–2008 Assistant (1992-1998), Associate (tenured) (1998-2001), Professor (tenured) (2001-2007), and Clinical (2007-2008) Dept. of Anesthesiology, University of WA, Seattle, WA
- 1991–2007 Attending Physician, Harborview Medical Center, Seattle, WA

Other Experiences, Service, and Professional Memberships:

- 1987–present American Society of Anesthesiologists
- 1987–present Washington State Medical License: License #: 24868
- 1990–present Society for Neuroscience
- 1992–present International Cannabinoid Research Society
- 1996–present NIH grant reviews: NTRC (2000-2004), NIDA-K (2011-2013), multiple ad hoc's for NIDA-B, NIDA PPG's and Centers, DBD, LAM, NMB, MNPS, NCATS, and various SEPs
- 1999–2014 Editorial board, *British Journal of Pharmacology*
- 1999–present Ad hoc grant reviewer for ANR (France), Wellcome Trust (UK), MRC (UK), Alzheimer's Association (US), ARC (UK), GAAV (Czech Republic), and Marsden (NZ), SNF (Swiss), etc.
- 2000–2004 Member AHA Molecular Signaling I IRG
- 2008–present Indiana State Medical License: 01065462A
- 2014–present Editorial board, *Molecular Pharmacology*
- 2015–present Editorial board, *Cannabis and Cannabinoid Research*
- 2016–present Editorial board, *Neuropharmacology*

2016-2021 Member, National Advisory Council on Drug Abuse (NIDA Council)

Honors:

1981–1982	Tau Beta Pi Fellowship
1985–1986	Individual NRSA
1991–present	Diplomate, American Board of Anesthesiology, Certification #: 19544
1992–1996	Clinical Investigator Development Award (K08-NINDS)
1996	Science in Medicine, Young Investigator Lecture (Univ. of Washington)
2008	Mechoulam award for lifetime contributions to cannabinoid research
2008–2016	Member, Faculty of 1000
2014, 2018	Top 1% cited researchers (by Thompson-Reuters/Clarivate Analytics)
2015	IUB Alumni Association Distinguished Faculty Award
2016-present	Fellow AAAS
2018-present	Distinguished Professor, Indiana University
2020	Indiana University Bicentennial Medal

C. Contributions to Science

1. CB1 modulation of ion channels:

I first became interested in the neuroscience of cannabinoids as a post-doc. The CB1 receptor had just been cloned and its effects on ion channels were a mystery. I optimized several expression systems for studying ion channel modulation by CB1 receptors and showed that these receptors inhibited several types of presynaptic calcium channels and activated inwardly rectifying potassium channels. Endocannabinoids target these calcium channels to elicit short-term, endocannabinoid-suppression of synaptic plasticity, while CB1 activation of those potassium channels is involved in a cell autonomous, endocannabinoid-mediated suppression of neuronal excitability. My lab has followed up those earlier studies in a number of directions, for example, disruption of CB1 signaling by protein kinase C-mediated phosphorylation, investigating the efficacy of various CB1 splice variants to modulate synaptic transmission, and determination of the function of different CB1 receptor domains.

- a. **Mackie, K.** and B. Hille (1992). *Cannabinoids inhibit N-type calcium channels in neuroblastoma glioma-cells*. Proceedings of the National Academy of Sciences USA **89**(9): 3825-3829.
- b. **Mackie, K.**, Y. Lai, R. Westenbroek and R. Mitchell (1995). *Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat-brain cannabinoid receptor*. Journal of Neuroscience **15**(10): 6552-6561.
- c. Twitchell, W., S. Brown and **K. Mackie** (1997). *Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons*. Journal of Neurophysiology **78**(1): 43-50.
- d. Garcia, D. E., S. Brown, B. Hille and **K. Mackie** (1998). *Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor*. Journal of Neuroscience **18**(8): 2834-2841.

2. Developmental effects of cannabinoids:

Somewhat surprisingly (given their prominent role in modulation of synaptic transmission), endocannabinoids play a major role in the prenatal development of the CNS, with their expression, subcellular localization, and function all tightly and dynamically regulated. In collaborative studies with Tibor Harkany and Hui-Chen Lu, we have found roles for endocannabinoids (acting through CB1 receptors) in axonal pathfinding and elongation as well as synapse specification. More recently we have become interested in the roles of endocannabinoids in postnatal development of the nervous system, particularly during adolescence, and how social cannabis use (or therapeutic manipulation of endocannabinoids signaling/levels) might impact these processes.

- a. Murphy, M.I., S. Mills, J. Winstone, E. Leishman, J. Wager-Miller, H.B. Bradshaw and **K. Mackie** (2017). *Chronic adolescent Δ⁹-tetrahydrocannabinol treatment of male mice leads to long-term cognitive and behavioral dysfunction, which are prevented by concurrent cannabidiol treatment*. Cannabis and Cannabinoid Res. **2**(1): 235-246. PMCID: PMC5655843.
- b. Scheyer, A.F., J. Wager-Miller, A.-L. Pelissier-Alicot, M.N. Murphy, **K. Mackie***, and O.J. Manzoni* (2020). *Maternal cannabinoid exposure during lactation alters the developmental trajectory of prefrontal cortex GABA-currents in offspring*. Biol. Psych. **87**:666-677. PMCID PMC7056509.

- c. Ao, Z., H. Cai, D.J. Havert, Z. Wu, Z. Gong, J.M. Beggs, **K. Mackie**, and F. Guo (2020). *One-stop microfluidic assembly of human brain organoids to model prenatal cannabis exposure*. *Anal. Chem.* **92**:4630-8. Non-US government support.
- d. Izaque, S.M, G.H.D. de Abreu, C.T. Johnson, R. Bondy, H.B. Bradshaw, **K. Mackie**, and H.C. Lu (2021). *Perinatal CBD or THC Exposure Results in Lasting Resistance to Fluoxetine in the Forced Swim Test: Reversal by Fatty Acid Amide Hydrolase Inhibition*. *Cannabis Cannabinoid Res.* 2021. doi: 10.1089/can.2021.0015. PMCID: PMC9225394.

*shared corresponding authorship.

3. CB2 receptor signaling: CNS actions and functional selectivity

The extent of CB2 expression in the CNS and its role in CNS function is a highly controversial topic: some investigators claim universally high levels of CB2 expression, while others maintain that CB2 has a more restricted distribution and role. Our contributions to this controversy are several and include: 1. Identifying brainstem CB2 receptors; 2. Clarifying that cultured hippocampal neurons do not express functional CB2 receptors, but that when CB2 receptors are transfected into these neurons they function in a fashion similar to CB1 receptors, including supporting endocannabinoid-mediated synaptic plasticity; 3. Discovering that CB2 receptor agonists (and inverse agonists) exhibit marked functional selectivity; 4. Finding (in collaboration with Dr. Hohmann's group) that a novel class of CB2 agonists provides long-lasting analgesia in the paclitaxel chemotherapy-induced neuropathy model without the involvement of CB1 receptors.

- a. Van Sickle, M. D., M. Duncan, P. J. Kingsley, A. Mouihate, P. Urbani, **K. Mackie**, N. Stella, A. Makriyannis, D. Piomelli, J. S. Davison, L. J. Marnett, V. Di Marzo, Q. J. Pittman, K. D. Patel and K. A. Sharkey (2005). *Identification and functional characterization of brainstem cannabinoid CB2 receptors*. *Science* **310**(5746): 329-332.
- b. Atwood, B.K., J. Wager-Miller, C. Haskins, A. Straiker, and **K. Mackie**, *Functional selectivity in CB(2) cannabinoid receptor signaling and regulation: implications for the therapeutic potential of CB(2) ligands*. *Mol Pharmacol*, 2012. **81**(2): p. 250-63. PMCID: PMC3263955
- c. Deng, L., J. Guindon, B.L. Cornett, A. Makriyannis, **K. Mackie**, and A.G. Hohmann, *Chronic Cannabinoid Receptor 2 Activation Reverses Paclitaxel Neuropathy Without Tolerance or Cannabinoid Receptor 1-Dependent Withdrawal*. *Biol Psychiatry*, 2015. **77**:475-87. PMCID: PMC4209205.
- d. Lin, X., A.S. Dhopeshwarkar, M. Huibregtse, **K. Mackie**, and A.G. Hohmann, *The slowly signaling G protein-biased CB2 cannabinoid receptor agonist LY2828360 suppresses neuropathic pain with sustained efficacy and attenuates morphine tolerance and dependence*. *Mol Pharmacol*, 2017. **93**:49-62. PMCID: PMC5749492.

4. Synthetic cannabinoids, THC, functional selectivity, and efficacy of CB1 receptor agonists:

GPCR signaling is complex: Agonist efficacy and functional selectivity (see below) are important determinants of the outcome of GPCR activation. Soon after anandamide was identified as an endogenous cannabinoid, we showed that it was a low-efficacy agonist. While characterizing the effects of endogenous cannabinoids during various forms of endocannabinoid-mediated synaptic plasticity in cultured autaptic hippocampal neurons, we found that THC is also a low-efficacy agonist in this functional assay. Interestingly, THC competes with the major endocannabinoid involved in synaptic plasticity, 2-arachidonoyl glycerol (2-AG), suggesting that some of THC's actions may be caused by antagonism of 2-AG signaling. Accumulating evidence for this concept includes the inability of CB1 antagonists to completely reverse CB1-mediated psychoactivity, and the profoundly different psychoactivity produced by highly efficacious CB1 agonists. An extension of this work showed that the cannabinoids found in synthetic cannabinoid, or "spice", preparations are highly efficacious CB1 receptor agonists, which may explain their more severe adverse effect profile.

- a. **Mackie, K.**, W. A. Devane and B. Hille (1993). *Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma-cells*. *Molecular Pharmacology* **44**(3): 498-503.
- b. Straiker, A. and **K. Mackie** (2005). *Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones*. *Journal of Physiology-London* **569**(2): 501-517.
- c. Atwood, B. K., J. Huffman, A. Straiker and **K. Mackie** (2010). *JWH-018, a common constituent of 'spice' herbal blends, is a potent and efficacious cannabinoid CB(1) receptor agonist*. *British Journal of Pharmacology* **160**(3): 585-593. PMCID: PMC2931559

- d. Atwood, B. K., D. Lee, A. Straiker, T. S. Widlanski and **K. Mackie** (2011). *CP47,497-C8 and JWH073, commonly found in 'spice' herbal blends, are potent and efficacious CB(1) cannabinoid receptor agonists.* European Journal of Pharmacology **659**(2-3): 139-145. PMCID: PMC3094488

5. Cannabinoid signaling and regulation of metabolism.

Partaking of cannabis is popularly associated with overconsumption of calorically dense food (a.k.a., "the munchies"). Indeed, the motivation for the clinical development of CB1 antagonists was that blockade of CNS CB1 receptors may decrease consumption of rich, fatty foods and lead to weight loss. As usual, the actual situation was more complex, with CB1 blockade initially suppressing food intake; however, the long-lasting effects of CB1 antagonism on weight and metabolism appeared to be driven through peripheral mechanisms. I became deeply involved in cannabinoids and metabolism in 2006 while assisting Sanofi with their attempts to have rimonabant approved in the US, and presented the basic science behind this drug at the FDA advisory committee meeting in 2007. Since that time we have continued to be interested in lipid signaling and metabolism. The work we propose with THC engaging GPR119 is the logical extension of these studies.

- a. Osei-Hyiaman, D., M. DePetrillo, P. Pacher, J. Liu, S. Radaeva, S. Batkai, J. Harvey-White, **K. Mackie**, L. Offertaler, L. Wang and G. Kunos (2005). *Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity.* J. of Clinical Investigation **115**(5): 1298-1305.
- b. Nesto, R. W. and **K. Mackie** (2008). *Endocannabinoid system and its implications for obesity and cardiometabolic risk.* European Heart Journal Supplements **10**(B): B34-B41.
- c. Cristina, L. G. Busetto, R. Imperatore, I. Ferrandino, L. Palomba, C. Silvestri, S. Petrosino, P. Orlando, M. Bentivoglio, **K. Mackie**, and V. Di Marzo (2013) *Obesity-driven synaptic remodeling affects endocannabinoid control of orexinergic neurons.* Proc Natl Acad Sci U S A. **110**:E2229-38. PMCID: PMC3683753
- d. Marcus, D.J., M.L. Zee, B.J. Davis, C.P. Haskins, M.J. Andrews, R. Amin, A.N. Henderson-Redmond, **K. Mackie**, T.A. Czyzyk, and D.J. Morgan. (2016) Mice expressing a "hyper-sensitive" form of the cannabinoid receptor 1 (CB1) are neither obese nor diabetic. PLoS One 11(8):e0160462. PMCID: PMC4976987.

Complete list of publications from PubMed (~350 publications, Web of Science h-index ~97, Google h-index ~117):

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1Pau59DliAAQh/bibliography/43782383/public/?sort=date&direction=asc>

BIOGRAPHICAL SKETCH

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

NAME: Sayeepriyadarshini Anakk

POSITION TITLE: Associate Professor

eRA COMMONS USER NAME (credential, e.g., agency login): SAYEEPRIYADARSHINI_ANAKK

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
BITS Pilani, Rajasthan, India	B.Ph. (honors)	06/1999	Pharmacology
BITS Pilani, Rajasthan, India	M.Sc. (honors)	06/1999	Biological Sciences
BITS Pilani, Rajasthan, India	M.Engg. (honors)	06/1999	Biotechnology
University of Texas	Ph.D.	05/2005	Biochemistry & Mol. Biology
Baylor College of Medicine	Post-doc	12/2012	Molecular & Cell Biology

A. Personal Statement

I am an Associate Professor with a long-term interest in investigating the role of nuclear receptors in regulating glucose and fat metabolism in normal as well as the diseased state. As a graduate student in Dr. Henry Strobel's laboratory at the University of Texas Health Science Center in Houston, I studied and characterized the gender-biased regulation of cytochromes P450 (CYP450). CYP450 enzymes are central to metabolizing endogenous and xenobiotic compounds. My graduate research focused on understanding how nuclear hormone receptors regulated xenobiotic/drug metabolism. This piqued my interest to explore the endogenous function and regulation of CYP450s namely CYP7A1- the rate-limiting enzyme in bile acid synthesis. As a post-doctoral fellow in Dr. David Moore's laboratory at Baylor College of Medicine, I identified the coordinated role for two nuclear receptors, Farnesoid X Receptor (FXR) and Small Heterodimer Partner (SHP), in maintaining bile acid homeostasis. I generated the *Fxr*^{-/-}, *Shp*^{-/-} double knockout (DKO) mice, which accumulated excess bile acids resulting in severe and juvenile onset cholestasis. Importantly, I discovered that BAs activate Yes Associated Protein (YAP), the final target of Hippo Kinases and can thus produce a strong mitogenic response and that IQGAP1 is a molecular link between BAs and YAP signaling. I have been working on liver metabolism for 17 years and BA signaling for the last 12 years. I have extensive training, expertise, and generated the tools required to understand BA signaling in regulating liver proliferation metabolism and growth. As we uncover new biology of BAs with cannabinoids in collaboration with Dr. Heather Bradshaw, I look forward to being an affiliate and utilize the BLMC of the *IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction* to advance my research questions. The following are some relevant studies for this collaboration:

- a. Erickson H.L. and **Anakk S.** Identification of IQ motif-containing GTPase Activating Protein 1 as a regulator of long-term ketosis. **JCI Insight.** 2018 Nov 2;3(21). pii: 99866. doi: 10.1172/jci.insight.99866.
- b. Desai MS*, Mathur B*, Eblimit Z, Vasquez H, Taegtmeier H, Karpen SJ, Penny DJ, Moore DD, **Anakk S.** Bile acid excess induces cardiomyopathy and metabolic dysfunctions in the heart.

Hepatology. 2017 Jan;65(1):189-201. doi: 10.1002/hep.28890. Epub 2016 Nov 29. *denotes equal contribution. PMCID: PMC5299964

- c. **Anakk S***, Bhosale M, Schmidt V, Johnson RL, Finegold MJ and Moore DD* (2013). Bile acids activate Yes Associated Protein (YAP) to promote liver carcinogenesis. **Cell Reports.** 5(4): 1060-9. *Corresponding authors. PMCID: PMC3961013
- d. **Anakk S**, Watanabe M, Ochsner SA, McKenna NJ, Finegold MJ and Moore DD (2011). Combined deletion of FXR and SHP results in juvenile onset cholestasis and induction of Cyp17A1. **Journal of Clinical Investigation.** 121(1): 86-95. PMCID: PMC3007143

Ongoing and Recent Research Support

- R01 DK113080 Anakk, S (PI) 07/01/2017 to 06/30/2022
Title: Understanding the mechanism(s) that regulate liver growth and function
The liver is constantly subjected to damage while metabolizing and detoxifying foreign compounds. This project is designed to understand the mechanism by which CAR, a xenobiotic sensor, coordinates hepatocyte proliferation with metabolic function.
- R01 DK113080 - Supplement Anakk, S (PI) 08/01/2019 to 05/31/2022 ACS132640-
RSG-18-230-01-TBE Anakk, S (PI) 01/01/2019 to 12/31/2022
Title: Elucidating mechanisms underlying gender-biased incidence of liver cancer
Hepatocellular carcinoma (HCC) is a male-predominant cancer, with 2.5 males diagnosed per female. Although multiple pathways are implicated, the mechanism underlying female protection is poorly understood. In this proposal we will examine the contributions of bile acids (BAs), gut microbial BA metabolites and estrogen signaling in protecting females from developing HCC.
- R01 DK130317 Anakk, S (PI) and Cecilia Leal (PI) 08/01/2021 to 07/30/2024
Title: "Biophysical and genetic cues regulating lipid droplet packaging and alterations in obesity" Fat is packed and stored in the adipose tissues as lipid droplets (LDs). The two major goals of this study are- (I) to characterize the biophysical and structural properties of the LDs and (II) to define the role for bile acids and genetic control of LD expansion during obesity.

B. Positions and Honors

Positions:

- 2000-05 Graduate student, University of Texas Health Science Center, Houston, TX
2006-12 Post-doc, Molecular & Cellular Biology, Baylor College of Medicine, Houston, TX
2013- Assistant Professor, Dept. of Molecular & Integrative Physiology, UIUC, IL
2013- Affiliate, Beckman Institute for advanced science and technology, UIUC, IL
2015- Assistant Professor, Div. of Nutritional Sciences, UIUC, IL

Honors:

- 2016 Outstanding Advisor Award, Medical Scholars Program, UIUC, IL
2017 David L Williams Runner up Award Kern Lipid Conference, Vail, Colorado

C. Contribution to Science

- 1. FXR and SHP coordinate bile acid levels and Excess bile acids drive YAP activation.** The realization that synthesis of bile acids is not merely a route for cholesterol clearance, and that they also act as key signaling molecules piqued my interest in studying their function in normal physiology and

disease (Kerr et al. 2013). Further, nuclear receptors, FXR and SHP, act as sensors of these cholesterol metabolites by repressing their excessive synthesis through a feedback mechanism.

I generated *Fxr*^{-/-}, *Shp*^{-/-} double knockout (DKO) mice and showed that they accumulate toxic levels of serum and hepatic bile acids due to their inability to control bile acid synthesis, circulation and transport (Anakk et al. 2011, (d)). Our results defined a coordinated fail-safe function for these two nuclear receptors in maintaining biliary homeostasis. While characterizing the hepatic tumors in *Fxr*^{-/-}, *Shp*^{-/-} DKO mice (Jiang et al., 2013), I identified an unanticipated mechanism for bile acid regulation of liver growth and tumorigenesis via the organ-size controlling Hippo signaling pathway (Anakk et al. 2012, 2013). I discovered that the DKO mice have increased liver size, hepatocyte proliferation and development of spontaneous HCC, which strongly resembled the phenotype of mammalian Hippo pathway Mst1/2 liver-specific knockouts (Anakk et al. 2012). Consistent with this overlap, I demonstrated that elevated bile acid levels are sufficient to activate Yes Associated Protein (YAP), the downstream effector of the Hippo signaling pathway (Anakk et al. 2013) via a cytoskeletal scaffolding protein IQ containing GTPase Activating Protein 1 (IQGAP1) (Anakk et al. 2013). We are currently focusing on dissecting the specific role(s) of IQGAP1 in integrating metabolic cues with signaling pathways and recently found a role for IQGAP1 in regulating ketogenesis (Erickson et al., 2018). We discovered that bile acids alter heart function and are one of the causal factors for heart failure in liver diseases (Desai, Mathur et al., 2017).

- e. Erickson H.L. and **Anakk S.** Identification of IQ motif-containing GTPase Activating Protein 1 as a regulator of long-term ketosis. **JCI Insight**. 2018 Nov 2;3(21). pii: 99866. doi:10.1172/jci.insight.99866. PMCID: PMC6238733
- f. Desai MS*, Mathur B*, Eblimit Z, Vasquez H, Taegtmeier H, Karpen SJ, Penny DJ, Moore DD, **Anakk S.** Bile acid excess induces cardiomyopathy and metabolic dysfunctions in the heart. **Hepatology**. 2017 Jan;65(1):189-201. doi: 10.1002/hep.28890. Epub 2016 Nov 29. *denotes equal contribution. PMCID: PMC5299964
- g. **Anakk S***, Bhosale M, Schmidt V, Johnson RL, Finegold MJ and Moore DD* (2013). Bile acids activate Yes Associated Protein (YAP) to promote liver carcinogenesis. **Cell Reports**. 5(4): 10609. *Corresponding authors. PMCID: PMC3961013

2. Role for FXR and SHP in Regulating Fat Metabolism

Bile acids are typically known for their ability to emulsify and help digest fat. Since FXR and SHP play a crucial role in maintaining bile acid homeostasis, we examined if the *Fxr*^{-/-}, *Shp*^{-/-} double knockout (DKO) mice exhibited differential response to high fat diet feeding. Excitingly, we found that the DKO mice did not gain weight despite similar food intake when challenged with high fat diet. In fact this was reflected in excellent glucose control and reduced accumulation of fat in the DKO mice. Our previous findings from *Shp*^{-/-} mice reveal that *Shp* loss protects against weight gain but predisposes to diabetes (Park, YJ et al., 2011). Additionally, we also identified that *Shp* is important to mediate cholesterollowering effect in response to vitamin D (Chow, EC et al., 2014). It is known that chronic FXR activation by its synthetic ligand GW4064 can worsen metabolic syndrome whereas in short term FXR agonism can be beneficial. Additionally, whole body *Fxr*^{-/-} mice exhibit poor glucose control unlike the DKO mice.

We identified a key role for hepatic *Shp* in regulating fatty liver (Akinrotimi, O et al., 2017).

- a. Akinrotimi O*, Riessen R*, VanDuyne P, Park JE, Lee YK, Wong LJ, Zavacki AM, Schoonjans K and **Anakk S.** *Shp* deletion prevents hepatic steatosis and when combined with *Fxr* loss protects against type 2 diabetes. **Hepatology** 2017 Jun 6. doi: 10.1002/hep.29305. *denotes equal contribution. PMCID: PMC5696047.

- b. Kim KH, Choi S, Zhou Y, Kim EY, Lee JM, Saha PK, **Anakk S**, Moore DD. Hepatic FXR/SHP axis modulates systemic glucose and fatty acid homeostasis in aged mice. **Hepatology**. 2017 Aug;66(2):498-509. doi: 10.1002/hep.29199. Epub 2017 Jun 26. PMID: 28378930
- c. Park YJ, Kim SC, Kim J, **Anakk S**, Lee JM, Tseng HT, Yechoor V, Park J, Choi JS, Jang HC, Lee KU, Novak CM, Moore DD, Lee YK (2011). Dissociation of diabetes and obesity in mice lacking orphan nuclear receptor small heterodimer partner. **J Lipid Res.** 52(12):2234-44. PMCID: PMC3220290.
- d. Chow EC, Magomedova L, Quach HP, Patel R, Durk MR, Maeng HJ, Irondi K, **Anakk S**, Moore DD, Cummins CL and Pang S (2014). Vitamin D receptor activation down regulates the small heterodimer partner and increases CYP7A1 to lower cholesterol. **Gasteroenterology**. 146(4): 1048-59. PMCID: PMC24365583

3. Molecular mechanisms controlling gender-specific drug metabolism

Cytochrome P450 3As (CYP3As) are broad spectrum phase I enzymes responsible for metabolizing more than 50% of clinical drugs. Expression of CYP3As is about two-fold higher in women than in men leading to a faster clearance of many therapeutic drugs in women. For my doctoral work, I studied the mechanisms underlying differential expression of CYP3As in males versus females. I systematically analyzed the hepatic and renal expression of individual CYP3A isozymes in mice and rats (Anakk et al. 2003a; 2007); defined the genomic structures, expression patterns and biological activities of three novel murine CYP3A isoforms CYP3A13, CYP3A41 and CYP3A44 (Anakk et al. 2003, 2004); demonstrated the role of estrogen signaling in directing female-specific CYP3A expression in pregnancy; and determined the role of four separate nuclear receptors - Estrogen Receptor (ER), Glucocorticoid Receptor (GR), Pregnan X Receptor (PXR), and Constitutive Androstane Receptor (CAR) in the gender-biased regulation of CYP3As under basal and induced settings (Anakk et al. 2004; 2007). This work resulted in four first author and four co-author publications and was awarded the "Best Thesis" prize in biology by the Sigma Xi Rice University-Texas Medical Center Chapter.

- a. **Anakk S**, Huang W, Staudinger JL, Tan K, Cole TJ, Moore DD and Strobel HW. Gender dictates the nuclear receptor-mediated regulation of CYP3A44. **Drug Metab Disp** 2007 Jan;35(1):36-42. PMID: 17020958, doi:10.1124/dmd.106.011270.
- b. **Anakk S**, Kalsotra A, Kikuta Y, Huang W, Zhang J, Staudinger JL, Moore DD, and Strobel HW. CAR/PXR provide directives for CYP3A41 gene regulation differently from CYP3A11. **Pharmacogenomics J.** 2004 ;4(2):91-101. PMID: 14770174, doi:10.1038/sj.tpj.6500222
- c. **Anakk S**, Ku C, Davies PJ and Strobel HW. Insights into gender bias: Role of CYP3A9. **J Pharmacol Exp Ther.** 2003a May. 305(2):703-9. PMID: 12606633, doi: 10.1124/jpet.102.048090

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1rujgfsgfjb5p/bibliography/45458082/public/?sort=date&direction=ascending>

BIOGRAPHICAL SKETCH

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

NAME: Atwood, Brady Kenneth

eRA COMMONS USER NAME (credential, e.g., agency login): ATWOODB

POSITION TITLE: Assistant Professor

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Brigham Young University, Provo, UT	B.S.	05/2005	Neuroscience/Molecular Biology
University of Washington, Seattle, WA (degree-granting institution); Indiana University, Bloomington, IN	Ph.D.	12/2010	Neurobiology & Behavior
National Institute on Alcohol Abuse and Alcoholism, NIH, Rockville, MD	Postdoctoral	12/2015	Synaptic Pharmacology & Behavior

A. Personal Statement

I am an Associate Professor of Pharmacology & Toxicology at Indiana University School of Medicine (IUSM). I have been working in the synaptic transmission field for over 19 years with my primary focus for much of that time on the impact that drugs of abuse have on synaptic plasticity. I am particularly skilled in brain slice electrophysiology, optogenetic manipulations of neural circuits, the use of transgenic mice, analyses of drug-related behaviors in mouse models, and the study of G protein coupled receptor signaling mechanisms and synaptic plasticity (e.g. Munoz et al., *Nature Communications* 2018). My laboratory employs a multidisciplinary approach to assess the role of opioid and cannabinoid receptors at specific synapses in the brain in modulating synaptic transmission and behaviors that are relevant to drug abuse and addiction as well as how addictive drugs selectively affect synaptic plasticity in the brain (e.g. Reeves et al. *Addiction Biology* 2020; Haggerty et al., *eLife* 2022). I am also interested in large scale proteomic assessments of brain circuitry associated with alcohol and opioid addiction/misuse how prenatal opioid exposure affects development.

In 2018 I formed a collaborative group of nine faculty members and their laboratories for a “Grand Challenge” project at IUSM. We were awarded a highly competitive 2-year grant to develop an animal model of prenatal opioid exposure and to begin to determine some of its long-term effects on physical and neurodevelopment and on brain function (*Consequences of Opioid Neonatal Abstinence Syndrome*, Indiana University, \$581,324). This was a large undertaking to accomplish in a very short time. I led this team of investigators (including Drs. Hui-Chen Lu and Edna Huang, co-PI and core leader, respectively of this P30 proposal) to successfully develop a mouse model of prenatal methadone exposure. Our model has the advantage of exposing offspring to methadone from conception through to weaning and achieves clinically relevant levels of methadone exposure to the fetus (often not even measured in most models). It also produces neonatal opioid withdrawal behaviors. We published this model, showing that prenatal methadone exposure impaired physical development and neurodevelopment (Grecco et al., *eLife* 2021). We used a multidisciplinary approach: behavioral assessments, microscopy, electrophysiology, magnetic resonance imaging, and even bone structure assessments. We plan to use the P30’s multiscale imaging core to perform cortical 2P imaging in these mice as our preliminary results suggest that perinatal opioid treatment perturbs the development of cortical networks.

Funding of particular relevance

R01 AA027214-03 (Atwood)

NIH/NIAAA

09/20/18—08/31/23

Synapse-specific interactions between ethanol and opioid receptor-mediated synaptic depression in dorsal striatum

This study determines the mechanisms whereby alcohol selectively alters opioid receptor-mediated plasticity at specific corticostriatal synapses and the behavioral relevance of those forms of plasticity.

Role: PI

IU Grand Challenges: Solving the Addictions Crisis (Atwood, Yamamoto)

11/1/18—6/30/20

Consequences of Opioid Neonatal Abstinence Syndrome

This is a multi-investigator project to develop a mouse model of in utero opioid exposure to determine the long-term behavioral, physiological, biochemical, and genetic consequences of that exposure.

Role: Co-PI

R00 AA023507-05 (Atwood) NIH/NIAAA

04/15/15—03/31/19

Dorsal striatal mu opioid receptor function and alcohol use

The goal of this study is to determine the effects of alcohol on mu opioid receptor-mediated synaptic modulation of excitatory and dopaminergic synapses in the dorsal striatum and the role of dorsal striatal mu opioid receptors in goal-directed and habitual alcohol consumption.

Role: PI

Publications of particular relevance

- a. Grecco, G.G., Mork, B., Huang, J.-Y., Metzger, C.E., Haggerty, D.L., Reeves, K.C., Gao, Y., Hoffman, H., Katner, S.N., Masters, A.R., Morris, C.W., Newell, E.A., Engleman, E.A., Baucum, II A.J., Kim, J., Yamamoto, B.K., Allen, M.R., Wu, Y.-C., Lu, H.-C., Sheets, P.L., **Atwood, B.K.** (2021). Prenatal Methadone Exposure Disrupts Behavioral Development and Alters Motor Neuron Intrinsic Properties and Local Circuitry. *eLife*. 10:e66230 (2021). PMCID: PMC7993998.
- b. Munoz, B., Fritz, B.M., Yin, F., **Atwood, B.K.** Alcohol exposure disrupts mu opioid receptor-mediated long-term depression at insular cortex inputs to dorsolateral striatum. *Nature Communications*. 9(1):1318 (2018). PMCID: PMC5882774.
- c. Haggerty, D.L., Munoz, B.M., Pennington, T., Grecco, G.G., **Atwood, B.K.** Anterior insular inputs to the dorsolateral striatum control binge drinking. *eLife* (2022). In press.
- d. Reeves, K.C., Kube, M.J., Grecco, G.G., Fritz, B.M., Muñoz, B., Yin, F., Gao, Y., Haggerty, D.L., Hoffman, H.J., **Atwood, B.K.** Mu opioid receptors on vGluT2-expressing glutamatergic neurons modulate opioid reward. *Addiction Biology*. Jul 20:e12942 (2020). PMCID: 7854952.

B. Positions, Scientific Appointments, and Honors

2022-Present	Associate Professor, tenure pending (effective 2023), Department of Pharmacology & Toxicology, IUSM, Indianapolis, IN
2019-Present	Director, Stark Neurosciences Research Institute Electrophysiology Core
2016-Present	Investigator, Indiana Alcohol Research Center, IUSM, Indianapolis, IN
2016-Present	Investigator, Stark Neurosciences Research Institute, IUSM, Indianapolis, IN
2016-Present	Investigator, Center for Diabetes and Metabolic Diseases, IUSM, Indianapolis, IN

Other Experience and Professional Memberships

2021-present	Editorial Board, Section on Neuropharmacology for Frontiers journals.
2019-present	Editorial Board, Frontiers in Synaptic Neuroscience
2015-present	Member, Research Society on Alcoholism
2012-present	Member, International Narcotics Research Conference
2008-present	Member, Society for Neuroscience

Honors and Awards

2021	IUSM's nominee for National Academy of Medicine Emerging Leaders in Health and Medicine
2015	NIH K99 Pathway to Independence Award
2015	International Narcotics Research Conference Travel Award
2013	Gordon Research Conference Travel Award (Cannabinoid Function in the CNS)
2013	International Narcotics Research Conference Travel Award
2012	International Narcotics Research Conference Travel Award

2011	Gordon Research Conference Travel Award (Cannabinoid Function in the CNS)
2009	NIDA Frontiers in Addiction Research Mini-convention Travel Award
2005	Office of Research and Creative Activities Award - Brigham Young University
2005	Pacific Cascades Chapter of Society for Neuroscience Travel Award
1999	Heritage Scholar - Brigham Young University

C. Contributions to Science

1. Long-term depression (LTD) is a form of synaptic plasticity that weakens neurotransmission between neurons and may underlie multiple components of learning and memory. As a postdoctoral fellow, I identified several novel forms of LTD in the dorsal striatum, a brain region critical for goal-directed and habitual action selection. These forms of LTD are mediated by the mu, delta and kappa opioid receptors. They are differentially expressed at distinct synaptic sites, as revealed using electrophysiological, pharmacological, and optogenetic tools. I also discovered that locally released endogenous opioid peptides could induce opioid receptor LTD and that opioid peptide release does not require intense stimulation as previously believed. I discovered that a single *in vivo* exposure to the opiate analgesic, oxycodone, could disrupt mu opioid receptor LTD for up to 3 days without impacting delta or kappa opioid receptor LTD. Oxycodone had a similar effect on CB1 cannabinoid receptor-mediated LTD, which supports my finding that mu opioid receptor LTD and CB1 receptor-mediated LTD intersect with one another. I performed additional work in collaboration with other members of the laboratory utilizing optogenetic and transgenic mouse tools to determine the role of CB1R LTD at specific synapses in the dorsal striatum and nucleus accumbens. Current work demonstrates that alcohol selectively affects mu opioid LTD exclusively at insular cortex inputs to dorsal striatum sparing opioid plasticity at other synapses. A high-fat, high-sugar western diet may have a similar effect on dorsal striatal circuits. My work lays the groundwork for understanding the role of opioid and cannabinoid receptors in determining the activity of the dorsal striatum and therefore potentially action selection behaviors. Because the functionality of these receptors is altered by drugs of abuse and highly palatable diets, these findings may open the door to better utilization of pharmacological tools in treating drug abuse and obesity, as well as other diseases or conditions that involve the dorsal striatum. Current work in my own laboratory continues to pursue these objectives and we are identifying novel circuits that are selectively impacted by drugs of abuse and diet.

- a. **Atwood, B.K.**, Kupferschmidt, D.A., Lovinger, D.M. Opioids induce dissociable forms of long-term depression of excitatory inputs to the dorsal striatum. *Nature Neuroscience*. 17(4): 540-548 (2014). PMCID: PMC4163916
- b. Gremel, C., Chancey, J., **Atwood, B.K.**, Luo, G., Neve, R., Ramakrishnan, C., Deisseroth, K., Lovinger, D.M., Costa, R. Endocannabinoid modulation of orbitostriatal circuits gates habit formation. *Neuron*. 90(6):1312-1324 (2016). PMCID: PMC4911264.
- c. Mateo, Y., Johnson, K., Covery, D.P., **Atwood, B.K.**, Wang, H.-L., Zhang, S., Gildish, I., Cachope, R., Bellocchio, L., Guzman, M., Morales, M., Cheer, J., Lovinger, D. M. Endocannabinoid Actions on Cortical Terminals Orchestrate Local Modulation of Dopamine Release in the Nucleus Accumbens. *Neuron*, 96(5):1112-1126 (2017). PMCID: PMC5728656
- d. Fritz, B.M., Munoz, B., Yin, F., Bauchle, C., **Atwood, B.K.** A high-fat, high-sugar 'western' diet alters dorsal striatal glutamate, opioid, and dopamine transmission in mice. *Neuroscience*. 372:1-15 (2018). PMCID: PMCPMC5809281.

2. As the opioid epidemic continues onward, there has been a dramatic increase in the incidence of babies born that undergo neonatal opioid withdrawal syndrome (NOWS, also known as neonatal abstinence syndrome) as a consequence of prenatal opioid exposure (POE). Many women with opioid use disorder are being treated with opioid maintenance therapies such as methadone and buprenorphine. Current clinical practice discourages opioid abstinence during pregnancy for women with opioid use disorder. Thus, there will continue to be many children born with POE. Little is known about the brain mechanisms that underlie the negative neurodevelopmental outcomes for children born with POE. My laboratory developed one of the most translationally relevant mouse models of prenatal methadone exposure (PME) in order to elucidate these mechanisms. Our model recapitulates many outcomes that are reminiscent of human NOWS. Offspring are exposed to high levels of methadone throughout the entire duration of pregnancy, experience opioid withdrawal effects upon birth, and show significant sensorimotor behavioral developmental delays. Mechanistically we find that local microcircuits within the primary motor cortex are altered by PME, which may underlie the disrupted motor development of PME offspring. We also found that adolescent males with PME drink more alcohol than controls and female PME offspring show greater locomotor behavioral responses to alcohol exposure. Given

opioids alter gut motility, and the gut microbiome can affect neurological function we also explored the composition of the microbiome of opioid-dependent mothers and their PME offspring. We found that opioid exposure produced an expansion in the diversity of the gut microbiome in both mother and offspring that were correlated with one another, possibly due to vertical transfer.

- a. Grecco, G.G., Gao, Y., Gao, H., Liu, Y., **Atwood, B.K.** Prenatal Opioid Administration Induces Shared Alterations to the Maternal and Offspring Gut Microbiome. *Drug and Alcohol Dependence*. 227:108914 (2021). PMCID: PMC8464518
- b. Grecco, G.G., Haggerty, D.L., Reeves, K.C., Gao, Y., Maulucci, D., **Atwood, B.K.** Prenatal Opioid Exposure Repograms the Behavioral Response to Future Alcohol Reward. *Addiction Biology*. 27(2):e13136 (2022). PMCID: PMC8896285
- c. Grecco, G.G., Munoz, B., Viana Di Prisco, G., Doud, E.H., Fritz, B.M., Maulucci, D., Gao, Y., Moseley, A.L., Baucum, A.J., **Atwood, B.K.** Prenatal opioid exposure impairs endocannabinoid and glutamate transmission in the dorsal striatum. *eNeuro*. ENEURO.0119-22.2022 (2022). PMCID: PMC9034757
- d. Grecco, G.G., Huang, J.Y., Munoz, B., Doud, E.H., Hines, C.D., Gao, Y., Rodriguez, B., Moseley, A.L., Lu, H.-C., **Atwood, B.K.** Sex-Dependent Synaptic Remodeling of the Somatosensory Cortex in Mice With Prenatal Methadone Exposure. *Advances in Drug and Alcohol Research*. (2022) In press.

3. As a graduate student I studied cannabinoid receptor pharmacology. The CB2 receptor is predominantly localized in immune cells, but it also has some expression in neurons. I found, contrary to the prevailing dogma at the time, that CB2 receptors do in fact couple to ion channels, but do so in a ligand-dependent manner. This led to a further study of functional selectivity of CB2 ligands. I found that specific classes of cannabinoids direct the CB2 receptor to signal through different pathways, thereby producing distinct consequences for cellular function. Neuronal CB2 receptors can inhibit synaptic transmission, which might account for some of the effects of CB2 ligands on behavior. This work may provide the background for targeted therapeutic research in treating pain and drug abuse, along with other diseases. I was also one of the first to explore the ability of cannabinoid compounds found in blends of the synthetic marijuana drug "Spice" to modulate neurotransmission and neuronal function. JWH018, JWH073, and CP47,497-C8, were all CB1 receptor agonists that inhibited synaptic transmission and activated other CB1 signaling pathways. These ligands are very potent and efficacious CB1 agonists, much more than delta-9-THC, the primary psychoactive compound found in marijuana. The presence of these highly efficacious CB1 agonists in Spice may account for the differing physiological responses to Spice than those responses typically produced by marijuana.

- a. **Atwood, B.K.**, Wager-Miller, J., Haskins, C., Straiker, A, Mackie, K. Functional selectivity in CB(2) cannabinoid receptor signaling and regulation: implications for the therapeutic potential of CB(2) ligands. *Molecular Pharmacology*. 81(2): 250-263 (2012). PMCID: PMC3263955.
- b. **Atwood, B.K.**, Straiker A., Mackie, K. CB(2) cannabinoid receptors inhibit synaptic transmission when expressed in cultured autaptic neurons. *Neuropharmacology*. 63(4): 514-523 (2012). PMCID: PMC3263955.
- c. **Atwood, B.K.**, Lee, D., Straiker, A., Widlanski, T.S., Mackie, K. CP47,497-C8 and JWH073, commonly found in 'Spice' herbal blends, are potent and efficacious CB(1) cannabinoid receptor agonists. *European Journal of Pharmacology*. 659(2-3): 139-145 (2011). PMID: 21333643
- d. **Atwood, B.K.**, Huffman, J., Straiker, A., Mackie, K. JWH018, a common constituent of 'Spice' herbal blends, is a potent and efficacious cannabinoid CB receptor agonist. *British Journal of Pharmacology*. 160(3): 585-593 (2010). PMID: 20100276

4. The risk for developing an Alcohol Use Disorder (AUD) is highly impacted by genetics, with a family history of AUDs greatly increasing that risk. Multiple animal models of family history of alcoholism have been developed, largely based on selectively breeding rats and mice to create lines that are either high alcohol preferring (HAP) or low alcohol preferring (LAP). As part of work performed with the Indiana Alcohol Research Center, we explored how the function of the dorsal striatum was impacted by this genetic selection process. We found that dorsal striatal medium spiny neurons were more excitable in HAP mice than in LAP mice and that GABA and glutamate release were upregulated in HAP mice. These data relate to the behavioral phenotypes of hyperactivity, impulsivity, and other behaviors associated with the increased alcohol preference that have been measured in these mice. In order to determine potential molecular mechanisms underlying these electrophysiological differences, we performed a multi-omics analysis of the dorsal striatum in these mice: transcriptomics (RNAseq), and global and phospho-proteomics. This was the first -omics analyses of these mice to be performed. We found that thousands of genes and hundreds of proteins and phosphorylation states differed between lines and these differences were largely related to synaptic function, metabolism, and cellular structure. This study will be useful

for anyone that seeks to correlate behavioral, genetic, and neuron function measures in order to determine mechanisms that underlie the increased risk for AUDs.

- a. Fritz, B.M., Muñoz, B., **Atwood, B.K.** Genetic Selection for Alcohol Preference in Mice Alters Dorsal Striatum Neurotransmission. *Alcoholism: Clinical and Experimental Research*. 43 (11):2312-2321 (2019). PMCID: PMC6824951.
 - b. Grecco GG, Haggerty DL, Doud E, Fritz BM, Hoffman H, Mosely A, Simpson E, Liu Y, Baucum AJ, **Atwood BK**. A Multi-Omic Analysis of the Dorsal Striatum in an Animal Model of Divergent Genetic Risk for Alcohol Use Disorder. *J. Neurochem.* (2020). 10.1111/jnc.15226. PMID: 33111353
5. Indiana University School of Medicine is home to many researchers that study Alzheimer's Disease (AD) and its related pathologies as well as multiple NIH-funded AD-related Centers, including the Indiana Alzheimer Disease, MODEL-AD, and Alzheimer's Disease Drug Discovery Centers. I have begun to contribute my expertise in electrophysiology to enhance many studies performed by AD researchers and members of these Centers. Thus far, I have helped to determine that hippocampal LTP is disrupted by an accumulation of a specific form of amyloid (Danish amyloid) implicated in a certain form of cerebral amyloid angiopathy, but that this amyloid protein fails to alter plasticity in the absence of tau, suggesting that amyloid itself does not alter plasticity, but does so through tau. We also discovered that a loss of Trem2, a microglial protein that is implicated in AD risk, produces a reduction in hippocampal glutamate transmission which provides functional validation of altered synapse structures. I continue to work with many of these AD researchers providing electrophysiological support.
- a. You, Y., Perkins, A., Cisternas, P., Muñoz, B., Taylor, X., You, Y., Garringer, H.J., Oblak, A.L, Ghetti, B., **Atwood, B.K.**, Vidal, R., Lasagna-Reeves, C. Tau as a mediator of neurotoxicity associated to Cerebral Amyloid Angiopathy. *Acta Neuropathologica Communications*. 7(1):26 (2019). PMCID: PMC6390363
 - b. Jay, T., von Saucken, V.E., Muñoz, B., Codocedo, J.F., **Atwood, B.K.**, Lamb, B.T., Landreth, G.E. The neurodegeneration-associated receptor TREM2 is required for microglial instruction of astrocytic synaptic engulfment in neurodevelopment. *Glia*. 67(10):1873-1892 (2019). PMCID: PMC7576303.
 - c. Jadhav, V.S., Lin, P.B., Xu, G., Pennington, T., Viana Di Prisco, G., Jacob, A., Moutinho, M., Puntambekar, S.S., Zhang, J., **Atwood, B.K.**, Bissel, S.J., Oblak, A.L., Landreth, G.E., Lamb, B.T. Trem2 Y38C mutation and loss of Trem2 impairs neuronal synapses with age. *Molecular Neurodegeneration*. 15(1):62 (2020). PMCID: PMC7594478.
 - d. Karahan H, Smith D, Kim B, El-Amin MM, Dabin L, Pennington T, Viana di Prisco G, McCord B, Lin P, **Atwood BK**, Oblak A, Kim J. Deletion of Abi3 gene exacerbates neuropathological features of Alzheimer's disease in a mouse model of A β amyloidosis. *Science Advances*. 7(45):eabe3954 (2021). PMCID: PMC8565913

Complete List of Published Works: [https://www.ncbi.nlm.nih.gov/myncbi/1FOJ6Ac9Hx-
kb/bibliography/public/](https://www.ncbi.nlm.nih.gov/myncbi/1FOJ6Ac9Hx-kb/bibliography/public/)

BIOGRAPHICAL SKETCH

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

NAME: Babalonis, Shanna

eRA COMMONS USER NAME (agency login): shanna.babalonis

POSITION TITLE: Assistant Professor, Regular Title Series

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)	Completion Date MM/YYYY	FIELD OF STUDY
West Virginia Univ., Morgantown, WV	BA	05/2002	Psychology, Behavior Analysis
University of North Carolina-Wilmington, Wilmington, NC	MS	05/2005	Behavioral Pharmacology
University of Kentucky, Lexington, KY	PhD	05/2010	Behavioral Neuroscience and Psychopharmacology
University of Kentucky College of Medicine, Lexington, KY	Postdoctoral Fellow	2013	Department of Behavioral Science, Center on Drug & Alcohol Research

A. Personal Statement

I am an Assistant Professor, Regular Title Series in the Department of Behavioral Science with an appointment in the Center for Drug and Alcohol Research and am the Director of our new Cannabis Center at the University of Kentucky. Over the past 10+ years, I have completed studies in the human laboratory and the clinic that focus on the assessment of novel pharmacotherapies for the treatment of opioid and cannabis use disorders. In the human laboratory, I have conducted studies on the abuse liability of several classes of medications, most commonly prescription opioids and cannabinoids (including pharmaceutical cannabinoids and smoked cannabis). I am currently the PI on three current NIH-funded projects that demonstrate my ability to conduct the proposed research: 1) a NIDA R01 that examines acute cannabis and opioid interactions in recreational, non-physically dependent participants to evaluate the effects of cannabis pre-treatment on opioid self-administration; 2) a NIDA R21 that evaluates an array of smoked cannabis doses (THC, CBD combinations) and alcohol administration on impairment outcomes and simulated driving performance; and 3) a NIDA R01 that examines the effects of cannabis on outcomes related to opioid use disorder (e.g., opioid withdrawal, reward and safety). The current pilot project plans to utilize enrollment in the cannabis/opioid use disorder study and examine the lipidomic profile at designated times during the study (e.g., baseline, opioid administration alone and in combination with cannabis, opioid withdrawal alone and during cannabis administration) to provide some of the first data on how endocannabinoids and lipids are modulated by these events. Overall, I am confident that my colleagues and I have the resources and necessary experience to engage in this exciting translational collaboration.

Ongoing and recently completed projects that I would like to highlight include:

R01 DA 054347

Babalonis (PI)

06/1/2022 – 05/30/2026

NIH/NIDA

Title: *Cannabis Modulation of Outcomes Related to Opioid Use Disorder: Opioid Withdrawal, Opioid Abuse Potential and Opioid Safety*

The proposed project examines current project aims to explore how both acute (Study 1) and repeated (Study 2) cannabis administration impacts 1) opioid abuse potential, 2) opioid withdrawal severity, and 3) opioid safety/physiological effects.

Role: PI

Kentucky State Legislature Babalonis (PI, Director) 07/01/22 – 06/30/24
The KY state legislature passed HB 604 which established the Kentucky Cannabis Center at the University of Kentucky. The goal of this appropriation is to accelerate clinical research on medical cannabis and examine its risk/benefit ratio for a variety of medical conditions.
Role: PI, Director

R01 DA 016718-12 Walsh (PI) 9/30/2018 - 7/31/2023

NIH/NIDA

Title: *Licit and Illicit Opioids: Comparative Studies in Humans*

These studies aim to fill critical knowledge gap by assessing agents with sedative properties that commonly are prescribed with opioids (i.e., benzodiazepines, gabapentin), abused in combination with opioids, and found in combination at autopsy (benzodiazepines, alcohol and gabapentin).

Role: Co-Investigator

R01 DA 047368-01 Lile (PI) 4/15/2019 - 2/29/2024

NIH/NIDA

Title: *A Translational Determination of the Mechanisms of Maladaptive Choice in Opioid Use Disorder*

This project will have a significant impact on the field by establishing the experimental application of reinforcement-learning theory to the study of maladaptive dynamic drug-use decision-making in opioid use disorder to reveal behavioral and neural mechanisms that can be targeted for future prevention and treatment development.

Role: Co-Investigator

Citations:

1. **Babalonis S**, Lofwall MR, Sloan PA, Nuzzo PA, Fanucchi LC, Walsh, SL (2019). Cannabinoid modulation of opioid analgesia and subjective drug effects in healthy humans. *Psychopharmacology* 236: 3341-3352. PMCID: PMC6832798
2. Lofwall MR, **Babalonis S**, Nuzzo PA, Elayi SC, Walsh SL (2016). Opioid withdrawal suppression efficacy of oral dronabinol in opioid dependent humans. *Drug Alcohol Depend* 164: 143-50. PMCID: PMC4910823
3. **Babalonis S**, Haney M, Malcolm RJ, Lofwall MR, Votaw VR, Sparenborg S, Walsh SL (2017). Oral cannabidiol does not produce a signal for abuse liability in frequent marijuana smokers. *Drug Alcohol Depend* 172: 9-13. PMCID: PMC5361620
4. Lofwall MR, **Babalonis S**, Nuzzo PA, Siegel A, Campbell C, Walsh SL. Efficacy of extended-release tramadol for treatment of prescription opioid withdrawal: a two-phase randomized controlled trial. *Drug Alcohol Depend*. 2013 Nov 1;133(1):188-97. PubMed PMID: 23755929; PubMed Central PMCID: PMC3786049.

B. Positions and Honors

Positions and Employment

- 2001-2002 Research Intern/Research Assistant, Center for Disease Control/National Institute for Occupational Safety and Health
- 2002-2005 Research Assistant and Course Instructor, University of North Carolina-Wilmington
- 2005-2010 Research Assistant and Pre-doctoral Fellow, University of Kentucky, Department of Behavioral Science
- 2010-2013 Postdoctoral Fellow, University of Kentucky, Department of Behavioral Science
- 2013-2018 Assistant Professor, Research Title Series; University of Kentucky, Department of Behavioral Science, Center on Drug and Alcohol Research
- 2018- Assistant Professor, Regular Title Series (Tenure Track); University of Kentucky, Department of Behavioral Science, Center on Drug and Alcohol Research
- 2022- Director, University of Kentucky Cannabis Research Center

Honors

- 2003 - 2005 Research Travel Awards, University of North Carolina Wilmington

2005	Graduate Teaching Excellence Award, University of North Carolina Wilmington
2006	NIDA Women & Gender Junior Investigator Travel Award CPDD Conference
2007	NIDA Pre-doctoral Trainee (T32 Grant Award)
2009	NIDA Women & Gender Junior Investigator Travel Award CPDD Conference
2009	NIDA Early Career Investigator Travel Award APA Conference
2009	Graduate Student Poster Presentation Award, Bluegrass Society for Neuroscience
2010 - 2012	NIDA Postdoctoral Trainee (T32 Grant Award)
2011 - 2013	National Institutes of Health Loan Repayment Award
2011	First Place Presentation and Travel Award, University of Kentucky College of Medicine Postdoctoral Scholars in Basic and Clinical Sciences
2012	NIDA Director's Travel Award CPDD Conference
2013 - 2014	National Institutes of Health Loan Repayment Award (Renewal Award)
2013	Pilot Project Award, Department of Behavioral Science, University of Kentucky
2014 - 2017	KL2 Scholar, University of Kentucky Center for Clinical and Translational Science
2015 - 2016	National Institutes of Health Loan Repayment Award (Renewal Award)
2015 - 2017	Junior Investigator Award, University of Kentucky Center for Clinical and Translational Science

C. Contribution to Science

1. **Examining the Abuse Liability of Medications in the Human Laboratory:** Prescription opioids are widely misused – in 2019, an estimated 10.1 million individuals reporting past-year misuse. The following studies examined the abuse potential and physiological effects of prescription medications under controlled inpatient laboratory settings. These studies collected some of the first controlled data on the relative abuse liability of IV and oral oxymorphone (Opana®) and the atypical opioid, tramadol (Ultram®) in current opioid users. Data from these studies provided important public health information and empirical evidence for regulatory decision-making regarding the relative abuse liability of these commercially available prescription medications.
 - a. **Babalonis S**, Comer SD, Jones JD, Nuzzo P, Lofwall MR, Manubay J, Hatton KW, Whittington RA, Walsh SL. Relative potency of intravenous oxymorphone compared to other μ opioid agonists in humans - pilot study outcomes. *Psychopharmacology (Berl)*. 2021 PMCID: PMC8514134.
 - b. **Babalonis S**, Lofwall MR, Nuzzo PA, Siegel AJ, Walsh SL. Abuse liability and reinforcing efficacy of oral tramadol in humans. *Drug Alcohol Depend*. 2013 Apr 1;129(1-2):116-24. PMCID: PMC3594406.
 - c. **Babalonis S**, Lofwall MR, Nuzzo PA, Walsh SL. Pharmacodynamic effects of oral oxymorphone: abuse liability, analgesic profile and direct physiologic effects in humans. *Addict Biol*. 2014 Jul 31;PubMed PMID: [25130052](#); PubMed Central PMCID: PMC4383736.
 - d. Walsh SL, Nuzzo PA, **Babalonis S**, Casselton V, Lofwall MR. Intranasal buprenorphine alone and in combination with naloxone: abuse liability and reinforcing efficacy in physically dependent opioid abusers. *Drug Alcohol Depend*. 2016 May 1;162:1908-8. PMCID: PMC 4833536.
2. **Exploring the Gender Differences and the Influence of Reproductive Hormones on the Behavioral Effects of Drugs of Abuse:** Medications and drugs of abuse often have very different effects in women and men, as demonstrated by a large body of animal and human research. My colleagues and I have conducted several studies to examine one key biological factor that may contribute to these observed sex differences, female reproductive hormones, which cross the blood-brain barrier, can be synthesized de novo in the brain and can act as neuromodulators. These studies demonstrated that both endogenous and exogenously administered estradiol and progesterone modified women's behavioral response to stimulant and sedative drugs, respectively.
 - a. Lile JA, Kendall SL, **Babalonis S**, Martin CA, Kelly TH. Evaluation of estradiol administration on the discriminative-stimulus and subject-rated effects of d-amphetamine in healthy pre-menopausal women. *Pharmacol Biochem Behav*. 2007 Jun-Jul;87(2):258-66. PMCID: PMC1991295.
 - b. **Babalonis S**, Emurian CS, Martin CA, Lile JA, Kelly TH. Modulation of the discriminative stimulus effects of triazolam across the menstrual cycle phase in healthy pre-menopausal women. *Drug Alcohol Depend*. 2008 Apr 1;94(1-3):276-80. PMCID: PMC2440678.

- c. **Babalonis S**, Lile JA, Martin CA, Kelly TH. Physiological doses of progesterone potentiate the effects of triazolam in healthy, premenopausal women. *Psychopharmacology (Berl)*. 2011 Jun;215(3):429-39. PubMed Central PMCID: PMC3137367.
 - d. **Babalonis S**, Lile JA, Martin CA, Kelly TH. Progesterone effects on the discriminative stimulus, subjective and performance effects of triazolam in healthy, premenopausal women. *Behav Pharmacol*. 2011 Sep;22(5-6):441-9. PMCID: PMC3172674.
3. *Examining the Therapeutic Utility and Abuse Potential of Cannabinoids*: Our research group has examined putative pharmacotherapeutic medications (e.g., cannabidiol, n- acetylcysteine) for cannabis use disorder, neither of which displayed a signal for efficacy in reducing cannabis use in the laboratory or in the clinic. We have also examined the therapeutic effects of cannabinoids for the treatment of opioid withdrawal and as an opioid adjuvant for the treatment of pain (using a series of experimental pain models in the laboratory) and have assessed the abuse potential of oral cannabidiol and opioid/cannabinoid combinations. Together, these studies demonstrate our ability to conduct careful assessments, with pharmaceutical cannabinoids and smoked cannabis, in both the clinic and the human laboratory that are clinically applicable and that provide data for public health improvement.
- a. Lofwall MR, **Babalonis S**, Nuzzo PA, Elayi SC, Walsh SL. Opioid withdrawal suppression efficacy of oral dronabinol in opioid dependent humans. *Drug Alcohol Depend*, 2016 Jul 1;164:143-50. PMCID: PMC4910823.
 - a. **Babalonis S**, Lofwall MR, Sloan PA, Nuzzo PA, Fanucchi LC, Walsh, SL (2019). Cannabinoid modulation of opioid analgesia and subjective drug effects in healthy humans. *Psychopharmacology* 236: 3341-3352. PMCID: PMC6832798.
 - b. **Babalonis S**, Haney M, Malcolm RJ, Lofwall MR, Votaw VR, Sparenborg S, Walsh SL (2017). Oral cannabidiol does not produce a signal for abuse liability in frequent marijuana smokers. *Drug Alcohol Depend* 172: 9-13. PMCID: PMC5361620.
 - c. Gray KM, Sonne SC, McClure EA, Ghitza UE, Matthews AG, McRae-Clark AL, Carroll KM, Potter JS, Wiest K, Mooney LJ, Hasson A, Walsh SL, Lofwall MR, **Babalonis S**, Lindblad RW et al. A randomized placebo-controlled trial of N-acetylcysteine for cannabis use disorder in adults. *Drug Alcohol Depend*. 2017 Aug 1;177:249-257. doi: 10.1016/j.drugalcdep.2017.04.020. Epub 2017 Jun 10. PMCID: PMC5535813.

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/shanna.babalonis.1/bibliography/48455048/public/?sort=date&direction=descending>

BIOGRAPHICAL SKETCH

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

NAME: Barna, László

eRA COMMONS USER NAME (credential, e.g., agency login): Ibarna

POSITION TITLE: Senior Research Scientist

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
University of Technology and Economics, Institute of Physics, Budapest, Hungary	MSc	06/1999	Physics
University of Technology and Economics, Faculty of Biomedical Engineering, Budapest, Hungary	MSc	02/2002	Biomedical Engineering
Semmelweis University, János Szentágothai Doctoral School of Neuroscience, Budapest, Budapest	PhD	07/2018	Neuroscience

A. Personal Statement

My scientific interest is focused on the development of new tools for neuroscience applications of state-of-the-art light microscopy imaging methods, especially STORM super-resolution microscopy. As the Head of the Nikon Center of Excellence for Neuronal Imaging at the Institute of Experimental Medicine in Budapest for 12 years, I had the unique opportunity already in 2010 to have access in our Center to the first STORM imaging setup installed in Europe. I led an innovation program with the dedicated aim to develop approaches for nanoscale molecular imaging in complex brain circuits. Our efforts culminated in a new workflow for brain sample preparation and to combine patch-clamp electrophysiology with STORM super-resolution molecular imaging. We also developed a novel methodology by building together a confocal microscope with a STORM setup to be able to perform nanoscale molecular imaging within a defined subcellular anatomical context. In terms of our collaboration with the Nikon microscopy division, several of our hardware and software developments have been integrated into the Nikon N-STORM system and have become widely available as off-the-shelf tools for the life science community. I have also developed freely available standalone software tools for data analysis, such as the VividSTORM software that has been downloaded from GitHub 1,619 times from 49 countries so far. My background in physics, in the structural biology of proteins and in small molecule docking simulations also helped the recent development of PharmacoSTORM, a version of STORM imaging using fluorescently labeled ligands. I published 22 papers and book chapters that received 1172 citations, my h-index is 13. In terms of service, I was involved in the theoretical microscopy training of several hundred established and young researchers and led practical training for more than 100 researchers and students in the last decade. I organized and managed the largest light microscopy core facility in Eastern Europe where I gained extensive experience in 7 large systems including confocal, spinning disc, STORM super-resolution, STED and multi-photon microscopes and their analysis software. I was invited to give lectures about our methodical developments at six international microscopy conferences and I frequently presented our work at several neuroscience meetings including the Society for Neuroscience Conference. I also organized several microscopy workshops in Hungary. In 2021, I spent 5 months at the Department of Psychological and Brain Sciences, Indiana University Bloomington as a Visiting Professor. I was responsible for the installation of the new correlated confocal and STORM super-resolution system established in the department, and I trained the local researchers for confocal and super-resolution microscopy use. In Bloomington, I experienced an outstanding innovation environment and the ambitious research goals helped my decision to give up my permanent position in Budapest and move to the United States in 2022. I work as a Senior Research Scientist in the laboratory of Istvan Katona since August 1st, where we are developing new methods for multi-scale imaging approaches to combine physiological, anatomical, and pharmacological measurements at the nanoscale level in intact brain circuits.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

- 2022 - Senior Research Scientist, Indiana University Bloomington, Bloomington, IN
2021 - 2021 Visiting Professor, Indiana University Bloomington, Bloomington, IN
2010 - 2022 Director, Nikon Center of Excellence for Neuronal Imaging, Institute of Experimental Medicine, Hungarian Academy of Science, Budapest, Hungary
2004 - 2010 Junior Research Fellow, Laboratory of Structural Biophysics, Institute of Enzymology, Budapest, Hungary
2001 - 2004 PhD student, Laboratory of Structural Biophysics, Institute of Enzymology, Budapest, Hungary

Honors

- 2018 Dennis Gabor prize, Dennis Gabor Foundation
2007 Young Bio-technologist Prize, Hungarian Biochemical Society

C. Contribution to Science

1. Correlated confocal and STORM super-resolution microscopy in neuroscience:

STORM super-resolution imaging, a version of single-molecule localization microscopy (SMLM), offers unprecedented molecular imaging opportunities. However, functional interpretation of the nanoscale molecular distribution data requires the visualization of the relevant anatomical context in complex tissues. To identify the cellular and subcellular profiles in brain circuits together with the quantitative measurement of target protein densities with nanoscale precision, we developed a correlative deconvolved confocal and STORM imaging methodology. We made important developments in tissue handling, in labeling approaches, such as application of small molecules for binding studies, and in aligned data acquisition. We also wrote an open-source software called VividSTORM to facilitate correlated confocal and super-resolution imaging data analysis including features for automatic, irregularly shaped region-of-interest selection, for molecular clustering, for 3D nanoscale distance measurements along cellular surface and for receptor internalization analysis. Using these new tools, we determined cell-type-specific nanoscale principles of CB₁ cannabinoid receptor distribution, we quantitatively measured Δ⁹-THC-induced molecular tolerance in axon terminals and we discovered that cariprazine, a novel anti-psychotic sold under the brand name Vraylar predominantly binds to D₃ dopamine receptors located along the axons of the granule cells in the Islands of Calleja.

Prokop S, Ábrányi-Balogh P, Barti B, Vámosi M, Zöldi M, **Barna L**, Urbán GM, Tóth AD, Dudok B, Egyed A, Deng H, Leggio GM, Hunyady L, van der Stelt M, Keserű GM, Katona I (2021) PharmacostORM nanoscale pharmacology reveals cariprazine binding on Islands of Calleja granule cells. *Nature Communications*, 12:6505. PMCID: PMC8586358.

Igarashi M, Nozumi M, Wu LG, Cella Zanacchi F, Katona I, **Barna L**, Xu P, Zhang M, Xue F, Boyden E (2018) New observations in neuroscience using superresolution microscopy. *The Journal of Neuroscience*, 38:9459-9467. PMCID: PMC6209844I

Barna L, Dudok B, Miczán V, Horváth A, László ZI, Katona I (2016) Correlated confocal and super-resolution imaging by VividSTORM. *Nature Protocols*, 11:163-83.

Dudok B*, **Barna L***, Ledri M, Szabó SI, Szabadits E, Pintér B, Woodhams SG, Henstridge CM, Balla GY, Nyilas R, Varga C, Lee SH, Matolcsi M, Cervenak J, Kacskovics I, Watanabe M, Sagheddu C, Melis M, Pistis M, Soltesz I, Katona I (2015) Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. *Nature Neuroscience*, 18:75-86. PMCID: PMC4281300

2. Molecular docking and dynamic simulation for drug discovery:

One of the most useful tools of modern drug discovery is rational molecule design. Saving time, money, and human resources, in silico pre-screening of millions of small molecules by computational approaches substantially improved high-affinity receptor ligand and enzyme substrate development. I used high-resolution protein structures and performed molecular dynamic simulations based on newly developed docking algorithms that helped to design and synthesize selective lead molecules for matrix metalloproteinases (MMPs) and for phosphodiesterase 5 (PDE5).

Bencsik P, Kupai K, Görbe A, Kenyeres É, Varga ZV, Pálóczi J, Gáspár R, Kovács L, Weber L, Takács F, Hajdú I, Fabó G, Cseh S, **Barna L**, Csont T, Csonka C, Dormán G, Ferdinandy P (2018) Development of Matrix Metalloproteinase-2 Inhibitors for Cardioprotection. *Frontiers in Pharmacology*, 9:296. PMCID: PMC29674965

Tömöri T, Hajdú I, **Barna L**, Lorincz Z, Cseh S, Dormán G (2012) Combining 2D and 3D in silico methods for rapid selection of potential PDE5 inhibitors from multimillion compounds' repositories: biological evaluation. *Molecular Diversity*, 16:59-72.

Dormán G, Cseh S, Hajdú I, **Barna L**, Kónya D, Kupai K, Kovács L, Ferdinandy P (2010) Matrix metalloproteinase inhibitors: a critical appraisal of design principles and proposed therapeutic utility. *Drugs*, 70:949-64.

Papp A, Szommer T, **Barna L**, Gyimesi G, Ferdinandy P, Spadoni C, Darvas F, Fujita T, Urge L, Dormán G (2007) Enhanced hit-to-lead process using bioanalogous lead evolution and chemogenomics: application in designing selective matrix metalloprotease inhibitors. *Expert Opinion on Drug Discovery*, 2:707-23.

3. Molecular modeling of enzyme activity:

Better understanding of the biophysical and structural properties of enzymes that fundamentally determine enzyme kinetics is necessary to develop more potent enzyme inhibitors with therapeutic potentials. I exploited molecular modeling and site-directed mutagenesis to determine the structural features of several enzymes and I contributed to the identification of novel structural information that contribute to enzyme thermostability, catalytic properties and auto-activation.

Kamondi S, Szilágyi A, **Barna L**, Závodszky P. (2008) Engineering the thermostability of a TIM-barrel enzyme by rational family shuffling. *Biochemical and Biophysical Research Communications*, 374:725-30.

Gál P, **Barna L**, Kocsis A, Závodszky P. (2007) Serine proteases of the classical and lectin pathways: similarities and differences. *Immunobiology*, 212:267-77.

Flachner B, Varga A, Szabó J, **Barna L**, Hajdú I, Gyimesi G, Závodszky P, Vas M. (2005) Substrate-assisted movement of the catalytic Lys 215 during domain closure: site-directed mutagenesis studies of human 3-phosphoglycerate kinase. *Biochemistry*, 44:16853-65.

Gál P, Harmat V, Kocsis A, Bián T, **Barna L**, Ambrus G, Végh B, Balczer J, Sim RB, Náray-Szabó G, Závodszky P (2005) A true autoactivating enzyme. Structural insight into mannose-binding lectin-associated serine protease-2 activations. *Journal of Biological Chemistry*, 280:33435-44.

BIOGRAPHICAL SKETCH

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

NAME: Bradshaw, Heather Bryte

ERA COMMONS USER NAME (credential, e.g., agency login): HBBRADSH

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Florida State University	BS	12/94	Nutrition
Florida State University	PhD	12/01	Neuroscience
Brown University	postdoctoral	01/04	Neuropharmacology
Indiana University	postdoctoral	07/07	Neuropharmacology

A. Personal Statement

After my graduate training focused on reproductive pain using behavioral and physiological techniques, I pursued training in the measurement of small molecule lipids associated with the endogenous cannabinoid. Today, my work combines the fields of neurophysiology, behavior, and lipid biochemistry to understand how lipid signaling drives changes in cellular communication through a systems neuroscience approach. A central focus of my research relies on lipidomics discovery and characterization of endogenous lipids using mass spectrometric techniques including understanding their biosynthesis and metabolism with a special emphasis on their roles in signaling. Ongoing investigations of the endogenous cannabinoid lipid signaling molecules, 2-arachidonoyl glycerol and N-arachidonoyl ethanolamine (Anandamide) have driven the discovery of more than 80 endogenous structural analogs, which are produced in the brain and periphery. Lipidomics screens are a targeted technique that provide novel information that drive hypotheses. Recently, my laboratory used lipidomics to discover that the phytocannabinoids delta 9 tetrahydrocannabinol (THC) and cannabidiol (CBD) differentially drive changes in lipids across the CNS. Through lipidomics screens using a range of KO models we were able to determine that the enzyme, NAPE-PLD was required for most changes in the CNS lipidome by CBD. Providing targeted lipidomics information for models of drugs to test and generate hypotheses is the focus of this proposal. Lipidomics is one of the current frontiers of research that promises to provided novel insights into to questions of CNS plasticity that becomes maladaptive with chronic drug use. Multiple lines of evidence indicate that the endocannabinoid system is an intersection of this phenomenon; therefore, bringing these lines of research together with lipidomics techniques will provide a unique opportunity to push these hypotheses forward.

Ongoing and recently completed projects that I would like to highlight include:

NIH-R01DA041208, (MPI Kamiya and Pletnikov: Bradshaw Co-I) 2016- 2021
Genetic alterations in astrocytes exacerbate cognitive effects of adolescent cannabis exposure
The major goal of this project is to understand that role of THC and eCB signaling in astrocytes plays in the signaling of DISC-1 that contributes to schizophrenia.

NIH-RO1DA043982, MPI (Manzoni, Mackie: Bradshaw Co-I) 2017-2022
Sex-specific critical periods determine the effects of cannabinoids on the mesocorticolimbic system
The major goals of this study are to determine the role of cannabinoid signaling on development.

NIH R01DA00668 (PI SK Dey; Bradshaw Co-I)

2015-2020

Endocannabinoid signaling in early pregnancy.

The main goals of this study are determining the involved of endogenous cannabinoid signaling in implantation events in the uterus.

Welcome Trust 223279: PI Dan Brierley, University College London; Bradshaw Co-I Jan 2022-Dec 2025
"Functional mapping of gut-brain neurocircuitry in health and obesity"

The major goal of the project for the Bradshaw lab is to perform lipidomics analysis on gut and brain tissue in animals with either low-fat or high-fat diets to determine the effect on endogenous signal lipids.

Citations related to my collaborative work using lipidomics that have driven novel hypotheses:

1. Maciel IS, de Abreu GHD, Johnson CT, Bondy R, **Bradshaw HB**, Mackie K, Lu HC. Perinatal CBD or THC Exposure Results in Lasting Resistance to Fluoxetine in the Forced Swim Test: Reversal by Fatty Acid Amide Hydrolase Inhibition. *Cannabis Cannabinoid Res.* 2021 Jun 28. doi: 10.1089/can.2021.0015. Online ahead of print. PMCID: PMC9225394
2. Bashashati M, Leishman E, **Bradshaw H**, Sigaroodi S, Tatro E, Bright T, McCallum R, Sarosiek I. Plasma endocannabinoids and cannabimimetic fatty acid derivatives are altered in gastroparesis: A sex- and subtype-dependent observation. *Neurogastroenterol Motil.* 2020 Aug 10:e13961. PMCID: PMC8018519 DOI: 10.1111/nmo.13961
3. Brierley DI, Harman JR, Giallourou N, Leishman E, Roashan AE, Mellows BAD, **Bradshaw HB**, Swann JR, Patel K, Whalley BJ, Williams CM. Chemotherapy-induced cachexia dysregulates hypothalamic and systemic lipoamines and is attenuated by cannabigerol. *J Cachexia Sarcopenia Muscle.* 2019 Aug;10(4):844-859. doi: 10.1002/jcsm.12426. Epub 2019 Apr 29. PMCID: PMC6711413

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2018-present Professor of Psychological and Brain Science at Indiana University

2016-2022 Director STARS (Science and Technology Research Scholars), College of Arts and Sciences

2013-2018 Associate Professor of Psychological and Brain Sciences at Indiana University

2007-2013 Assistant Professor of Psychological and Brain Sciences at Indiana University

Other Experience and Professional Memberships:

2020-present	Associate Editor, Cannabis and Cannabinoid Research
2018-present	Editorial Board, <i>Frontiers in Physiology: Lipids</i>
2016-2019	President and BOD member of the International Cannabinoid Research Society
2012-2016	Editorial Board, <i>British Journal of Pharmacology</i>
2001-present	International Cannabinoid Research Society
1995-present	Society for Neuroscience

Honors

2019	Florida State University Psychology Department Outstanding Graduate Award
2016	National Academy of Sciences Kavli Scholar
2016	Indiana University Trustee Teaching Award
2007	Organization for the Study of Sex Differences Young Investigator travel award
2006	Outstanding achievement in a lecture: International Cannabinoid Research Society
2006	Society for Neuroscience Women in Neuroscience travel award
2003-2006	Individual NRSA (NIDA)

C. Contributions to Science 103 peer-reviewed papers; 5 chapters; H-Index 47, 5994 citations

Hormonal contributions to neuronal processing and behavioral changes to noxious stimuli

During PhD studies with Karen Berkley at Florida State University I used behavioral and electrophysiological methods to answer questions about how hormones drive changes in these parameters. These data showed that changes in the hormonal milieu drive changes in neuronal and behavioral responses to noxious visceral stimuli; however, the molecular mechanism for these modifications were unknown. I have continued to build upon this work to understand some of the molecular mechanisms of how hormones play a

role in lipid signaling and have shown that levels of endogenous cannabinoids in the brain change across the hormonal cycle, are different in males and females, and are regulated during mating.

- 1) **Bradshaw, HB**, Temple, JL, Wood, E, Berkley KJ. Estrous variations in behavioral responses to vaginal and uterine distention in the rat. *Pain*. 1999 Aug;82(2):187-97.
- 2) **Bradshaw HB**, Berkley KJ. Estrous changes in responses of rat gracile nucleus neurons to stimulation of skin and pelvic viscera. *J Neurosci*. 2000 Oct 15;20(20):7722-7727.
- 3) **Bradshaw HB**, Rimmerman N, Krey JF, Walker JM. Sex and hormonal cycle differences in brain levels of pain-related cannabimimetic lipid mediators. *Am J Physiol Regul Integr Comp Physiol*. 2006 Aug;291(2):R349-58. Epub 2006 Mar 23
- 4) Stuart JM, Paris J, Frye C, **Bradshaw HB**. Brain levels of prostaglandins, endocannabinoids and related lipids are affected by mating strategies. 2013 International Journal of Endocrinology Volume 2013, Article ID 436252, 14 pages, <http://dx.doi.org/10.1155/2013/436252>. PMCID: PMC3863470.

Analysis of endogenous cannabinoids in a wide variety of model systems

Post-doctoral training in the lab of J Michael Walker centered on the development of lipidomics mass spectrometric techniques for the endogenous cannabinoids. I have become a world expert on measuring these endogenous cannabinoids in all tissue types. These lipids are routinely measured in my lab from small volumes of brain tissue, retina, bone, cartilage, lung, liver, kidney, uterus, placenta, skin, and plasma. The ability to modify extraction and analysis techniques has allowed me to work with colleagues from all over the world and in different model systems. This provides both a unique perspective as well as a growing data set with which to mine for systems biology.

- 1) Guindon J, Lai Y, Takacs SM, **Bradshaw HB**, Hohmann AG. Alterations in endocannabinoid tone following chemotherapy-induced peripheral neuropathy: Effects of endocannabinoid deactivation inhibitors targeting fatty-acid amide hydrolase and monoacylglycerol lipase in comparison to reference analgesics following cisplatin treatment. *Pharmacol Res*. 2013 Jan;67(1):94-109. doi: 10.1016/j.phrs.2012.10.013. PMCID: PMC352590.
- 2) Cha J, Bartos A, Egashira M, Haraguchi H, Saito-Fujita T, Leishman E, **Bradshaw H**, Dey SK, Hirota Y. Combinatory approaches prevent preterm birth profoundly exacerbated by gene-environment interactions. *2013 J Clin Invest*. Sep 3;123(9):4063-75. doi: 10.1172/JCI70098. PMCID: PMC3754274.
- 3) Crowe MS, Leishman E, Banks ML, Gujar R, Mahadevan A, **Bradshaw HB**, Kinsey SG. Dual inhibition of monoacylglycerol lipase and cyclooxygenases synergistically reduces neuropathic pain in mice. *Br J Pharmacol*. 2014 Nov 13. doi: 10.1111/bph.13012. PMCID: PMC4376450.
- 4) Leishman E, Kunkler PD, Manchanda M, Sangani K, Stuart JM, Oxford GS, Hurley JS, and **Bradshaw HB**. Acrolein Exposure Alters Levels of Endogenous Lipids, Including TRP Agonists: A Potential Molecular Mechanism for Headache Driven by TRPA1 activation. *Neurobiology of Pain*, 2017:1:28-36. PMCID: PMC5802349.

Lipidomics mass spectrometric techniques as a tool for identifying novel lipids and signaling systems

Developing a variety of extraction and mass spectrometric paved the way for the discovery of other structurally similar endogenous lipids. This growing class of lipids is derived from the conjugation of a fatty acid and an amine and is collectively called *N*-acyl amides or lipoamines. To date, my group has identified and characterized over 80 of these novel lipids in the mammalian brain and other model systems such as drosophila. I worked with collaborators at Hebrew University to discover a novel lipid; *N*-oleoyl serine, that plays a pivotal role in the maintenance of bone density and recently published that this lipid is present in high abundance in olive oil. Recently, we also published the largest scale lipidomics analysis of the effects of the deletion of endogenous cannabinoid metabolic enzymes on the levels of these lipids in the brain. These data show that endogenous cannabinoids are biochemically linked to potentially hundreds of lipids in the brain and body; therefore, the regulation of these by exogenous Cannabis compounds potentially has broader effects than ever realized.

- 1) Smoum R, Bar A, Tan B, Milman G, Attar-Namdar M, Ofek O, Stuart JM, Tam J, Kram V, O'Dell D, Walker MJ, **Bradshaw HB**, Bab I, Mechoulam R. Oleoyl serine, an endogenous *N*-acyl amide, modulates bone remodeling and mass. *Proc Natl Acad Sci U S A* 2010 Oct 12. 107:17710-5. PMCID: PMC2955099.
- 2) Tortoriello G, Rhodes BP, Takacs SM, Stuart JM, Basnet A, Harkney T, **Bradshaw HB**. Targeted Lipidomics in Drosophila melanogaster Identifies Novel 2-Monoacylglycerols and *N*-acyl Amides. 2013 PLoS ONE 8(7): e67865. doi:10.1371/journal.pone.0067865 PMCID: PMC3708943.
- 3) Leishman E, Cornett B, Spork K, Straker A, Mackie, K, and **Bradshaw HB**. Broad impact of deleting endogenous cannabinoid hydrolyzing enzymes and the cannabinoid receptor CB1 on the endogenous

cannabinoid-related lipidome in eight regions of the mouse brain. *Pharmacol Res.* 2016 Apr 22. pii: S1043-6618(16)30344-9. doi: 10.1016/j.phrs.2016.04.020. [Epub ahead of print] PMCID: PMC4914450.

- 4) Leishman, E, Mackie, K, Luguet S, and **Bradshaw HB**. Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain. *Biochim Biophys Acta.* 2016 Jun;1861(6):491-500. doi: 10.1016/j.bbalip.2016.03.003. Epub 2016 Mar 5. PMCID: PMC4909477.

Identification of a novel GPCR that is activated by THC as well as its endogenous ligands

Mass spec lipidomics is complemented in my lab with functional assays designed to determine the signaling role at GPCRs of these newly discovered lipids. Through a series of functional assays including cellular migration, proliferation, MAPK, and immunohistochemistry we showed NAGly is an endogenous ligand that activates GPR18 and that this signaling system plays a role in both microglial and human endometrial migration through similar intracellular cascades. In addition, our data provided evidence that the phytocannabinoid, Cannabidiol and the endogenous lipid, *N*-arachidonoyl serine, both act as antagonists at the GPR18 receptor. Finally, our data also provided compelling evidence that the phytocannabinoid, Δ^9 THC, is a potent ligand at GPR18, which mimics the signaling properties of NAGly. These data have set the foundation for the identification of an additional cannabinoid receptor (GPR18) that will aid in the scientific understanding of how cannabis works in the brain and body.

- 1) McHugh D, Hu S S-J, Rimmerman N, Vogil Z., Walker JM, **Bradshaw HB**. *N*-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. *BMC Neurosci* 2010 Mar 26;11:44. PMCID: PMC2865488.
- 2) McHugh D, Wager-Miller J, Page J, and **Bradshaw HB**. siRNA knockdown of GPR18 receptors in BV-2 microglia attenuates *N*-arachidonoyl glycine induced cell migration. *J Mol Signal.* 2012 Jul 26;7(1):10. PMCID: PMC3493281.
- 3) McHugh D, Page J, Dunn E, **Bradshaw HB**. $\Delta(9)$ -THC and *N*-arachidonoyl glycine are full agonists at GPR18 and cause migration in the human endometrial cell line, HEC-1B. *Br J Pharmacol* 2012 Apr;165(8):2414-24. doi: 10.1111/j.1476-5381.2011.01497.x. PMCID: PMC3423258..
- 4) McHugh D, Roskowski D, Xie S, and **Bradshaw HB**. $\Delta9$ -THC and *N*-arachidonoyl glycine regulate BV-2 microglial morphology and cytokine release plasticity. *Front Pharmacol.* 2014 Jan 2;4:162. doi: 10.3389/fphar.2013.00162. eCollection 2014. PMCID:PMC3877838.

Novel endogenous ligand identification of TRP receptors

An important emerging field of signaling is focused on the transient receptor potential (TRP) channels. There are 28 known variants in mammalian species and the endogenous ligands for most are unknown. Recently, we identified 20 lipoamines with activity at TRPV1-4 and showed that at least 8 of these were up or down regulated in an acute model of peripheral pain. In collaboration with Sven Jordt we also showed that those that activate TRPV4 were present in lung and differentially regulated with edema. Recently, we collaborated with Andrea Hohmann to discover that mice lacking the ability to metabolize many of these endogenous TRP activators had an elevated response to noxious stimuli. This same enzyme metabolizes the endogenous cannabinoid, Anandamide, which illustrates how these signaling systems are interconnected.

- 1) Huang SM, Lee h, Chung M-K, Yu YY, **Bradshaw HB**, Coulombe PA, Walker JM, Caterina MJ. Overexpressed transient receptor potential vanilloid 3 ion channels in skin keratinocyte modulate pain sensitivity via prostaglandin E2. *J Neurosci* 2008 Dec 17;28(51):13727-37. PMCID: PMC2676929.
- 2) Raboune S, Stuart JM, Leishman E, Takacs SM, Rhodes B, Basnet A, Jameyfield E, McHugh D, Widlanski T, **Bradshaw HB**. Novel endogenous *N*-acyl amides activate TRPV1-4 receptors, BV-2 microglia, and are regulated in brain in an acute model of inflammation. *Front Cell Neurosci.* 2014 Aug 1;8:195. doi: 10.3389/fncel.2014.00195. eCollection 2014. PMCID: PMC4118021.
- 3) Balakrishna S, Song W, Achanta S, Doran SF, Liu B, Kaelberer MM, Yu Z, Sui A, Cheung M, Leishman E, Eidam HS, Ye G, Willette RN, Thorneloe KS, **Bradshaw HB**, Matalon S, Jordt SE. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2014 Jul 15;307(2):L158-72. doi: 10.1152/ajplung.00065.2014. Epub 2014 May 16. PMCID: PMC4152165.
- 4) Carey L, Slivicki R, Leishman E, Cornett B, Mackie K, **Bradshaw HB**, Hohmann A. A pro-nociceptive phenotype unmasked in mice lacking fatty-acid amide hydrolase. *Mol Pain.* 2016 May 13;12. pii: 1744806916649192. PMCID: PMC4956176.

Complete List of Published Work in MyBibliography

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1TKIVdk1nxAl/bibliography/46507251/public/?sort=date&direction=ascending>

BIOGRAPHICAL SKETCH

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

NAME: **Bruchas, Michael R.**ERA COMMONS USER NAME (credential, e.g., agency login): **MBRUCHAS**POSITION TITLE: **Professor**

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Creighton University	B.S.	08/1995	Biology
Creighton University School of Medicine	Ph.D	08/2004	Pharmacology
University of Washington, Seattle	Post-Doc	2004-2010	Neuroscience

A. Personal Statement This proposal is ideally suited for my laboratory and our multidisciplinary, multi-PI research team. In my graduate and post-doctoral training (11yrs) I received research training in multidisciplinary approaches of GPCR pharmacology, physiology, and behavioral neuroscience. This includes pharmacological studies G-protein coupled receptors, signal transduction mechanisms, and mouse behavioral approaches including aversion, preference, anxiety, and depression animal models. In my own laboratory we actively merge the interface between signal transduction, anatomical, bioengineering, and behavioral analysis to understand neuromodulator (i.e. monoamine, neuropeptide) neural circuits and signaling pathways related to the neuromodulation and motivated behaviors. My laboratory routinely uses optogenetics, *in vivo* physiology, and *in vivo* imaging as a means to interact and perturb neuronal function in freely moving animals. I have a strong track recording of breakthroughs and cutting-edge innovation in neuroscience. As a post-doctoral fellow in the Department of Pharmacology at the University of Washington I discovered several novel neuropeptide, monoamine, dependent signaling pathways, circuits, and behavioral effects which are now being widely pursued by a number (~100) of leading academic and industry laboratories. This highlights how my research has molded the future direction of research in the field. I was awarded the INRC-Young Investigator Award in 2014, largely for these efforts. Since the lab's founding we have taken on several important and innovative new directions in the fields of optogenetics and bioengineering (some are highlighted below) for real time dissection of neural circuits and neuromodulation in behavior. The lab has numerous recent publications highlighting our ability to innovate and to use *in vivo* chemogenetics, optogenetics, to dissect affective behavioral circuits (Al-Hasani et al., 2015, *Neuron*; McCall et al., 2015, McCall et al., 2017, *eLife*; *Neuron*; Jeong et al., *Cell*, 2015; Siuda et al., 2016 *NPP*). These papers describe chemogenetic, optogenetic and physiological dissection of neural circuits in motivated behaviors, as well as novel device development for wireless optogenetics, wireless pharmacology and photometry, opsins. The laboratory's scope is neurobiological basis of neuromodulation in affective behavior, in a variety of circuits, including reward and aversion pathways, with a focus on neuropeptides and monoamines. We also have parallel efforts in neuroscience tool development and its implementation and have recent studies where we have mastered the use of photometry and single cell GRIN lens calcium and biosensor imaging methods (Parker et al., *Cell* 2019, Al-hasani /Gowrishankar et al., 2021; and Lu et al., *PNAS* 2018; Xia et al., 2017, *Cell Reports*; Seo et al, *Neuron*, 2021, Castro et al., 2021, *BioRxiv/Nature*, in press).

Selected Relevant Recent Publications:

1. D. C. Castro, C. S. Oswell, E. T. Zhang, C. E. Pedersen, S. C. Piantadosi, M. A. Rossi, A. Hunker, A. Guglin, J. A. Morón, L. S. Zweifel, G. D. Stuber, **M. R. Bruchas** (2021) An endogenous opioid circuit determines state-dependent reward consumption. *Nature*, Oct;598(7882):646-651. PMID: 34646022. PMC8858443.

2. R. Al-Hasani*, R. Gowrishankar*, G. P. Schmitz*, C. E. Pedersen, D. J. Marcus, S. E. Shirley, T. E. Hobbs, A. J. Elerding, S. J. Renaud, M. Jing, Y. Li, V. A. Alvarez, J. C. Lemos, **M. R. Bruchas** (2021) Ventral tegmental area GABAergic inhibition of ventral accumbens shell cholinergic interneurons promotes reward reinforcement. *Nature Neuroscience*, 24(10),1414-1428. PMID: 34385700. PMC8823543.
3. B. A. Copits, R. Gowrishankar, P. R. O'Neill, JN. Li, K. S. Girven, J.J. Yoo, X. Meshik, K. E. Parker, S. M. Spangler, A. J. Elerding, B. J. Brown, S. E. Shirley, K. K. L. Ma, A. M. Vasquez, M. C. Stander, V. Kalyanaraman, S.K. Vogt, V.K. Samineni, T. Patriarchi, L. Tian, N. Gautam, R. K. Sunahara, R. W. Gereau, **M. R. Bruchas**. (2021). A photoswitchable GPCR-based opsin for presynaptic inhibition. *Neuron*, 109(11), 1791-1809.e11. PMID: 33979635. PMC8194251.
4. K.E. Parker, A.M. Gomez, S.M. Spangler, M. Walicki, S. Feng, R. Al-Hasani, J.G. McCall, B. Copits ,W.J. Planer, T.J. Kash, J. Dougherty, G.D. Stuber, **M.R. Bruchas** (2019) A Paraniagral VTA Nociceptin Circuit that Constrains Motivation for Reward. *Cell*, Jul 25;178(3):653-671. PMID: 31348890. PMC7001890.

*featured commentary in *Neuron* 2019, and *Nature Neuroscience* 2019

Selected Ongoing Research Support (Summary)

R37 DA033396-06 Bruchas (PI) 8/01/2018-4/30/2023 *(2028, MERIT AWARD)

Dissecting Dynorphin-Kappa Opioid Mediated Reinstatement of Nicotine Preference

Determine the role of dynorphin/KOR activity in serotonergic and dopaminergic circuits as necessary and sufficient for stress-induced nicotine preference using viral rescue ("gain of function").

P50: Project 4 Circuit-level Approaches for Dissecting Approach/Avoidance Behaviors Mediated by Nociceptin Systems in Mice.

P50MH119467 PI, Project 4 Bruchas

Period 4/1/2020-3/31/2025

The research aims of this 5-year project are: (1) to determine the anatomical and functional characteristics of nociceptin expressing neurons within the ventral midbrain and identify behavioral conditions (acute vs chronic stress, motivation and Approach-Avoidance) that are modulated by this system; (2) to identify and characterize pnVTA nociceptin neurons and their afferents involved in motivated behavior that drive negative affective behavior. The project will use novel and validated mouse cre-driver models that allow unparalleled access to PNOC+ neurons in the VTA, combined with optogenetic, chemogenetic, calcium imaging, viral tracing, and behavior to uncover circuit mechanisms that underlie how these neurons are regulated by aversive stimuli.

R61/R33 Optopharmacology and Sensors for Dissecting Opioid Action In Vivo

1R61DA051489-01 MPI, Bruchas and Gu (UW Biochemistry) Period 8/31/21-7/30/25

We will utilize a series of cutting-edge approaches to: 1) develop novel opioid sensors for in vivo, sub-second measures of fentanyl, morphine, and methadone and opioid peptides, 2) demonstrate the utility of optopharmacological approaches for dissecting opioid action, and 3) apply the sensors and optopharmacological approaches to perform in vivo precision pharmacological experiments to modulate pain and reward circuits related to drug abuse.

University of Washington Center of Excellence in Opioid Addiction Research

P30 DA048736 (NIDA P30 Center) Co-I, Bruchas

Co-Director of the Imaging and Neural Circuits Core with Dr. Garret Stuber.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

8-2018	Professor, University of Washington, Seattle. Departments of Anesthesiology, Pharmacology, and the Center for the Neurobiology of Addiction, Pain and Emotion
9-2017	Installed as Henry E. Mallinckrodt Professor of Anesthesiology and Neuroscience
7-2015-	Associate Professor, with tenure, Departments of Anesthesiology and Neuroscience, Washington University, St. Louis, MO
2014-	Asst. Professor, Dept. of Biomedical Engineering, Washington University, St. Louis, MO
2011-	Member of Division of Biology and Biomedical Sciences Washington University: Programs in Neuroscience, Biochemistry, Molecular-Cell Biology
11-2010-	Assistant Professor, Departments of Anesthesiology and Neurobiology, Washington University School of Medicine, St. Louis, MO
2009-2010	Acting Instructor, Department of Pharmacology, University of Washington School of Medicine Seattle WA.
2004-2009	Senior Fellow, Department of Pharmacology, University of Washington School of Medicine

Seattle, WA.
2000-2004 Ph.D Student, Department of Pharmacology, Creighton University School of Medicine, Omaha, NE.

Honors:

- 2021 John J. Abel Award in Pharmacology, ASPET.
2018 Jacob P. Waletsky Award for Addiction Research, Society for Neuroscience
2018 Mahoney Neuroscience Institute, Rising Star Award, University of Pennsylvania
2017 Henry E. Mallinckrodt Endowed Professorship
2016-2021 NIH BRAIN Initiative (3 Awards, Optogenetics, and Wireless Photometry)
2014 DECODE Award (Deciphering the Circuit Basis of Disease using Microendoscopy)
2014 INRC Young Investigator Award (International Narcotics Research Conference)
2013 NIH EUREKA Award
2012 NIH Director's Award, Transformative R01
2009- NIH, Young Investigator "Pathway to Independence" Award (K99-R00)

C. Contributions to Science [* = co-first or co-senior/corresponding authors]

Complete List of my work can be found at: <http://www.ncbi.nlm.nih.gov/pubmed/?term=Bruchas+M>

1. Optogenetic interrogation of neural circuits in behavior and novel tool development

Optogenetics, the ability to selectively control and manipulate neuronal function has recently transformed neuroscience, and has allowed for unprecedented discoveries in the neural circuit basis of behavior. While these approaches of using light-sensitive ion channels and pumps have proved very useful they have been limited in i) their abilities in complex behaviors (stress, home cage, etc), as well as ii) in their ability to engage neuromodulatory signaling networks with spatiotemporal control. Therefore, our group established novel techniques and approaches on two related fronts: 1) optogenetic bioengineering techniques for dissecting neural circuit function in behavioral models. We published a novel wireless optogenetic approaches in *Science* and *Nature Protocols* whereby we developed novel multimodal wireless optogenetic devices and examined reward seeking behavior in both operant, anxiety and real time conditioned preference behaviors. We recently developed a novel method for wireless *in vivo* pharmacology with optogenetics that is published in *Cell* and show that this can be used for dissecting peptide and monoamine neural circuits. This technology is under patent review, and currently available via *Neurolux* to facilitate widespread adoption in the field. 2) We have been actively developing novel optically-sensitive GPCRs for control of neuromodulation in real time, *in vivo*. Two papers using optogenetic approaches, cell-type selective targeting, real time aversion, operant, Opto-XRs, and *in vivo* optogenetic behavioral modulation are under revision, and one whereby we developed a novel opsin-neuropeptide (mu-opioid) GPCR was just published in *Neuron*. In all these cases, the goal is to utilize these tools to better understand how neuromodulation effects of affective behavior.

a. B. A. Copits, R. Gowrishankar, P. R. O'Neill, Jun-Nan Li, K. S. Girven, J.J. Yoo, X. Meshik, K. E. Parker, S. M. Spangler, A. J. Elerding, B. J. Brown, S. E. Shirley, K. K. L. Ma, A. M. Vasquez, M. C. Stander, V. Kalyanaraman², S. K. Vogt, V.K. Samineni, T. Patriarchi, L. Tian, N. Gautam, R. K. Sunahara, Robert W. Gereau IV, **M. R. Bruchas**. (2021). A photoswitchable GPCR-based opsin for presynaptic inhibition. *Neuron*, May 10:S0896-6273(21)00307-X PMC8194251.

b. T.-I. Kim, J.G. McCall, Y.H. Jung, X. Huang, E.R. Siuda, Y. Li, J. Song, Y.M. Song, H.A. Pao, R.-H Kim, C. Lu, S.D. Lee, I.S. Song, G.C. Shin, R. Al-Hasani, S. Kim, M.P. Tan, Y. Huang, F. Omenetto, J.A. Rogers* and **M.R. Bruchas*** (2013) Injectable, Cellular-Scale Optoelectronics with Applications for Wireless Optogenetics. *Science*, 340:211-216. *Co-senior/co-corresponding author. PMC3769938

c. G. Shin, A. M. Gomez, R. Al-Hasani, Y. Jeong, J. Kim, Z. Xie, A. Banks, S. M. Lee, S. Y. Han, C. J. Yoo, J. Lee, Se. H. Lee, J. Kurniawan, J. Tureb, Z. Guo, J. Yoon, S. Park, S. Y. Bang, Y. Nam, M. C. Walicki, V. K. Samineni, A. D. Mickle, K. Lee, S. Y. Heo, J. G. McCall, T. Pan, L. Wang, X. Feng, T. Kim, J. K. Kim, Y. Li, Y. Huang, R. W. Gereau IV, J. S. Ha, **M. R. Bruchas***, and J. A. Rogers. (2017) Flexible near field wireless optoelectronics as subdermal implants for broad applications in optogenetics. *Neuron*, 93:509-521. PMC5377903 * co-corresponding, lead contact. *Featured Paper for the issue, and featured paper of 2017.

d. Qazi R.* , Gomez A.* , Zhou Z., Sim J., Xiong Y., Abdo J., Kim C., Anderson A., Lohner F., Chul Lee B., Jang K., Xiao J., **Bruchas M.R.***, Jeong JW.* . (2019) Chronic, Smartphone-enabled Wireless In Vivo Neuropharmacology and Optogenetics., *Nature Biomedical Engineering*, Aug;3(8):655-669.

*co-corresponding author, featured paper.

2. Reward, Addiction, and Motivated Behaviors

Understanding the neurobiological mechanisms by which drugs of abuse and stress pathways interact to promote drug-seeking and reinstatement is a fundamental research interest. As a post-doctoral fellow, I uncovered novel neural circuits, cell types, and kappa-opioid signaling pathways that mediate stress-induced reinstatement of cocaine, opioid, and nicotine place preference. My laboratory has recently identified locus coeruleus noradrenergic circuits and hippocampal mechanisms that are required for reinstatement of cocaine and morphine place preference. A selection of these recent papers is below.

a. G.S. Portugal, R. Al-Hasani, A. Fakira, J. Gonzalez Romero, Z. Meylan, J.G. McCall, **M.R. Bruchas***, and J.A. Morón Concepcion*. (2014) Hippocampal long-term potentiation (LTP) is disrupted during expression and extinction but is restored following reinstatement of morphine place preference. *J. Neurosci*, 34:527-538. PMC3870935 ***corresponding author / equal contribution**

b. R. Al-Hasani, A.M. Foshage, J.G. McCall, **M.R. Bruchas** (2013) Locus Coeruleus Kappa Opioid Receptors Modulate Reinstatement of Cocaine Place Preference through a Noradrenergic Mechanism. *Neuropsychopharmacology*, 38:2484-97. PMC3799068

c. S. Nygard, N. Hourgettes, W.A. Carlezon, **M.R. Bruchas**. (2016) Stress-induced reinstatement of nicotine preference requires Dynorphin/Kappa Opioid activity in the basolateral amygdala. *J. Neuroscience*, 36(38): 9937-48. PMC5030354

d. L Xia*, SK Nygard*, GG Sobczak, NJ Hourgettes and **MR Bruchas** (2017) Dorsal-CA1 hippocampal neuronal ensembles encode nicotine-reward contextual associations. *Cell Reports*, Jun 6;19(10):2143-2156 PMC5550275

3. GPCR Signaling and Functional Selectivity

As a graduate student, post-doc, and PI I have been actively researching GPCR signaling in endogenous receptor systems. I have specifically focused on the role of GPCR signaling in activation of mitogen-activated protein kinase pathways through both G-protein and non-canonical arrestin pathways. My work as a post-doc uncovered novel arrestin-mediated signaling pathways that engage p38 and JNK MAPK pathways. We found that these pathways are critically activated in stress and result specific behavioral consequences including dysphoria-like behaviors, opening the field for potential investigation of opioid biased ligands (now pursued by numerous laboratories). Recent studies in my own laboratory have extended this work on GPCR signaling bias and functional selectivity to the nociceptin/orphaninFQ receptor system, as well as towards the development of optogenetic tools to control specific GPCR signaling outputs in awake, freely moving mice. We have also been actively been developing and characterizing Opto-XRs for in vivo manipulation of signaling.

a. E. R. Siuda, J.G. McCall, R. Al-Hasani, G. Shin, S. I. Park, M.J. Schmidt, S.L. Anderson, W.J. Planer, J.A. Rogers, and **M. R. Bruchas**. (2015b). Optodynamic simulation of Beta-adrenergic signaling. *Nature Communications*, Sep 28;6:8480. PMC4588095.

b. S.D. Chang, S.W. Mascarella, S.M. Spangler, H.A. Navarro, V.Gurevich, F. Carroll, and **M.R. Bruchas** (2015). Quantitative Signaling and structure-activity analyses reveal functional selectivity at the nociceptin/orphaninFQ opioid receptor. *Molecular Pharmacology*, Sep;88(3):502-11. PMC4551045

c. B. A. Copits, R. Gowrishankar, P. R. O'Neill, Jun-Nan Li, K. S. Girven, J.J. Yoo, X. Meshik, K. E. Parker, S. M. Spangler, A. J. Elerding, B. J. Brown, S. E. Shirley, K. K. L. Ma, A. M. Vasquez, M. C. Stander, V Kalyanaraman², S. K. Vogt, V.K. Samineni, T. Patriarchi, L. Tian, N. Gautam, R. K. Sunahara, Robert W. Gereau IV, **M. R. Bruchas**. (2021). A photoswitchable GPCR-based opsin for presynaptic inhibition. *Neuron*, May 10:S0896-6273(21)00307-X. PMC8194251

d. E.R. Siuda, M. Schmidt, B. Copits, M. Baird., R. Al-Hasani, J. McCall, W. Planer, R. Gereau, **M.R. Bruchas**. Spatiotemporal control of opioid signaling and behavior. (2015) *Neuron*, May 20;86(4):923-35. PMC4441608

4. Stress neural circuits, neuropeptides and signaling in reward and motivation

In my post-doctoral training and in the Bruchas laboratory, we are actively engaged in dissecting the neural circuits and signal transduction processes, by which neuromodulators control affective behaviors following stress. This includes studies to understand how stress is encoded in the brain in specific regions, as well as, how neuromodulators such as neuropeptides (opioids, CRF, galanin, etc) or monoamines (dopamine, norepinephrine, serotonin) act in specific regions and cell types. Using mouse genetics, optogenetics, physiology and behavioral pharmacology we have uncovered novel roles of kappa-opioid receptor signaling to MAPK pathways in neural circuits for motivated behaviors including dysphoria, social defeat, aversion, and

reinstatement of drug seeking. Our recent work had uncovered specific locus coeruleus noradrenergic system circuits and the role of central amygdala corticotropin releasing hormone (CRH) circuits in stress-induced anxiety.

a. J.G.McCall, R. Al-Hasani, E. R. Siuda, D. Y. Hong, C.P. Ford, and **M.R. Bruchas**. (2015) CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. *Neuron*, Aug 5;87(3):605-20. PMC4529361.

b. **Bruchas M.R.***, Schindler A.G, Shankar H., Messinger D.I., Miyatake M., Land B.B., Lemos, J.C., Hagan C., Neumaier J., Quintana A., Palmiter R., Chavkin C.* (2011) p38alpha MAPK deletion in serotonergic neurons produces stress-resilience in models of depression and addiction. *Neuron*, 72, 498-511. PMC3155685

•Cover article and featured preview publication, *co-corresponding

c. Luskin AT, Bhatti DL, Mulvey B, Pedersen CE, Girven KS, Oden-Brunson H, Kimbell K, Blackburn T, Sawyer A, Gereau RW 4th, Dougherty JD, **Bruchas MR**. (2021) Extended amygdala-parabrachial circuits alter threat assessment and regulate feeding. *Science Advances*. Feb 26;7(9):eabd3666. doi: 10.1126/sciadv.abd3666. PMC7909877.

d. R Al-Hasani*, J.-M T. Wong*, O. S. Mabrouk, J. G. McCall, G. P. Schmitz, K. A. Porter-Stransky, B. J. Aragona, R. T. Kennedy, **M. R. Bruchas** (2018) *In vivo* detection of optically-evoked neuropeptide release. *eLife*, Sep 3;7. pii: e36520. doi: 10.7554/eLife.36520. PMC6135606.

5. Pain and Interoception

As a post-doctoral fellow and PI I have been actively interesting in understanding both the peripheral and central mechanisms of chronic pain. We dissected the role of kappa and mu-opioid systems in chronic pain responses, and identified critical MAPK signaling pathways that mediate opioid mediated chronic pain-like behaviors.

a. Ippolito D.L.* , Xu, M.* , **Bruchas, M.R.**, Wickman, K. Chavkin, C. (2005) Tyrosine phosphorylation of K(ir)3.1 in spinal cord is induced by acute inflammation, chronic neuropathic pain, and behavioral stress. *J. Biol. Chem.* 280:41683-93. PMC2392895

b. Norris AJ, Shaker JR, Cone AL, Ndiokho IB, **Bruchas MR**. (2021) Parabrachial opioidergic projections to preoptic hypothalamus mediate behavioral and physiological thermal defenses. *eLife*. 2021 Mar 5;10:e60779. doi: 10.7554/eLife.60779. PMC7935488.

c. S. I. Park, D. Brenner , G. Shin , C. Morgan , B. Copits , H. Chung , M. Pullen , K. Nim Noh , S. Davidson , S. Ju Oh , J. Yoon , K-I Jang , V. Samineni , M. Norman , J. Grajales-Reyes , S. Vogt , S. Sudaram , K. Wilson , J. S. Ha , R. Xu , T. Pan , T. Kim , Y. Huang , M. Montana , J. Golden , **M.R. Bruchas**, R. Gereau, J. Rogers (2015). Soft, stretchable, fully implantable miniaturized optoelectronic systems for wireless optogenetics. *Nature Biotechnology*, Dec;33(12):1280-1286. PMC26551059.

d. N. Massaly, A. Wilson-Poe, Hipolito, T. Markovic, L. Shiwei, D. L. Bhatti, B. M. Walker, R. Neve, C. M. Cahill, K. Shoghi, R. Al-Hasani, **M. R. Bruchas*** and Jose A. Morón* (2019) Pain-induced Negative Affect is mediated via Recruitment of the Nucleus Accumbens Dynorphin-Kappa Opioid System. *Neuron*, doi: 10.1016/j.neuron.2019.02.029. PMC6509001 *co-corresponding author

BIOGRAPHICAL SKETCH

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

NAME: CHEER, Joseph, François

ERA COMMONS USER NAME (credential, e.g., agency login): JCHEER

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Universidad de los Andes, Bogotá, Colombia	B. S.	1996	Biology (Chem/Math minor)
University of Nottingham, Nottingham, UK	Ph.D.	2000	Neuroscience
Wake Forest University, Winston-Salem, USA	Postdoc	2000-2002	Neuroscience

A. Personal Statement

I am an expert on the neurobiology of reward and motivation, by virtue of my expertise in systems neuroscience. Namely, the exploration of neural circuits involved in the control of motivated behavior, and how different neuromodulator molecules modulate the neural substrates that may dysregulated in mental illness. I have conducted experiments for over 15 years addressing neural activity within the mesolimbic reward pathway. We have refined the electrochemical measurement of dopamine, endocannabinoids and other transmitters, allowing their measurement on the subsecond time scale. We complement this line of research with the use of ensemble recordings (using electrophysiology or calcium imaging) of neuronal activity in the brain paired with time-resolved cell-specific manipulation techniques such as optogenetics and fiber photometry.

I have been involved in education of graduate and medical students for over 15 years and of postdoctoral fellows for nearly as long. I have trained 10 postdoctoral fellows most of whom have gone on to secure independent positions. I have also trained 9 graduate students as well as two physician scientists, who are now attending physicians. Additionally, I have trained over 30 undergraduates who have successfully gone on to complete their Ph.D. or M.D.s.

B. Positions, Scientific Appointments, and Honors**Employment**

- Laboratory Manager, 1996 - 1997
Laboratory of Microsurgery and Experimental Neurology, Colombian Neurological Institute, Colombia
- Graduate Student in Neuroscience, 1997 - 2000
Neuroscience Section, School of Biomedical Sciences, University of Nottingham, Nottingham, UK (C.A. Marsden)
- Post-doctoral Research Fellow, 2000 - 2002
Department of Physiology and Pharmacology, Wake Forest University, Winston-Salem, NC (S.A. Deadwyler)
- Research Associate 2002 - 2005
Department of Chemistry and Neuroscience Center, University of North Carolina, Chapel Hill, NC (R.M. Wightman)
- Research Assistant Professor 2005 - 2006

Department of Psychology, University of North Carolina, Chapel Hill, NC

- Assistant Professor (tenure-track) 2006 - 2008
Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, NY
- Assistant Professor (tenure-track) 2008 - 2012
Department of Anatomy and Neurobiology, University of Maryland Medical School, Baltimore, MD
- Associate Professor (tenured) 2012 - 2017
Department of Anatomy and Neurobiology, University of Maryland Medical School, Baltimore, MD
- Full Professor (tenured) 2017 - present
Department of Anatomy and Neurobiology, University of Maryland Medical School, Baltimore, MD

Professional membership

- 1997 – 2000 British Association of Psychopharmacology
- 1997 – 2000 British Pharmacological Society
- 1999 – present Society for Neuroscience
- 2000 – present International Cannabinoid Research Society

Awards

- 1996 Pre-doctoral Scholarship from the Colombian Science Foundation
1997 Glaxo-Wellcome Ph.D. Studentship
1997 British Pharmacological Society travel award
1997 British Association of Psychopharmacology travel award
1998 British Association of Psychopharmacology award
1999 Brain ®/IBRO award
1999 British Pharmacological Society travel award
2001 Coy Waller Merit Award on Cannabinoid Research
2001 International Cannabinoid Research Society/NIDA travel award
2003 10th International Conference on In Vivo Methods travel award
2004 Cover, *The Journal of Neuroscience*, Volume 24, Number 18
2004 Smallwood Award for Teaching Undergraduate Research, UNC Chapel Hill
2005 International Cannabinoid Research Society/NIDA travel award
2006 Winter Conference on Brain Research Fellowship
2007 Albany Medical College Junior Faculty Award
2008 NARSAD Young Investigator Award
2010 Top reviewer for "Neuropharmacology"
2012 Cachope et al., *Cell Reports*, Faculty of 1000, *Neuron* commentary
2012 Oleson et al., *Neuron*, *Neuron* spotlight
2013 Plenary speaker for the Carolina Cannabinoid Collaborative Annual meeting
2014 Accepted into the American College of Neuropsychopharmacology
2014 Hernandez et al., *Biological Psychiatry*, featured article and commentary
2015 Invited Visiting Professorship (UNICA VP), University of Cagliari, Italy
2016 Covey et al., *The Journal of Neuroscience*, featured article
2017 Sterling Drug Lecturer, Boston University
2017 Plenary Speaker, International Cannabinoid Research Society (declined)
2017 GPILS/OPS Postdoctoral Mentor Award at UMSOM
2018 STAR-PREP Excellence in Mentoring Award
2018 Accepted for full membership at the American College of Neuropsychopharmacology
2019 Plenary Speaker, Gordon Research Conference on Cannabinoid Function in the CNS
2019 Plenary Speaker, Spanish Cannabinoid Society
2019 Biomedical Distinguished Lecturer, The Frank Reidy Center for Bioelectronics, Old Dominion University

Editorial boards: *Frontiers in Neuropharmacology* (2011-present), *Journal of Neuroscience* (2015-present), *Cannabis and Cannabinoid Research* (2016-present), *Journal of Cannabis Research* (2018-present), *eLife* (2017-present)

Ad hoc reviewer peer-reviewed journals

Science (2011-present)
Neuron (2010-present)
Journal of Neuroscience (2006-present)
Proceedings of the National Academy of Sciences of the USA (2008-present)
Neuropharmacology (2006-present)
Neuroscience (2004-present)
Neuropsychopharmacology (2007-present)
Behavioral Brain Research (2009-present)
Cerebral Cortex (2010-present)
Journal of Neurochemistry (2008-present)
Experimental Neurology (2009-present)
Behavioral Neuroscience (2010- present)
European Journal of Neuroscience (2006-present)
Journal of Neurophysiology (2008-present)
PLOS One (2010-present)
Basal Ganglia (2010-present)
Biological Psychiatry (2010-present)

C. Contribution to Science

The nucleus accumbens as a limbic motor integrator in motivation

The nucleus accumbens is a brain region with an important role in processing reward information and the selection of behaviors appropriate to the context. It has been proposed that one mechanism by which context guides response selection in the nucleus accumbens is via a synaptic interaction that has been characterized as a gating mechanism; hippocampal inputs are believed to be necessary for other inputs to drive action potential firing in the nucleus accumbens. Although the gating hypothesis has received further support from several studies, novel data indicate that the nucleus accumbens could disengage from its hippocampal inputs and follow prefrontal activity during epochs in which selection of a behavior is required. We proposed that the nucleus accumbens behaves a switchboard where response selection takes place by the interactions among multiple afferents. Here we have found that it is critical for decision-making and selection of the appropriate behavioral response to a given context in two different operant tasks. Furthermore, strong prefrontal afferent activation attenuates the impact of hippocampal afferents and this interaction is dependent on local GABA or dopamine release.

Morra JT, Glick SD, **Cheer JF**. (2012) Cannabinoid receptors mediate methamphetamine induction of high frequency gamma oscillations in the nucleus accumbens. *Neuropharmacology*. 63: 565-574 PMID: 22609048

Hernandez G, **Cheer JF**. (2012) Effect of CB1 receptor blockade on food-reinforced responding and associated nucleus accumbens neuronal activity in rats. *J Neurosci* 32: 11467-11477 PMID: 22895729

Oleson EB, Beckert MV, Morra JT, Lansink CS, Cachope R, Abdullah RA, Loriaux AL, Schetters D, Patti T, Roitman MF, Lichtman AH, **Cheer JF**. (2012) Endocannabinoids shape accumbal encoding of cue-motivated behavior via CB1 receptor activation in the ventral tegmentum. *Neuron*. 73: 360-373. PMID: 22284189

Covey DP, **Cheer JF**. (2019) Accumbal Dopamine Release Tracks the Expectation of Dopamine Neuron-Mediated Reinforcement. *Cell Rep*. 27: 481-490 PMID: 30970251

Endogenous cannabinoids are canonical mediators of motivation

Phasic dopamine release in the nucleus accumbens has traditionally been associated with motivated behaviors driven by primary rewards such as food. However, there has been less investigation regarding how aversively conditioned cues engage motivational networks. This is notable since avoidance of aversive stimuli can be highly motivating and because the nucleus accumbens is a limbic-motor interface connected to several key nodes of the fear network. Recent work from our laboratory supports an important role for phasic accumbal dopamine release in the avoidance of punishment. Specifically, release is suppressed during cues associated with fear and enhanced upon cues linked to active avoidance. It is theorized that dopamine contributes to these processes by modulating the invigorating action of learned associations on instrumental responding, a mechanism essential for behavior to occur. We see that patterns of release seen during active avoidance conform to theories involving dopamine and they causally influence behavior. We interfered with or facilitated endocannabinoid tone in the ventral tegmentum while additional optogenetic control of dopamine neurons has allowed explicit tests of current hypotheses related to endocannabinoid function.

Hernandez G, Oleson EB, Gentry RN, Abbas Z, Bernstein DL, Arvanitogiannis A, **Cheer JF**. (2014) Endocannabinoids promote cocaine-induced impulsivity and its rapid dopaminergic correlates. *Biol Psychiatry*. 75: 487-498. PMID: 24138924

Oleson EB, Cachope R, Fitoussi A, Tsutsui K, Wu S, Gallegos JA, **Cheer JF**. (2014) Cannabinoid receptor activation shifts temporally engendered patterns of dopamine release. *Neuropsychopharmacology*. 39: 1441-1452 PMID: 24345819

Wenzel JM, Oleson EB, Gove WN, Cole AB, Gyawali U, Dantrassy HM, Bluett RJ, Dryanovski DI, Stuber GD, Deisseroth K, Mathur BN, Patel S, Lupica CR, **Cheer JF**. (2018) Phasic Dopamine Signals in the Nucleus Accumbens that Cause Active Avoidance Require Endocannabinoid Mobilization in the Midbrain. *Curr Biol*. 28: 1392-1404 PMID: 29681476

Frau R, Miczán V, Traccis F, Aroni S, Pongor CI, Saba P, Serra V, Sagheddu C, Fanni S, Congiu M, Devoto P, **Cheer JF**, Katona I, Melis M. (2019) Prenatal THC exposure produces a hyperdopaminergic phenotype rescued by pregnenolone. *Nat Neurosci*. 22: 1975-1985 PMID: 31611707

Harnessing the power of endocannabinoid signaling to predict Huntington's disease

Huntington's disease (HD) is a neurodegenerative disorder caused by a mutation in the huntingtin gene. Among the most predominant symptoms, motor and cognitive alterations have some of the most detrimental effects on the individual's life. The striatum is the first region affected by the progressive neurodegeneration that spreads through the cortex and other subcortical areas during late stages of the disease. While accepted medication is based on reducing dopaminergic function, inconsistent data report diminished dopaminergic function in some HD patients, as well as in animal models of the disease. Therefore, the elucidation of endophenotypes with appropriate predictive validity for hypodopaminergic activity as well as disease progression is required. Importantly, loss of cannabinoid type 1 receptors (CB1) is a critical pathogenic factor in HD. Indeed, several components of the endogenous cannabinoid (eCB) system, including altered synthetic and degradative enzyme as well as expression of CB1 receptors, have been reported. We are performing a detailed characterization of physiological markers of eCB function in contemporary animal models of Huntington's disease, such as the recently generated knock-in Z_Q175_KI mouse.

Covey DP, Dantrassy HM, Zlebnik NE, Gildish I, **Cheer JF** (2016) Compromised dopaminergic encoding of reward accompanying suppressed willingness to overcome high effort costs is a prominent prodromal characteristic of the Q175 mouse model of Huntington's disease. *J. Neurosci.*, 36: 4993-5002 PMID: 27147652

Covey DP, Dantrassy HM, Yohn SE, Castro A, Conn PJ, Mateo Y, **Cheer JF**. (2018) Inhibition of endocannabinoid degradation rectifies motivational and dopaminergic deficits in the Q175 mouse model of Huntington's disease. *Neuropsychopharmacology*. 43: 2056-2063 PMID: 29925886

Zlebnik NE, Gildish I, Sesia T, Fitoussi A, Cole EA, Carson BP, Cachope R, **Cheer JF**. (2019) Motivational Impairment is Accompanied by Corticoaccumbal Dysfunction in the BACHD-Tg5 Rat Model of Huntington's Disease. *Cereb. Cortex*. 29: 4763-4774 PMID: 30753343

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/collections/bibliography/40754008/>

BIOGRAPHICAL SKETCH

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

NAME: Dalkilic, Mehmet Miray

ERA COMMONS USER NAME (credential, e.g., agency login): Dalkilic (Person ID: 14089715)

POSITION TITLE: Professor of Computer Science

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Indiana University, Bloomington	BA	05/1988	Chemistry
Indiana University, Bloomington	MS	05/1996	Computer Science
Indiana University, Bloomington	PhD	05/2000	Computer Science

A. Personal Statement

I am a Professor in Computer Science, the Data Science Program, and an Adjunct in the Statistics Department. I work in the area of data science. Specifically, I work on improving AI and ML algorithms to work with big data; I work in specific STEM areas: astronomy, geosciences, and recently material science. I was the first faculty at the School of Informatics, Computing, and Engineering. I created the introductory curriculum, initially built the various research areas (graduate computational biology program), and most recently created a new introductory computer science/data science/statistics class that serves as the major for three departments. After graduating with chemistry, I was in Indiana University's MD/PhD program (Neurobiology then switched to Biochemistry). I began taking computer science classes and discovered it to be the vocation that was best suited for me.

B. Positions, Scientific Appointments, and Honors

2021-date Professor
 2019-date Adjunct Statistics Department/Undergraduate Director Data Science
 2017-date Visiting Scientist, Crane NSWC
 2007-date Associate Professor, Computer Science
 2002-2007 Assistant Professor, School of Informatics
 2000-2002 Visiting Assistant Professor, School of Informatics, Indiana University
 1999-2000 Lecturer, Computer Science, Rose-Hulman Institute of Technology
 1989-1998 Associate Instructor, Computer Science, Indiana University

C. Contributions to Science

I graduated the first PhD in Computation Biology and our work was the first to demonstrate the power of integrating experimental data for *D. melanogaster*

Costello, Dalkilic MM, Beason SM, Gehlhausen JR, Patwardhan R, Middha S, Eads BD, Andrews JR, "Gene networks in *Drosophila melanogaster*: integrating experimental data to predict gene function". *Genome Biology*, 16 Sep 2009, 10(9):R97

Expanding to larger data sets we produced what would be the beginning of a novel characterization of heaps and a new concept of "data expression"

Mark Jenne, Owen Boberg, Hasan Kurban, Mehmet Dalkilic. Studying the Milky Way Galaxy Using ParaHeap-k September 2014 Computer 47(9):26-33

This lead to a new, more powerful extension of expectation maximization that utilized convergence on structures allowing for much larger data sets:

Hasan Kurban, Mark Jenne and Mehmet M. Dalkilic: Using data to build a better EM: EM* for big data. International Journal of Data Science and Analytics (JDSA), 4(2), pp. 83-97. (2017)

Currently we are working in material science focusing on nanoparticles:

1. Hasan Kurban, Mustafa Kurban, Parichit Sharma and Mehmet M. Dalkilic . "Predicting Atom Types of Anatase TiO₂ Nanoparticles with Machine Learning". *Key Engineering Materials*, vol.880, pp.89-94, 2021
2. Mustafa Kurban, Hasan Kurban and Mehmet M. Dalkilic. "Controlling structural and electronic properties of ZnO NPs". *Bilge International Journal of Science and Technology Research*, 3(0), 35-39, 2019.
3. Hasan Kurban, Mustafa Kurban, and Mehmet M. Dalkilic. "Rapidly predicting Kohn-Sham total energy using data centric AI. *Sci Rep.* 2022 Aug 24;12(1):14403. doi: 10.1038/s41598-022-18366-7. PMCID: PMC9402589
4. Malec M, Kurban H, Dalkilic M. "cclImpute: an accurate and scalable consensus clustering based algorithm to impute dropout events in the single-cell RNA-seq data." *BMC Bioinformatics*. 2022 Jul 22;23(1):291. doi: 10.1186/s12859-022-04814-8. PMCID: 9306045

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Dani, John A.

eRA COMMONS USER NAME (credential, e.g., agency login): JADANI

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
University of Michigan	B.S.	1975	Atm. & Ocean Science
University of Minnesota	Ph.D.	1980	Physiology
University of Washington	Postdoc	1982	Physiol. & Biophysics,
UCLA	Postdoc	1984	Physiology

A. Personal Statement

My lab's efforts arise from the hypothesis that fundamental mechanisms underlie the communication and adaptability of nervous system circuitry. Cellular and circuit mechanisms that normally serve the brain (particularly those that underlie neural plasticity and learning) are damaged, altered, and misdirected during addiction. Therefore, fundamental mechanisms underlying neuronal function offer points of entry for pharmacological, physiological, and genetic methods aimed at relieving or preventing addictions. From this research perspective, my laboratory has made contributions toward our understanding of ion channel biophysics, synaptic plasticity, learning, and memory, particularly as those mechanisms underlie addictive drug use.

My lab's recent focus arises from the finding that stress, which signals via common neural circuitry with addictive drugs, alters midbrain circuitry and increases the vulnerability for heavier drug self-administration. We discovered that stress acts via the glucocorticoid system to downregulate the transporter, KCC2, which defines the Cl⁻ gradient of some CNS neurons, including midbrain GABA neurons. Most recently, we have found that cocaine, acting via a different cellular mechanism, also causes functional downregulation of KCC2, altering midbrain GABAergic circuitry, and increasing cocaine self-administration itself. We have been captivated by these findings because after decades of outstanding research advances, there are still no FDA-approved therapies to decrease cocaine use disorder. KCC2 is a very accessible potential therapeutic target, which has been targeted for peripheral nerve pain. Therefore, our recent work aims to examine KCC2's regulation of cocaine self-administration and use multiple approaches for potential therapies to mitigate cocaine abuse.

To understand the consequences of KCC2 downregulation on cocaine self-administration, we apply a wide range of techniques: ex vivo brain slice electrophysiology, in vivo electrophysiology, local-infusion for in vivo pharmacology and genetic manipulations, and diverse behavioral approaches along with addictive drug self-administration. In the strong research environment provided by the neurosciences at Penn and with outstanding lab personnel and local collaborators, we provide a very strong training environment. I am committed to pre-doctoral and post-doctoral training since I have trained 8 pre- and 30 post-doctoral fellows. With respect to this application I can provide research training in the area of in vitro and vivo electrophysiology, diverse in vivo neuronal manipulations, and many diverse behavior paradigms.

B. Positions and HonorsPositions and Employment

2013-present	Chair, Dept. of Neuroscience, Perelman School of Medicine, University of Pennsylvania Director, Mahoney Institute for Neurosciences, University of Pennsylvania
2010-2013	Director, Center on Addiction, Learning, Memory, BCM
2007-2010	Merit Appointment, Michael E. DeBakey VA Medical Center, Houston, TX
2005-2013	Prof., Graduate Program in Translational Biology & Molecular Medicine, BCM (2ndary)
2004-2013	Prof., Menninger Dept of Psychiatry & Behavioral Sciences, BCM (2ndary)

1999-2013	Professor, Dept of Neuroscience, BCM (primary appointment)
1999-2011	Exec Committee of Grad Prog Structural and Computational Biology and Molecular Biophysics
1991-1999	Associate Professor, Dept of Neuroscience, BCM
1987-1991	Assistant Professor, Dept of Physiology & Biophysics, Baylor College of Medicine (BCM)
1984-1987	Research Associate Scientist, Section of Molecular Neurobiology, Yale University

Selected Honors

2017	Distinguished Lecturer, School of Medicine, University of Virginia
2012	Keynote Speaker, Motivated behavior, stress and addiction, Federation Neurosci. Soc., Chile
2011	Frank G. Standaert Lectureship in Pharmacology, Georgetown University
2005	Killam Lecturer, Montreal Neurological Institute, McGill University
2004	Distinguished Lecturer, Biological Sciences Training Program, Yale University Medical School
2003	Wiersma Visiting Professorship, Dept of Biology, California Institute of Technology
2000	Jacob Javits Neuroscience Award from NIH, National Inst. of Neurological Disorders & Stroke
1998-1999	Visiting Scholar, Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology
1998	DeBakey Award for outstanding research, BCM
1984-1987	NIH New Investigator Research Award
1982	Bacaner Basic Science Award for excellence in research, Mn Medical Foundation
1975	Distinguished Achievement Award; and Graduated <i>summa cum laude</i> , Univ. of Michigan, Col. of Engineering

Selected National Scientific Participation and Editorial Boards

2020, June	NIH grant review, Special Study Section, ZRG1 IFCN E02
2019, Nov	NIH grant review, Special Emphasis Panel, Tobacco Regulatory Science
2018, Nov	NIH grant review, Special Emphasis Panel, Tobacco Regulatory Science
2017, Dec	NIH grant review, ZRG1 BBB-Y Biobehavioral Applications on Substance Abuse and Reward
2016, Nov	NIH grant review, ZRG1 IFCN-L 02 M, Neuroendocrinology, Sleep, Stress, and Alcohol
2016, Feb	NIH grant review, ZRG1IFCN-C(02), Special Emphasis Panel: Alcohol, Drugs, Neurotoxicology
2015-present	Editorial Board for International Archives of Addiction Research and Medicine
2015	NIH grant review, ZRG1 IFCN-C, Special Emphasis Panel: Drugs, Alcohol and Heavy Metals.
2015	External reviewer, University of Texas Biomed. Sci. Neuroscience Program
2015	NIH grant review, ZRG1 IFCN-C(02), Special Emphasis Panel: Alcohol, Neurotoxicology, Drugs.
2015	Review panel, Ford Foundation Fellowship Program
2014-present	Editorial Board of Journal of Neurology and Brain Disorders, Ommega Publishers
2014-present	Editorial Board for Journal of Neuroscience and Neuroeconomics
2014	NIH grant review, NINDS NST-2 F31 and K99 applications
2013, '15	Co-Editor with Bertrand and Donnelly-Roberts, Special Issue, "nAChRs as Therapeutic Targets
2013	NIH grant review, FDA-NIH Tobacco related applications
2012-present	Editorial Board for Journal of Clinical & Experimental Pharmacology
2012	Chaired, NIH grant review, ZRG1 MDCN-F (50) P; & NIH review, Director's Pioneer Awards
2012	NIH grant review, ZRG1 IFCN-C (02) M Special Emphasis Panel: Drugs,
2011-present	Editorial Board for World Journal of Pharmacology
2011-present	Editorial Member, Frontiers in Bioscience
2011, '13	Advisory Board, nAChRs as Therapeutic Targets, 3rd & 4th Satellite Meeting to SfN
2011	Advisory Board, Wellcome Trust Conference, Nicotinic Acetylcholine Receptors
2011	NIH grant review, IFCN-C, Neurotoxicology and Alcohol
2011	NIH grant review, Special Emphasis Panel/Scientific Review Group, ZDA1 JXR-D
2011	NIH grant review, P01s, Medications Development program projects
2011	Chaired, NIH grant review, PPG, Neural Plasticity program projects
2010-present	Editorial Board for Journal of Addiction Research & Therapy
2010	NIH grant review, NIDA CEBRA, ZDA1 GXM-A
2010	NIH grant review, Special Emphasis Panel, ZRG1, IFCN-H
2009-present	Editorial Board for Frontiers in Neuroscience
2009-present	Honorary Editorial Board of Journal of Experimental Pharmacology
2009	NIH grant review, Scientific Areas of Integrated Review Groups, IRG [MDCN]
2009	NIH grant review, Challenge Grants

2008,'13'	Section Editor, Kosten, Dani, Gorelick, ASAM, Principles of Addiction Medicine, 4th & 5th ed
2008	NIH, NIAAA, Mechanism of Behavior Change Initiative
2008	VA Merit Review Subcommittee for Mental Health
2007	NIH study section for NGDDG Drug Discovery, ZMH1-ERB-Y02
2006, Mar	NIH study section ad hoc, MDCN-F 02, Biophysics & Protein Interact., Special Emphasis Panel
2006	NIH study section ad hoc for section NTRC
2005	NIH study section ad hoc for Neurobiology of Motivated Behavior
2004	NIH NIDA ad hoc reviewer for Cutting-edge Basic Research Awards
2003-2016	Contributing member for Faculty of 1000
2003	NIH, NIAAA Workshop on Multiscale Systems Neuroscience
2003	NIH, NIDA Binational Workshop on Drug Abuse and Addiction Research
2002	Guest Editor with D Berg for a special issue of Journal of Neurobiology
2001	NIH study section ad hoc for MDCN-4; and NIH study section ad hoc for MDCN-5
2000	NIH study section review, NIDA, Microarray-Based Res. on Drug Abuse
2000	NIH NIDA, Cell Biology of Addiction, development of future funding
2000	Member, Scientific Review of Biostatistics Branch and Tenure, NIH NIEHS, NC
2000	NIH study section ad hoc outside opinion, NINDS council ZRG1 MDCN-5
1999	NIH study section review, NIDA, Collaborative Site Grants for Addiction
1998	NIH study section acting chair, Neurological Sciences 2
1998-2010	Editorial Board for the Journal of General Physiology
1997-1998	Reviewing Editor of Editorial Board for The Journal of Neuroscience
1991-1998	NIH study section special session review for Physiology Section; Neurological Sciences 2
1990-1996	Editorial board for The Journal of Theoretical Biology

C. Contributions to Science

1. Stress exposure induces increased drug self-administration in rats: Our studies revealed a complex combination of mechanisms that act through the stress axis to influence synaptic plasticity, midbrain circuitry, cellular receptor systems, and drug co-morbidity of alcohol, nicotine, and other addictive drugs. For example, alcohol and nicotine reinforcement and stress involve common neural circuitry, including the mesolimbic system. We demonstrated in rats that stress boosts corticosterone levels and acting via glucocorticoid receptor activity causes downregulation of the K⁺, Cl⁻ cotransporter, KCC2, located on midbrain GABA neurons. We also showed that adolescent drug exposure produces a long-lasting change in midbrain GABAergic circuitry, but adult exposures to drug or stress produce only short-term changes before the circuitry spontaneously returns to the initial state. In both cases, this functional downregulation of KCC2 alters midbrain GABAergic circuitry leading to increased drug self-administration. Blocking stress hormone receptors prior to stress or drug exposure or enhancing KCC2 activity prevented the influence over midbrain GABAergic circuitry and normalized alcohol self-administration. These results indicated that, like stress, addictive drugs can recruit neuroendocrine systems to influence neurotransmission and behavior associated with drug use. A pre-exposure to stress produces alterations in CNS signaling via glucocorticoid receptors such that alcohol is more highly self-administered.

- a. Ostroumov A, Thomas AM, Kimmey BA, Karsch JS, Doyon WM, Dani JA (2016) Stress Increases ethanol self-administration via a shift toward excitatory GABA signaling in the ventral tegmental area. **Neuron** 92(2):493-504. PMID: 27720487
- b. Thomas AM, Ostroumov A, Kimmey BA, Taormina MB, Holden WM, Kim K, Brown-Mangum T, Dani JA. (2018) Adolescent Nicotine Exposure Alters GABAA Receptor Signaling in the Ventral Tegmental Area and Increases Adult Ethanol Self-Administration. **Cell Reports** 23(1):68-77. PMID: 29617674
- c. Doyon WM, Dong Y, Ostroumov A, Thomas AM, Zhang TA, Dani JA (2013) Nicotine decreases ethanol-induced dopamine signaling and increases self-administration via stress hormones. **Neuron** 79:530-40. PMID: 23871233
- d. Kimmey BA, Ostroumov A, Dani JA (2019) 5-HT2A receptor activation normalizes stress-induced dysregulation of GABAergic signaling in the ventral tegmental area. **Proc Natl Acad Sci U S A**. pii: 201911446. doi: 10.1073/pnas.1911446116. PMID: 31806759

2. Addiction, plasticity, learning, and memory: We have shown that addictive drugs (i.e., cocaine, methylphenidate, nicotine) induce synaptic changes in the brain that are comparable to those caused during learning and memory. We provided evidence showing that environmental stimuli repeatedly linked to addictive drugs become learned associations, and those stimuli come to elicit memories or sensations that motivate

continued drug use. Applying *in vivo* recording techniques to freely moving mice or rats, we showed that physiologically relevant concentrations of the addictive drug directly cause *in vivo* hippocampal synaptic potentiation of the kind that underlies learning and memory. The drug-induced long-term synaptic plasticity required local hippocampal circuitry signals combined with long-range afferents into the hippocampus. Disrupting general dopamine signaling prevented the drug-induced synaptic plasticity and various linked behaviors. These results indicated that dopaminergic signaling serves as a functional label of salient events by enabling and scaling synaptic plasticity that underlies drug-induced associative memory. Cues related to those memories then are able to shape behavior. Normally this association of environmental input and memory works to cue successful behaviors, but memories associated with addictive drugs act to perpetuate continued drug use.

- a. Broussard et al., (2016) Dopamine regulates aversive contextual learning and associated *in vivo* synaptic plasticity in the hippocampus. **Cell Reports** 14(8):1930-9. PMID: 26904943.
- b. Yang K, Dani JA (2014) Dopamine D1 and D5 receptors modulate spike timing-dependent plasticity at medial perforant path to dentate granule cell synapses. **J Neuroscience** 34(48):15888-97. PMID: 25429131
- c. Doyon WM, Ostroumov A, Ontiveros T, Gonzales RA, Dani JA (2021) Ethanol produces multiple electrophysiological effects on ventral tegmental area neurons in freely moving rats. **Addiction Biology** 26(2):e12899. doi: 10.1111/adb.12899. PMID: 32255261
- d. Ostroumov A, Wittenberg RE, Kimmey BA, Taormina MB, Holden WM, McHugh AT, Dani JA (2020) Acute Nicotine Exposure Alters Ventral Tegmental Area Inhibitory Transmission and Promotes Diazepam Consumption. **eNeuro** 7(2). PMID: 32102779

3. *Cocaine use is regulated by nicotinic mechanisms and midbrain synaptic potentiation:* Nicotinic acetylcholine receptors (nAChRs) potently regulate dopamine (DA) release in the striatum and alter cocaine's ability to reinforce behaviors. We found that biologically relevant concentrations of cocaine can mildly inhibit nAChR-mediated currents in midbrain DA neurons and consequently alter DA release in the dorsal and ventral striatum. Furthermore, our results showed that partial inhibition of nAChRs by cocaine reduces tonic DA release. This diminution of DA release via nAChR inhibition more strongly influences release evoked at low or tonic stimulation frequencies than at higher (phasic) stimulation frequencies, particularly in the dorsolateral striatum. This cocaine-induced shift favoring phasic DA release may contribute to the enhanced saliency and motivational value of cocaine-associated memories and behaviors linking nicotine and cocaine use. Animal models also show that potentiation of excitatory synaptic transmission onto ventral tegmental area (VTA) DA neurons is a critical component of sustained drug seeking. We identified that translational control by eIF2α phosphorylation (p-eIF2α) regulates cocaine-induced potentiation in the VTA. Then, we found that in mice with reduced p-eIF2α-mediated translation, cocaine induces persistent potentiation in VTA DA neurons. Moreover, selectively inhibiting eIF2α-mediated translational control with a small molecule, ISRB, or knocking down oligophrenin-1 (a mRNA whose translation is controlled by p-eIF2α) in the VTA also prolongs cocaine-induced synaptic potentiation. This persistent synaptic potentiation is mediated by the insertion of GluR2-lacking AMPARs into the synaptic membrane. Collectively, our findings suggest that eIF2α-mediated translational control regulates the progression from transient to persistent cocaine-induced potentiation and prolonged enhanced use.

- a. Placzek AN, Prisco GV, Khatiwada S, Sgritta M, Huang W, Krnjević K, Kaufman RJ, Dani JA, Walter P, Costa-Mattioli M (2016) eIF2α-mediated translational control regulates the persistence of cocaine-induced LTP in midbrain dopamine neurons. **eLife**. 5: e17517. doi: 10.7554/eLife.17517. PMID: 27960077
- b. Acevedo-Rodriguez A, Zhang L, Zhou F, Gong S, Gu H, De Biasi M, Zhou FM, Dani JA (2014) Cocaine inhibition of nicotinic acetylcholine receptors influences dopamine release. **Frontiers in Synaptic Neuroscience** 6(19). PMID: 25237305
- c. Huang W, Placzek AN, Viana Di Prisco G, Khatiwada S, Sidrauski C, Krnjević K, Walter P, Dani JA, Costa-Mattioli M. (2016) Translational control by eIF2α phosphorylation regulates vulnerability to the synaptic and behavioral effects of cocaine. **eLife**. 5. pii: e12052. doi: 10.7554/eLife.12052. PMID: 26928234
- d. Placzek AN, Molfese DL, Khatiwada S, Viana Di Prisco G, Huang W, Sidrauski C, Krnjević K, Amos CL, Ray R, Dani JA, Walter P, Salas R, Costa-Mattioli M. (2016) Translational control of nicotine-evoked synaptic potentiation in mice and neuronal responses in human smokers by eIF2α. **eLife**. 5. pii: e12056. doi: 10.7554/eLife.12056. PMID: 26928076

4. Regulation of dopamine (DA) signaling via local activity: We found that nicotinic cholinergic activity potently regulates endogenous DA release throughout the CNS, but especially in the nucleus accumbens. This complex and potent regulation of DA release by endogenous nicotinic activity and other locally acting factors has broad implications for memory mechanisms and potential therapies for degenerative decline of cholinergic or dopaminergic systems. We showed that inhibition of ongoing nicotinic activity can decrease evoked dopamine release by up to 80% in some areas of the brain, and under those circumstances, only phasic burst firing of DA neurons can produce meaningful dopamine release. We also showed that drugs used to treat Alzheimer's disease via cholinergic mechanisms also enhance DA release and in that way contribute to the positive influences of Alzheimer's disease therapies.

- a. Zhou FM, Liang Y, Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. **Nature Neuroscience** 4:1224-1229. PMID: 11713470
- b. Zhang L, Doyon WM, Clark JJ, Phillips PE and Dani JA (2009) Controls of tonic and phasic dopamine transmission in the dorsal and ventral striatum. **Molecular Pharmacology** 76(2):396-404. PMID: 19460877.
- c. Zhou FM, Liang Y, Salas R, Zhang L, De Biasi M, Dani JA. (2005) Corelease of dopamine and serotonin from striatal dopamine terminals. **Neuron** 46:65-74. PMID: 15820694 (Highlighted for Preview by Neuron)
- d. Zhang L, Dong Y, Doyon WM, Dani JA (2012) Withdrawal from chronic nicotine exposure alters dopamine signaling dynamics in the nucleus accumbens. **Biological Psychiatry** 71(3):184-91. PMID: 21872847

5. Nicotine mechanistically acts upon dopamine centers underlying addiction: We were the first to show that nicotine acts mechanistically by directly activating midbrain dopamine neurons and producing a DA signal indicative of all psychostimulant addictive drugs. At the time of the Nature publication, the class action court case against the tobacco companies was underway. Our evidence garnered national and international attention, producing direct evidence that nicotine acts mechanistically like all other well-known addictive drugs by activating DA neurons. We furthermore showed that this activity caused broad neuroadaptive and maladaptive effects on the central nervous system, including forms of receptor regulation and synaptic plasticity that underlie the addiction process. The work also revealed endogenous nicotinic mechanism that contribute to and regulate synaptic plasticity underlying learning and memory.

- a. Pidoplichko V, DeBiasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. **Nature** 390:401-404. PMID: 9389479
- b. Gray R, Rajan AS, Radcliffe KA, Yakehiro M, Dani JA. (1996) Hippocampal synaptic transmission enhanced by low concentrations of nicotine. **Nature**. 1996 Oct 24;383(6602):713-6.
- c. Zhang T, Tang J, Pidoplichko VI, Dani JA (2010) Addictive nicotine alters local circuit inhibition during the induction of in vivo hippocampal synaptic potentiation. **J Neuroscience** 30:6443-53. PMID: 20445070
- d. Ostroumov A, Dani JA (2018) Convergent Neuronal Plasticity and Metaplasticity Mechanisms of Stress, Nicotine, and Alcohol. **Annu Rev Pharmacol Toxicol.** 58:547-566. PMID: 28977763

Complete List of Published Work, MyBibliography: <http://www.ncbi.nlm.nih.gov/pubmed?term=dani%20ja>

BIOGRAPHICAL SKETCH

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

NAME: ADITI DAS

eRA COMMONS USER NAME (credential, e.g., agency login): ADITI_DAS

POSITION TITLE: Associate Professor of Chemistry and Biochemistry, Georgia Institute of Technology

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
St. Stephen's College, Delhi University, India	B.S.	07/1996	Chemistry
Indian Institute of Technology, Kanpur, India	M.S.	07/1998	Chemistry
Princeton University, New Jersey	Ph.D.	11/2005	Chemistry
University of Illinois Urbana Champaign, Illinois	Postdoctoral	05/2011	Biochemistry/Bio physics

A. Personal Statement

My expertise is in the area of membrane protein biochemistry and targeted lipidomics of lipid metabolites. My current research focus is on the biochemistry of eicosanoid synthesizing cytochrome P450 (CYP) epoxygenases that are implicated in the generation of anti-inflammatory lipid metabolites from the metabolism of dietary omega-3 and omega-6 fatty endocannabinoids and phytocannabinoids.

Research Training: I obtained my Ph.D. at Princeton University in Chemistry. I was awarded FMC graduate fellowship, first year science and engineering fellowship and was in the Dean's list for research excellence during my graduate studies. My research focused on functional studies of *de novo* designed heme proteins. I pursued post-doctoral research with Prof. Stephen Sligar where I studied the modulation of the redox potential of cytochrome P450 by the lipid bilayers of Nanodiscs. I obtained NSEC-NSF postdoctoral fellowship from Northwestern University. I was awarded the "Outstanding Researcher Award" for pioneering several new nanodisc techniques to study membrane proteins on engineered surfaces.

Independent Research: My research on eicosanoid synthesizing epoxygenases was initiated independently in my research laboratory. I have published multiple papers, of which ~35 have been published and/or submitted from my independent laboratory, which focuses on lipid metabolism and protein structure/function of CYP2J2 and other related proteins (*h-index* =27). One of my papers on drug metabolism was recipient of Colin Wraight best paper award from the Department of Biochemistry, Gordon Hammes Runners Up at ACS Biochemistry and recommended by the Faculty of 1000. I am currently a standing study section member of Biophysics and Biochemistry of Membranes (BBM). I received two National awards from American Society for Nutrition (ASN) 2019 Mary Swartz Rose Young Investigator Award for outstanding research on the safety and efficacy of bioactive compounds for human health and 2021 E.L.R Stokstad Award for outstanding fundamental research in nutrition. I have also received the National Award 2019 Eicosanoid Research Foundation Young Investigator award (Biochemistry molecular pharmacology). Additionally, I was awarded Zoetis Research Excellence Award from the College of Veterinary Medicine. I was also awarded El Sohly award 2022 for excellence in Cannabis science by American Chemical Society. Recently, I joined School of Chemistry and Biochemistry at Georgia Tech to strengthen my research program in bioactive lipids.

Feasibility of the proposed studies: I have been consulting with Dr. Heather Bradshaw for several years. We have exchanged scientific ideas and discussed about lipid and metabolite detection. With the funding of this proposal, our interactions will involve detection of several metabolites both in the cannabinoid and endocannabinoid area.

Ongoing projects that I wish to highlight

1R01GM115584-01A1 NIH (PI: Das) 04/01/2017-01/31/2023

Biochemical Mechanism of Eicosanoid Synthesizing Enzymes

Goal: To characterize the cytochrome P450 enzymes that are responsible for the metabolism of omega-3 fatty acids and their endocannabinoid derivatives

1R21AT010761-01 NIH (MPI: Das & Sarlah) 09/15/2019-08/31/2022

Systematic Investigation of Rare Cannabinoids with Pain Receptors.

Goal: To study the molecular pharmacology of rare cannabinoids

1R21NS121741 NIH (PI: Steelman, Co-I: Das) 09/01/2021-08/31/2023

Upper-respiratory infection, oligodendrocyte plasticity and behavior

This project will examine the role of microglia activation after infection on changes to oligodendrocytes and myelin

B. Positions, Scientific Appointments and Honors

Positions and Employment

2022-Present, Associate Professor, School of Chemistry and Biochemistry, Georgia Tech

2019-2022, Associate Professor, Department of Comparative Biosciences, UIUC

2015-Present, Affiliate Associate Professor, Neuroscience Program, UIUC

2015- Present, Affiliate Associate Professor, Division of Nutritional Sciences, UIUC

2015- Present, Affiliate Associate Professor, Center for Biophysics and Quantitative Biology, UIUC

2012- Present, Affiliate Associate Professor, Department of Bioengineering, UIUC

2011- 2019, Assistant Professor, Department of Comparative Biosciences, UIUC

2011-Present, Affiliate Associate Professor, Department of Biochemistry, UIUC

2011- Present, Affiliate Associate Professor, Beckman Institute for Advanced Science and Technology, UIUC

2006- 2011, NSF-NSEC-Postdoctoral Research Associate, University of Illinois Urbana-Champaign (UIUC)

2001-2005, Research Associate, Graduate Student in Hecht Laboratory, Princeton University

Honors and Awards (Selected)

2022 EI Sohly Award for Excellence in Cannabis Research by American Chemical Society

2021 E.L.R Stokstad Award from American Society for Nutrition (ASN)

2021 BBM study section member (Standing member)

2020 List of Teachers Ranked as Excellent for VM602

2019 Review Editorial board of *Frontiers in Pharmacology*

2019 List of Teachers Ranked as Excellent

2019 Mary Swartz Rose Young Investigator Award from American Society for Nutrition (ASN)

2019 Eicosanoid Research Foundation – YIA (Biochemistry & Molecular Pharmacology).

2019 Zoetis Research Excellence Award from the College of Veterinary Medicine

2018 Faculty of 1000 recommendation for Doxorubicin Paper in Biochemistry

2018 NIH Study Section, Macromolecular Structure and Function A Study (MSFA) Section (*Ad Hoc*)

2017 Colin Wraight best paper award from the Department of Biochemistry

2015 Editorial Board, *ChemistrySelect* published by Wiley-VCH, ChemPubSoc Europe

2015 National Scientist Development Grant, American Heart Association

2014 NIEHS Award at International Winter Eicosanoid Meeting 2014

2013 Moog Lecturer at Woodward Hauptmann Institute

2010 ACS Biological Division Travel Award to Attend ACS National meeting

2007 Outstanding Researcher Award, NSF-Nanoscale Science and Engineering Center.

2005 FMC Corporation Graduate Fellowship in Chemistry, Princeton (Top biochemistry student).

2004 Dean's List Finalist for Honorific Fellowship, Princeton (Top 12 in the Ph.D. graduating class).

2001 First year Science and Engineering Fellowship, Princeton University (Top incoming students).

C. Contributions to Science

Discovery of Novel Endocannabinoid Epoxides and Receptor Activation Studies: This is an emerging field of research in our laboratory. We are exploring the complex cross talk between endocannabinoid, endovannilloid and epoxygenase pathways. This cross-talk generates lipid mediators that can interact with both endocannabinoid and epoxygenase pathway receptors. The goal is to find novel anti-inflammatory endocannabinoid epoxides that are new immunomodulatory lipids that can be used as scaffold to develop new therapeutics. We have discovered DHA-ethanolamide epoxides in the brain that potently activate cannabinoid receptor 2 and are anti-inflammatory in microglial cells and are vasoactive. Recently, we discovered another class of endovannilloid epoxides that are TRPV1 antagonist and CB1 agonist and show favorable pharmacology towards the development of anti-pain therapeutics.

- Arnold, W., Carnevale, L., Xie, Z., Baylon, J., Tajkhorshid, E., Hu, H., **Das, A.***, "Anti-inflammatory dopamine- and serotonin-based endocannabinoid epoxides reciprocally regulate cannabinoid receptors and the TRPV1 channel" *Nature Communications*, 2021, 12, 926. PMID: 33568652 PMCID: PMC7876028
- Watson, J., Kim, J. and **Das, A*** "Emerging class of omega-3 fatty acid endocannabinoids & their derivatives", *Prostaglandins and Other Lipid Mediators (POLM)*, 2019, 11, 143 (PMID: 31085370, PMCID: PMC6685292)
- Roy, J., Watson, J., Hong, I., Fan, T., **Das, A.*** Anti-tumorigenic Properties of Omega-3 Endocannabinoid Epoxides. *J Med Chem.* 2018 Jul 12;61(13):5569-5579. doi: 10.1021/acs.jmedchem.8b00243. (PMID: 29856219, PMCID: PMC6685292)
- McDougle, D.R., Watson, J., Abdeen,A., Adili, R., Caputo, M., Krapf, J., Johnson, R., Kilian, K., Holinstat, M., **Das, A***, "Anti-inflammatory Omega-3 Endocannabinoid Epoxides", *Proc Natl Acad Sci U S A. (Direct Submission)* 2017 Jul 25;114(30):E6034-E6043. doi: 10.1073/pnas.1610325114. (PMID: 28687674: PMCID: PMC5544256)

Biochemical Mechanism of Membrane Bound Cytochrome P450 epoxygenases in Nanodiscs: A specific focus of my laboratory is on elucidating the biochemical mechanism of cytochrome P450 epoxygenases in Nanodiscs. While there are reports of their physiological relevance in cerebrovascular diseases, there is absence of any biochemical studies on epoxygenases that generate anti-inflammatory lipid epoxide mediators. With my strong background in P450 biochemistry, I am uniquely placed to study these enzymes. I have expressed the primary P450 in the heart, CYP2J2, in *E. coli*. I have also expressed CYP2C8, CYP2C9, the other epoxygenases in the endothelial cells and CYP2D6 in brain. Furthermore, I have developed several challenging novel lipidomics methods to assay the lipid epoxides and other hydroxylated lipid mediators.

- **Das A***, Weigle AT, Arnold WR, Kim JS, Carnevale LN, Huff HC. "CYP2J2 Molecular Recognition: A New Axis for Therapeutic Design." *Pharmacology and Therapeutics*, 2020, doi:10.1016/j.pharmthera.2020.107601. (PMID: 32534953, PMCID: PMC6685292)
- Arnold, W., Meling, D., Zelasko, S. and **Das, A.***. "CYP2C8 polymorphisms causes disruption of the electron transfer between CYP and CPR", *International Journal of Molecular Science*, 2019, doi: 10.3390/ijms20184626.(PMID: 31540428 PMCID: PMC6685292)
- Carnevale, L., Arango, A., Arnold, W., Tajkhorshid, E*. **Das, A.***"Endocannabinoid Virodhamine Inhibits Cardiovascular CYP2J2 epoxygenase", *Biochemistry*, 2018 Nov 20; 57(46):6489-6499. DOI: 10.1021/acs.biochem.8b00691 (PMID: 30285425 PMCID: PMC6262108).
- Arnold, W., Baylon, J., Tajkhorshid, E.*. **Das, A.***. "Arachidonic Acid Metabolism by Human Cardiovascular CYP2J2 is Modulated by Doxorubicin". *Biochemistry*, 2017 Dec 12. doi:10.1021/acs.biochem.7b01025. (PMID: 29200270 PMCID: PMC5743546)

Modulation of Membrane Protein Function by the Membrane and Crowded Environment: A major thrust of my research program is studying the biochemistry of membrane proteins and using targeted lipidomics to detect metabolites of fatty acid substrates. My work seeks to discover the basic principles of enzyme function and protein-lipid interactions in membrane environment, using biophysical methods. I have used Nanodiscs as membrane bilayer mimics to show that membranes modulate the function of membrane-bound proteins by (a) changing the electrostatics at the active site, (b) controlling the depth of insertion of the protein into membrane and (c) facilitating protein-protein interaction in membranes.

- Huff, H., Maroutsos, D., and **Das, A***, "Macromolecular Crowding and Lipid Composition Controls CYP2J2 activity." **Protein Science**, 2019, 28,928-940.
- Roy, J., Dibaeinia, P., Fan, T., Sinha, S. **Das, A.***, "Global Analysis of Osteosarcoma Lipidomes Reveal Altered Lipid Profiles in Metastatic vs. Non-Metastatic Cells." **Journal of Lipid Research**, 2019, 60, 375-387 (PMID: 30504231)
- Roy J, Pondenis H, Fan TM, **Das A***. "Direct Capture of Functional Proteins from Mammalian Plasma Membranes into Nanodiscs" **Biochemistry**. 2015;54:6299-302.
- **Das, A.**, Grinkova, Y. and Sligar, S.G. "Redox Potential Control by Drug Binding to Cytochrome P450 3A4." **J. Am. Chem. Soc.** 2007, 129 (45), 13778. (PMID: 17948999)

Nanotechnological uses of Membrane Protein in Nanodiscs: Membrane proteins are major drug targets. I am very interested to adapt nanotechnology tools for ultra-sensitive detection of drug binding to membrane bound proteins, using cytochrome P450 as prototype protein system. We discovered a new phenomenon of "resonance plasmon coupling" that can be used to detect small molecule binding to proteins with color adsorbate. This research has led to successful projects that are now being used to develop patentable technologies for detection of drug binding to membrane proteins. I pioneered the use of Nanodiscs to stabilize membrane proteins on nanoparticle surfaces.

- Lim, SJ, McDougle, D., Das, A* and Smith, A.* , "Lipoprotein Nanoplatelets: Fluorescent, Zwitterionic Probes for Molecular and Cellular Imaging." **J. Am. Chem. Soc.**, 2016, 138, 64-67. (PMID: 26687504)
- Plucinski, L., Gartia, M., Arnold, W., Ameen, A., Chang, T., Hsiao, A., Liu, G.* , Das, A.* , "Substrate Binding to CYP2J2- Nanodiscs Detected by Nanoplasmonic Lycurgus Cup Arrays" **Biosensors and Bioelectronics**, 2016, 337. (PMID 26334592)
- Das, A., Zhao, J. Van Duyne, R. and Sligar, S.G. "Screening of Type I and II Drug Binding to Human Cytochrome P450-3A4 in Nanodiscs by Localized Surface Plasmon Resonance Spectroscopy", **Anal. Chem.**, 2009, 3754. (PMID: 19364136)
- Zhao*, J., Das, A.* , Zhang, X, Schatz, G., Sligar, S., Van Duyne, R. "Resonance Surface Plasmon Spectroscopy: Low Molecular Weight Substrate Binding to Cytochrome P450" **J. Am. Chem. Soc.** 2006, 11004. (* Co-first authors) (PMID: 16925400).

Complete List of Published Work at Pubmed:

<https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/53931671/?sort=date&direction=ascending>

BIOGRAPHICAL SKETCH

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

NAME Sudhansu K. Dey	POSITION TITLE Lova Riekert Chair and Professor of Pediatrics		
eRA COMMONS USER NAME (credential, e.g., agency login) DEY_SK			
EDUCATION/TRAINING (<i>Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.</i>)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Presidency College, Calcutta, India University of Calcutta, India University of Calcutta, India	B. Sc M. Sc Ph.D.	1965 1967 1972	Physiology Physiology Reproductive Physiology

A. Personal Statement

S. K. Dey's life-long research mission is to understand the endocrine, paracrine, autocrine and juxtacrine signaling networks that influence uterine biology and embryo-uterine interactions during pregnancy. His lab was the first to discover that uterine cyclooxygenase-2 (COX-2) is critical to ovulation, fertilization and implantation, and that COX-2 derived prostacyclin (PGI₂) mediates embryo implantation via PPAR δ . These studies had a profound impact on the understanding of female fertility and in other biological systems, including cancer biology. Dey's group was also the first to discover that G-protein coupled cannabinoid receptor CB1 and its endogenous ligand anandamide are critical to uterine biology and embryo implantation. This study showed that while higher anandamide levels adversely affect implantation, lower levels stimulate embryo growth and implantation via CB1. These observations led to studies in humans, showing that higher anandamide levels cause spontaneous abortion in women. His group has shown that aberrant cannabinoid signaling impairs oviductal embryo transport, which has a clinical relevance to ectopic pregnancy in women. The group's discovery that estrogen levels are critical in specifying the window of uterine receptivity for implantation is drawing the attention of many IVF programs. In brief, Dr. Dey's career has been dedicated to defining a roadmap for embryo-uterine interactions during implantation at the molecular and genetic level and his research has made a lasting impact in the field of female fertility.

His belief that life and death are connected by a common thread influenced his interest in cancer biology, especially in ovarian and uterine cancers. His group has shown that several signaling pathways that are tightly regulated during pregnancy are dysregulated during tumorigenesis. Recent studies by him and his colleagues Dr. Daikoku have shown that while Cox1 is the predominant isoform which is upregulated in epithelial ovarian cancer (EOC) and inhibition of Cox1 attenuates EOC, Cox2 is overexpressed in mouse and human models of endometrial cancers. The unique and overlapping research infrastructures, expertise and resources of these investigators provide an extraordinary opportunity to address the proposed themes and research programs in this application. A better understanding of periimplantation biology could potentially alleviate female infertility and develop novel contraceptives. In this respect, most of our preclinical studies were subsequently proven to be clinically relevant. Our program has published **314** regular research articles, **52** review and symposium articles and **9** book chapters and book reviews. My research expertise, the long history of our program in uterine biology and periimplantation events, and the significant contributions made by our group speak for our credence in pursuing the proposed research. My commitment and enthusiasm for this field remain undiminished after more than four decades of devoted research.

B. Positions and Honors

Professional Experience

- 1973-75 Postdoctoral Fellow in Reproductive Biology, Ford Foundation, University of Kansas Medical Center
- 1975-77 Research Associate in Reproductive Biology, NIH, University of Kansas Medical Center
- 1977-81 Assistant Professors of OB/GYN and Physiology, University of Kansas Medical Center
- 1981-84 Associate Professors of OB/GYN and Physiology, University of Kansas Medical Center
- 1984-94 Professors of OB/GYN and Physiology, University of Kansas Medical Center
- 1995-02 Professor of Molecular and Integrative Physiology, University of Kansas Medical Center
- 2002-08 Professor of Pediatrics and

Professor of Cell and Developmental Biology
Professor of Pharmacology
Vanderbilt University Medical Center
2008-present Professor of Pediatrics, Cincinnati Children's Research Foundation, Cincinnati Children's Hospital Medical Center, University of Cincinnati
2008-2021 Director, Division of Reproductive Sciences, Cincinnati Children's Research Foundation, Cincinnati Children's Hospital Medical Center, University of Cincinnati
2008-present Professor, Division of Developmental Biology
2021-Present *Co-Director, Center for Reproductive Sciences, Division of Developmental Biology*

Honors

Senior Investigator Award (University of Kansas Medical Center)
Chancellor's Club Award (University of Kansas Medical Center)
Dolph C. Simon, Sr. Award (University of Kansas Medical Center)
University Distinguished Professor (University of Kansas Medical Center)
Concurrent MERIT Awards from NICHD/NIH (1999) and NIDA/NIH (2003)
Investigator Research Award, Society for the Study of Reproduction, 2001
Carl G. Hartman Award (Life-time Achievement Award), Society for the Study of Reproduction, 2008
IVI Schering-Plough Award in Reproductive Medicine, 2009
Elected to AAAS Fellow, 2017
Member, Human Embryology and Development Study Section, NIH, 1983-1984
Member, Population Research Committee, NICHD/NIH, 1993-1997
Member, Cellular, Molecular and Integrative Reproduction (CMIR) Study Section, NIH, 2015-2018
Elected Distinguished Fellow, SSR, 2022

Editorial Boards

Biology of Reproduction (1985-1989; 2004-2010)
Endocrinology (1999-2004)
Mol Reproduction & Dev (2001-2005)
Prostaglandins and Other Lipid Mediators (2003-2012)
Reproduction, Associate Editor (2004-2013)
Molecular Human Reproduction, Associate Editor (2007-2013)
J Clinical Investigation (2004-present),
Member, Advisory Board – Science Translational Medicine, AAAS (2013-present)

Memberships

The Society for the Study of Reproduction, American Physiological Society, The Endocrine Society, American Society of Biochemistry and Molecular Biology, American Association for the Advancement of Science, International Cannabinoid Research Society.

C. Contributions to Science

1. Embryonic factors influence embryo growth

We discovered that cooperative interactions among preimplantation embryos promote their own growth via paracrine interactions via growth factors secreted by them. This study exemplified that embryos cultured in groups in a small volume of medium have superior growth than those cultured singularly. Many human IVF programs have adopted this concept to improve embryo growth.

- a. Paria BC and Dey SK. Preimplantation embryo development in vitro: Cooperative interactions among embryos and role of growth factors. **Proc Natl Acad Sci USA** 87:4756- 4760, 1990. *PMCID: PMC54196*
- b. Paria BC, Dey SK and Andrews GK. Antisense c-myc effects on preimplantation mouse embryo development. **Proc Natl Acad Sci USA** 89(21):10051-10055, 1992. *PMCID: PMC50275*
- c. Paria BC, Das SK, Andrews GK and Dey SK. Expression of the epidermal growth factor receptor gene is regulated in mouse blastocysts during delayed implantation. **Proc Natl Acad Sci USA** 90(1):55-59, 1993. *PMCID: PMC45598*

- d. Paria BC, Klaus E, Klagsbrun M and Dey SK. Heparin-binding EGF-like growth factor interacts with mouse blastocysts independently of ErbB1: A possible role for heparan sulfate proteoglycans and ErbB4 in blastocyst implantation. **Development** 126(9):1997-2005, 1999.

2. Molecular signaling in fertility regulation

Our group discovered that uterine cyclooxygenase-2 (COX-2) is critical for ovulation, fertilization and implantation, and COX-2 derived prostaglandins (PG) mediate embryo implantation via PG receptors and peroxisome proliferator activated (PPAR) receptors. These studies have profound impact on female fertility and have raised concerns regarding chronic consumption of NSAIDS or COX-2 inhibitors by women during their reproductive life.

- a. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM and Dey SK. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. **Cell** 91(2):197-208, 1997. *PMID: 9346237*
- b. Lim H, Gupta RA, Ma WG, Paria BC, Moller DE, Morrow, JD, DuBois RN, Trzaskos JM and Dey SK. Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPAR δ . **Genes & Dev** 13:1561-1574, 1999. *PMCID: PMC316805*
- c. Reese J, Paria BC, Brown N, Zhao X, Morrow, JD and Dey SK. Coordinated regulation of fetal and maternal prostaglandins directs successful birth and postnatal adaptation in the mouse. **Proc Natl Acad Sci USA** 97(17):9759-9764, 2000. *PMCID: PMC16938*
- d. Daikoku T, Cha J, Sun X, Tranguch S, Xie H, Fujita T, Hirota Y, Lydon J, DeMayo F, Maxson R, Dey SK. Conditional Deletion of MSX Homeobox Genes in the Uterus Inhibits Blastocyst Implantation by Altering Uterine Receptivity. **Developmental Cell** 21(6): 1014-25, Dec 2011. *PMCID:PMC3241866*

3. Cannabinoids/endocannabinoid signaling influences female reproduction

We discovered that G-protein coupled cannabinoid receptor CB1 and CB2 and their endogenous ligand anandamide are critical to embryo implantation. This study showed that amplification and silencing of cannabinoid/endocannabinoid signaling both adversely affect various aspects of early pregnancy. These observations led to studies in humans showing that higher endocannabinoid levels cause spontaneous abortion in women. Our group has shown that aberrant cannabinoid/endocannabinoid signaling impairs oviductal embryo transport, which has clinical relevance to ectopic pregnancy in women.

- a. Wang H, Guo Y, Wang D, Kingsley PJ, Marnett LJ, Das SK, DuBois RN and Dey SK. Aberrant cannabinoid signaling impairs oviductal transport of embryos. **Nat Med** 10(10):1074-1080, 2004. *PMID: 15378054*
- b. Sun X, Deng W, Li Y, Tang S, Leishman E, Bradshaw H, Dey SK. Sustained Endocannabinoid Signaling Compromises Decidual Function and Promotes Inflammation-induced Preterm Birth. **J Biol Chem**. Feb 2016. *PMID 26900150*
- c. Li Y, Dewar A, Kim Y, Sun X, Dey SK. Pregnancy success in mice requires appropriate cannabinoid receptor signaling for primary decidua formation. **eLife** 2020. doi:10.7554/eLife.617662
- d. Kim, YS; Li, Y; Yuan, J; Borg, JP; Sun, X; Dey, SK. Cannabinoid and planar cell polarity signaling converges to direct placentation. **Proc Natl Acad Sci USA**. 2021; 118.

4. Defective implantation causes adverse ripple effects with poor pregnancy outcome.

We have shown that a short delay in timing of implantation or defective implantation creates an adverse ripple-effect throughout the course of pregnancy, leading to defective feto-placental development and poor pregnancy outcome. This constitutes a new concept that embryo-uterine interaction prior to and during implantation set up the subsequent developmental programming. This is consistent with clinical data showing that implantation beyond the normal window of receptivity leads to pregnancy losses in women.

- a. Cha J, Bartos A, Egashira M, Haraguchi H, Saito-Fujita T, Leishman E, Bradshaw H, Dey SK, and

- Hirota Y. Combinatory approaches prevent preterm birth profoundly exacerbated by gene-environment interactions. **J Clin Invest** 123 (9): 4063-75. Sept 2013. PMCID:PMC3754274
- b. Deng W, Cha J, Yuan J, Haraguchi H, Bartos A, Leishman E, Viollet B, Bradshaw HB, Hirota Y, Dey SK. p53 coordinates decidual sestrin 2/AMPK/mTORC1 signaling to govern parturition timing. **J Clin Invest** 2016 Aug 1; 126(8):2941-54. PMID: 27454290
 - c. Yuan J, Cha J, Deng W, Bartos A, Sun X, Ho HH, Borg JP, Yamaguchi TP, Yang Y, Dey SK. Planar cell polarity signaling in the uterus directs appropriate positioning of the crypt for embryo implantation. **Proc Natl Acad Sci USA** 2016 Nov 28. PMID: 27911818
 - d. Yuan J, Deng W, Cha J, Sun X, Borg JP, Dey SK. Tridimensional visualization reveals direct communication between the embryo and glands critical for implantation. **Nature Comm.** Feb 2018. PMID: 29426931

5. Cox-1 and Cox-2 play a major role in molecular signaling leading to gynecological cancer.

We were the first to show that COX-1 and COX-2 are major triggers for reproductive cancers, and that COX-1 is the primary factor in ovarian cancer, which replaced the standing belief that COX-2 was the operative prostaglandins (PGs). This work was followed by a search for the cause of uterine cancer using mouse models. We found that COX-2 and mTORC1 signaling promote endometrial cancer. Combination therapies targeting these pathways have shown success in blocking the progression of cancer in mice and, as these pathways are conserved in humans, leads us to possible therapies for human women.

- a. Gupta RA, Tejada LV, Tong BJ, Das SK, Morrow JD, Dey SK and DuBois RN. Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. **Cancer Res** 63(5):906-911, 2003.
- b. Daikoku T, Tranguch S, Trofimova IN, Dinulescu DM, Jacks T, Nikitin AY, Connolly DC and Dey SK. Cyclooxygenase-1 is overexpressed in multiple genetically engineered mouse models of epithelial ovarian cancer. **Cancer Res** 66(5):2527-2531, 2006.
- c. Blaisdell A, Crequer A, Columbus D, Daikoku T, Mittal K, Dey SK, Erlebacher A. Neutrophils Oppose Uterine Epithelial Carcinogenesis via Debridement of Hypoxic Tumor Cells. **Cancer Cell**. Dec 2015. PMID: 26678340
- d. Liang X, Daikoku T, Terakawa J, Ogawa Y, Joshi AR, Ellenson LH, Sun X, Dey SK. The uterine epithelial loss of Pten is inefficient to induce endometrial cancer with intact stromal Pten. **PLoS Genet**. 2018 Aug 24;14 (8):e1007630. Doi:10.1371/journal.pgen.1007630. eCollection 2018 Aug. PMID: 30142194

The List of my published work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1hMRE7NYYmLk2/bibliography/41157030/public/?sort=date&direction=ascending>

D. Research Support

- 1) **1R01HD068524-04** (S.K. Dey, PI): 09/26/2011-06/30/2027
NIH/NICHD
Molecular signaling in uterine receptivity to implantation
The goal of this project is to map the molecular roadmap to embryo implantation
- 2) **2R01HD103475** (S.K. Dey, PI) : 04/01/2015-03/31/2025
NIH/NICHD
Endocannabinoid Signaling during Early Pregnancy
The goal of this project is to explore the role of ligand-receptor signaling of endocannabinoid signaling in pregnancy events.

OVERLAP

No scientific overlap

BIOGRAPHICAL SKETCH

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

NAME: DUNAEVSKY, ANNA

ERA COMMONS USER NAME (credential, e.g., agency login): ADUNAEVSKY

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Hebrew University	BS	MM/1990	Biology/Psychology
Hebrew University, Hadassah Medical School	MS	MM/1992	Neurobiology
University of Massachusetts, Amherst	PHD	MM/1997	Neurobiology

A. Personal Statement

My research is aimed towards examining the mechanisms that both refine neural circuitry in the developing normal and diseased mammalian CNS and reemerge during learning in the adult CNS. My research program utilizes multidisciplinary approaches to the study of synapse development and remodeling in health and disease, and includes anatomical, molecular biological, biochemical, imaging and electrophysiological methods. My group has been utilizing cellular and molecular approaches in conjunction with multiple microscopy approaches to study dynamic properties of dendritic spines, their interaction with astrocytic processes and presynaptic terminals and localization of synaptic proteins during development. In the past decade my lab has shifted towards *in vivo* models where we have been combining behavioral analysis with *in vivo* imaging of cortical cells. We apply these methods to study neuronal and astrocytic development and function in genetic and environmental models of neurocognitive disorders, such as the Fragile X Syndrome and Maternal Immune Activation. My research program has been continuously funded by the NIH for over 20 years. I serve as a permanent member on the Developmental Brain Disorders for NIH, attesting to my expertise in neuroscience. I am also a member of the advisory board for the UNMC neuroscience T32 graduate training, a senior member of the Neuroscience graduate program, and a Full Professor in the Department of Neurological Sciences, where I serve as the Vice-Chair for Research of the Division of Developmental, Degenerative, and Regenerative Neuroscience. I have been serving as the Director of the Cognitive Neuroscience of Development and Aging (CoNDA) COBRE since November 2020. Mentoring is an important component of the Center and I have been awarded the UNMC Outstanding Mentor of Junior Faculty Award because of my dedication towards mentoring young investigators including students. I will continue this activity including providing an inclusive and supportive working environment and providing students the necessary tools to develop into rigorous and successful scientists.

Ongoing and recently completed projects that I would like to highlight:

R01NS109381 NIH
Dunaevsky, Anna (PI)
01/01/19-12/31/23
The Role of Astrocytes in the Fragile X Pathogenesis

The goal of this study is to determine how loss of FMRP in astrocytes impacts astrocytic signaling and synaptic plasticity.

R21 NS122157 NIH

Dunaevsky, Anna (PI)

8/1/21 – 1/31/23

Developing an Astroglial Model for Fragile X Syndrome

The goal of this study is to identify functional impairments in astrocytes derived from FXS hiPSC.

P20 GM130447 NIH

Dunaevsky, Anna (PI)

03/13/20-1/31/25

Cognitive Neuroscience of Development and Aging (CONDA) Center

The goal of this Center of Biomedical Research Excellence (COBRE) is to expand neuroimaging and cognitive neuroscience research in Nebraska, with an emphasis on lifespan development.

Completed Research Support

R01 MH107223 NIH

Dunaevsky, Anna (PI)

08/01/15-05/31/19

Maternal Immune Activation in a Genetic Mouse Model of ASD

Nebraska Stem Cell Grant

Dunaevsky, Anna (PI)

10/1/17-12/31/18

Modeling Fragile X syndrome with stem cells

R21 MH107029 NIH

Dunaevsky, Anna (PI)

09/25/15-07/31/18

The Role of Astrocytic signaling in Synaptic Plasticity

R01 HD067218

Dunaevsky, Anna (PI)

03/15/12-02/28/18

Mechanisms of Motor Skill Learning in the Fragile X Mouse Model

Citations:

1. Padmashri R, Ren B, Oldham B, Jung Y, Gough R, Dunaevsky A. Modeling Human-specific Interlaminar Astrocytes in the Mouse Cerebral Cortex. *J Comp Neurol.* 2021 PubMed PMID: [32639590](#) PubMed Central PMCID: [PMC7818222](#)
2. Coiro P, Padmashri R, Suresh A, Spartz E, Pendyala G, Chou S, Jung Y, Meays B, Roy S, Gautam N, Alnouti Y, Li M, Dunaevsky A. Impaired synaptic development in a maternal immune activation mouse model of neurodevelopmental disorders. *Brain Behav Immun.* 2015 Nov;50:249-58. PubMed PMID: [26218293](#); PubMed Central PMCID: [PMC4955953](#).
3. Padmashri R, Reiner BC, Suresh A, Spartz E, Dunaevsky A. Altered structural and functional synaptic plasticity with motor skill learning in a mouse model of fragile X syndrome. *J Neurosci.* 2013 Dec 11;33(50):19715-23. PubMed PMID: [24336735](#); PubMed Central PMCID: [PMC3858638](#).
4. Dunaevsky A, Tashiro A, Majewska A, Mason C, Yuste R. Developmental regulation of spine motility in the mammalian central nervous system. *Proc Natl Acad Sci U S A.* 1999 Nov 9;96(23):13438-43. PubMed PMID: [10557339](#); PubMed Central PMCID: [PMC23966](#).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020-	Vice Chair of Translational and Basic Research, Department of Neurological Science, University of Nebraska Medical Center
2018-	Professor, Department of Neurological Sciences, University of Nebraska Medical Center
2016 – 2018	Professor, Department of Developmental Neuroscience, Monroe-Meyer Institute, University of Nebraska Medical Center
2016 -	Member, Developmental Brain Disorders NIH study section
2011 -	Reviewing Editor, PLOS One
2010 - 2016	Associate Professor (with tenure), Department of Developmental Neuroscience, Monroe- Meyer Institute, University of Nebraska Medical Center
2002 - 2010	Assistant Professor, Department of Neuroscience, Brown University
2002 –	Ad Hoc reviewer, NIH, NSF, ISN, Wellcome Trust
2002 -	Member, Society for Neuroscience
1997 - 2001	Postdoctoral Fellow, Department of Pathology and the Center for Neurobiology and Behavior, Columbia University

Honors

2020	University of Nebraska Medical Center Outstanding Mentor of Junior Faculty Award
2015	University of Nebraska Medical Center Distinguished Scientist Award
2004	Award, Whitehall Foundation
1999	Postdoctoral National Research Service Award, NIH
1995	Graduate School Fellowship, University of Massachusetts, Amherst
1990	Graduate School Fellowship, The Hebrew University-Hadassah Medical School

C. Contributions to Science

1. Structural synaptic plasticity

I am most identified with my work on the dynamic properties of dendritic spines using live multiphoton imaging approaches. My papers were among the very first to establish that dendritic spines are highly dynamic in young developing mice and that motility diminishes as neurons mature. My work was one of the very first to image in live preparations both pre and postsynaptic components as well as combine ultrastructural EM analysis with multiphoton imaging, an approach now widely used. Our studies set the baseline for study of spine motility in brain slices and were the precursors to the dendritic spine *in vivo* imaging studies.

- a. Harms KJ, Rioult-Pedotti MS, Carter DR, Dunaevsky A. Transient spine expansion and learning-induced plasticity in layer 1 primary motor cortex. *J Neurosci*. 2008 May 28;28(22):5686-90.
PubMed PMID: [18509029](#); PubMed Central PMCID: [PMC2793590](#).
- b. Dunaevsky A, Blazeski R, Yuste R, Mason C. Spine motility with synaptic contact. *Nat Neurosci*. 2001 Jul;4(7):685-6. PubMed PMID: [11426220](#).
- c. Dunaevsky A, Tashiro A, Majewska A, Mason C, Yuste R. Developmental regulation of spine motility in the mammalian central nervous system. *Proc Natl Acad Sci U S A*. 1999 Nov 9;96(23):13438-43. PubMed PMID: [10557339](#); PubMed Central PMCID: [PMC23966](#).

2. Mechanisms of neurodevelopmental disorders

My laboratory has applied this understanding of plasticity to animal models of autism and neurodevelopmental disorders. By combining behavioral paradigms with functional and structural analysis of synapses my studies contributed to the understanding of how synapses are modified by motor-skill learning. Moreover, by performing these studies in a mouse model of the Fragile X Syndrome, these studies elucidate the role of FMRP in regulating synaptic plasticity with learning. Recent studies have also examined synaptic impairments in the Maternal Immune Activation model of neurodevelopmental disorders.

- a. Pendyala G, Chou S, Jung Y, Coiro P, Spartz E, Padmashri R, Li M, Dunaevsky A. Maternal Immune Activation Causes Behavioral Impairments and Altered Cerebellar Cytokine and

- Synaptic Protein Expression. *Neuropsychopharmacology*. 2017 Jun;42(7):1435-1446. PubMed PMID: [28102228](#); PubMed Central PMCID: [PMC5436129](#).
- b. Suresh A, Dunaevsky A. Relationship Between Synaptic AMPAR and Spine Dynamics: Impairments in the FXS Mouse. *Cereb Cortex*. 2017 May 24: 1-13 ;PubMed PMID: [28541473](#). PubMed Central PMCID: [PMC6057510](#)
 - c. Coiro P, Padmashri R, Suresh A, Spartz E, Pendyala G, Chou S, Jung Y, Meays B, Roy S, Gautam N, Alnouti Y, Li M, Dunaevsky A. Impaired synaptic development in a maternal immune activation mouse model of neurodevelopmental disorders. *Brain Behav Immun*. 2015 Nov; 50:249-58. PubMed PMID: [26218293](#); PubMed Central PMCID: [PMC4955953](#).
 - d. Padmashri R, Reiner BC, Suresh A, Spartz E, Dunaevsky A. Altered structural and functional synaptic plasticity with motor skill learning in a mouse model of fragile X syndrome. *J Neurosci*. 2013 Dec 11;33(50):19715-23. PubMed PMID: [24336735](#); PubMed Central PMCID: [PMC3858638](#).

3. Neuron-Glia interactions

I also contributed to the study of the role of astrocytes, in regulating neuronal development, synaptic plasticity and ultimately behavior. We were among the first to be able to perform time-lapse imaging of growing dendrites along astrocytes and to determine the dynamic properties of astrocytic processes as they ensheathe dendritic spines. We have also been studying the role of astrocytic calcium signaling in synaptic plasticity and learning. Most recently we have been studying astrocytes derived from hiPSC.

- a. Padmashri R, Ren B, Oldham B, Jung Y, Gough R, Dunaevsky A. Modeling Human-specific Interlaminar Astrocytes in the Mouse Cerebral Cortex. *J Comp Neurol*. 2021
- b. Padmashri R, Suresh A, Boska MD, Dunaevsky A. Motor-Skill Learning Is Dependent on Astrocytic Activity. *Neural Plast*. 2015;2015:938023. PubMed PMID: [26346977](#); PubMed Central PMCID: [PMC4539503](#).
- c. Lippman JJ, Lordkipanidze T, Buell ME, Yoon SO, Dunaevsky A. Morphogenesis and regulation of Bergmann glial processes during Purkinje cell dendritic spine ensheathment and synaptogenesis. *Glia*. 2008 Oct;56(13):1463-77. PubMed PMID: [18615636](#); PubMed Central PMCID: [PMC2637407](#).
- d. Lordkipanidze T, Dunaevsky A. Purkinje cell dendrites grow in alignment with Bergmann glia. *Glia*. 2005 Aug 15;51(3):229-34. PubMed PMID: [15800897](#).

Complete List of Published Work in My Bibliography:

As the director of the Cognitive Neuroscience of Development and Aging Center (P20 GM130447) some citations associated with the center are listed in My Bibliography although I have not contributed to the studies.

<https://www.ncbi.nlm.nih.gov/myncbi/anna.dunaevsky.1/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: Dunn, Kenneth William

ERA COMMONS USER NAME (credential, e.g., agency login): kendunn

POSITION TITLE: Professor of Medicine

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
UC Santa Cruz	BA	06/1976	Biology
SUNY Stony Brook	PhD	06/1986	Biology
Columbia Univ., College Phys. and Surgeons	NIH fellow	05/1991	Cell Biology

A. Personal Statement

The research of my laboratory is broadly focused on the development and application of methods of microscopy, particularly intravital microscopy, to the study of cell biology and physiology. Beginning around 2001, I began to work with members of the IUSOM Nephrology Division to develop methods of intravital microscopy, culminating in funding of the Indiana NIDDK OBrien Center for Advanced Renal Microscopy, whose mission is to develop and disseminate methods of intravital and 3D microscopy of the kidney. Current OBrien Center projects in my laboratory include development of methods of digital image processing to measure microvascular flow in vivo (Clendenon et al., 2019) and deep-learning-based methods of segmentation to support automated cytometry of centimeter-scale biological tissues (Dunn et al., 2019). In the past few years, my laboratory has focused on developing novel methods of intravital microscopy to address the effects of drugs and disease on liver function including collaborations with the IU Biocomplexity Institute to characterize acute acetaminophen hepatotoxicity (Dunn et al., 2020), with Amgen to characterize drugs associated with idiosyncratic drug-induced liver injury (Ryan et al., 2018), and with Lilly Labs to analyze the organ and cellular disposition of bifunctional antibody therapeutics. Finally, for the past 25 years I have directed the Indiana Center for Biological Microscopy and currently direct microscopy cores of the P30 NIDDK Indiana Diabetes Center and the U54 Indiana Comprehensive Center of Excellence in Hematology, whose goals are to develop and apply novel methods of microscopy for the study of diabetes and hematology, respectively.

Dunn, KW, Martinez, M., Wang, Z., Mang, H., Clendenon, S., Sluka, J., Glazier, J. and J. Klaunig. 2020. Mitochondrial depolarization and repolarization in early stages of acetaminophen hepatotoxicity in mice. Toxicology. 439:PMID: 32315716.

Clendenon, S., Fu, X., Von Hoene, R., Clendenon, J., Sluka, J., Winfree, S., Mang, H., Martinez, M., Filson, A., Klaunig, J., Glazier, J. and **K. Dunn**. 2019. A simple automated method for continuous fieldwise measurement of microvascular dynamics. Microvascular Res. 123: 7-13. PMID: 30502365

Ryan, J., Morgan, R., Chen, Y., Volak, L., Dunn, R. and **K. Dunn**. 2018. Intravital multiphoton microscopy with fluorescent bile salts in rats as an in vivo biomarker for hepatobiliary transport inhibition. 2018. Drug Metab. Disp. 46:704-718. PMID:29467212

Dunn, KW, Fu, C., Ho, DJ, Lee, S., Salama, P. and EJ Delp. 2019. DeepSynth: Three dimensional nuclear segmentation of biological images using neural networks trained with synthetic data. Sci Rep. 9:18295. PMID: 31797882

Ongoing and Completed Research support

Ongoing

NIH/NIDDK P30 DK 079312-11 (Molitoris, P.I.). O'Brien Center, Center for Advanced Renal Microscopic analysis". Associate Director, Core director. 07-02 to 06-07, 06-07 to 06-12, 7-12 to 6-17, 07-17 to 06-22. Center for investigation of renal physiology utilizing cutting edge approaches in cell biology and light microscopy.

NIH/NIDDK P30 DK097512-01A1 (Evans-Molina, PI). Indiana Diabetes Research Center. Core director. 05/01/15 – 05/31/25. Develop and conduct advanced methods of intravital microscopy.

NIH/NIDDK. U54DK106846-01 (Broxmeyer, PI). Hematopoietic stem and progenitor cell regulation for enhanced clinical efficacy. Core director. 08/15 – 07/25. Develop and conduct advanced methods of intravital microscopy.

NIH/NIDDK R01 DK091623-06 (Molitoris, PI). Proximal Tubule Albumin Transport in Disease States 09/30/11 – 05/31/22

NIH/NIDDK UG3 DK114923 01 (Dagher, PI) Nephron sub-segmental omics and quantitative 3D imaging of human kidney. 09/15/19 – 06/30/22

NIH/NIDDK R01 DK122147-01 (Serezani, PI) Prostaglandin E2 Actions and Enhanced Susceptibility to Skin Infection in Diabetic Mice. 04/01/20 – 01/31/23

B. Positions, Scientific Appointments, and Honors

Professional positions

2014 – Professor of Medicine, Indiana University Medical Center, Indianapolis, IN

2001 – Assoc. Professor of Medicine, Indiana University Medical Center, Indianapolis, IN

1995 to 2001 - Asst. Professor of Medicine, Indiana University Medical Center, Indianapolis, IN

1991-1995 - Asst. Professor of Clinical Pathology, Columbia University College of Physicians and Surgeons

Awards and other professional activities

1994-1997 American Heart Association, NYC Affiliate, Investigatorship Award

1993-1995 National Kidney Found. Young Investigator Grant

1992-1993 Cystic Fibrosis Found. Pilot Grant, K. Dunn and T. McGraw, co-P.I.s

1987 NIH Postdoctoral Research Award

National invited presentations:

Annual meeting of the American Society for Clinical Pharmacology, April, 2021

Intravital multiphoton microscopy as a tool for evaluating drug distribution and transport

Indiana Clinical and Translational Sciences Institute, Purdue Univ., 2020

Tissue cytometry as a tool in renal research – engineering challenges and opportunities

World Pharma Congress, June, 2013

In vivo evaluation of drug effects in the kidney and liver using intravital multiphoton microscopy

American Association of Pharmaceutical Sciences, March, 2013

Intravital multiphoton microscopy as a tool for evaluating drug distribution and transport

World Pharma Congress, June, 2012

Characterizing hepatocellular cholestasis with intravital multiphoton microscopy

Non-linear Microscopy Symposium (Purdue University), June, 2011

Intravital microscopy of the kidney

Frontiers in Intravital Microscopy (NIH) 2010, 2011

Intravital microscopy of the kidney

Association of Biomedical Research Facilities, March, 2009

Intravital microscopy in a core imaging facility

American Society of Cell Biology Symposium – Epithelial membrane traffic - December 2007

Role of Rab10 in epithelial cell biology

Editorial boards

American Journal of Physiology (Cell) – Editorial Board - 2002 - 2015

American Journal of Physiology (Renal) – Editorial Board

Frontiers in Cell and Developmental Biology- Associate Editor

Study section service

2019 – NIH, National Technology Research Resource Award

2018 – NIDDK, P01 Bladder Physiology Special Emphasis Panel

2017 – NIH, NIAID – Emerging Science in and Technologies in Transplantation Research

2015 – NIH,NIGMS- EBIT Study section

2014 – NIDDK, Cystic Fibrosis Research and Translation Core Review panel

2010, 2011, 2012, 2013, 2014, 2015 – IMST, Cell Biology and Imaging Study Section

2010 – NIDCR – Board of Scientific Counselors Review

2008, 2009, 2017, 2019 – NCRR, Shared Instrumentation Grant Study Section

2008 – NIDDK, Molecular Therapy Core Center Study Section

2005, 2006, 2015 – NIDDK, Digestive Disease Research Core Center Study Section

2003, 2004 – NIH/NCI

2002 – NIDDK, General Medicine B Study Section

2001, 2003 - NSF

Also: Human Frontiers Science Program (1999) US-Israel Binational Science Foundation (2002, 2004),

UNC Biotechnology Center (2007, 2010)

C. Contributions to Science

1. Default mechanisms of membrane traffic. The organization and physiology of cells depends upon tightly orchestrated processes of membrane traffic, in which different proteins are trafficked to specific destinations with high fidelity. The diversity of endocytic ligands and receptors argues that the common pathways may be based upon default processes, rather than specific signals. My first major contribution derived from work done as a post-doctoral NIH fellow at Columbia University, in the laboratory of Fred Maxfield. My project resulted in the publication of two seminal papers describing the role of default mechanisms in endocytic membrane traffic. The first (Dunn et al., 1989, 364 citations) demonstrated that the efficient recycling of membrane receptors to the plasma membrane is accomplished in an iterative process in which early endosomes fuse repeatedly with endocytic vesicles, while at the same time continuously budding off recycling vesicles. This observation was consistent with a model of endocytic trafficking in which efficient sorting of membrane proteins away from the lysosomal pathway is accomplished by the repetition of a low efficiency process based solely upon the surface-area-to-volume ratio of endosomes and recycling vesicles. The second paper (Dunn et al., 1992, 216 citations), demonstrated that the delivery of endocytic ligands to lysosomes likewise occurs through a default pathway, in which ligands remaining in the early endosome at the end of the fusion-competent period, are transported to lysosomes as early endosomes mature into late endosomes. Thus, lysosomal targeting of an internalized ligand is determined simply by the disposition of a ligand into the volume of an endosome.

Dunn, K., McGraw, T. and F. Maxfield. 1989. Iterative fractionation of recycling receptors from lysosomally destined ligands in an early sorting endosome. *Journal of Cell Biology*, 109:3303-3314; PMCID:PMC2115921

Dunn, K. and F. Maxfield. 1992. Delivery of ligands from sorting endosomes to late endosomes occurs by maturation of sorting endosomes. *Journal of Cell Biology*. 117:301-310: PMCID:PMC2289412

2. Role of endocytosis in polarized epithelial cells. Epithelial function depends upon the polarized distribution of transporters and receptors to either the apical or basolateral membrane domain. My first project conducted as an independent researcher addressed the mechanisms and role of endocytic sorting in epithelial membrane polarity. In this NIH-funded project, I developed methods for microscopic imaging of living, polarized epithelial cells to support quantitative studies of endocytic membrane traffic in Madin-Darby Canine Kidney and other epithelial cells. This work resulted in three publications over the course of two years demonstrating a novel apical recycling compartment that excludes basolateral membrane proteins (Brown et al., 2000, 186 citations), demonstrating extensive intermixing of apical and basolateral membrane proteins in a set of acidic common endosomes (Wang et al., 2000, 142 citations) and demonstrating that disruption of

endocytic sorting with BrefeldinA resulted in a rapid and complete loss of membrane polarity (Wang et al., 2001, 48 citations). Together these papers demonstrated conclusively and for the first time, that membrane proteins from the apical and basolateral poles are continuously and thoroughly intermixing in endosomes and thus that the maintenance of epithelial polarity depends upon continuous sorting in endosomes. Using these same techniques, my laboratory subsequently demonstrated that Rab10 mediates an intermediate step in basolateral recycling (Babbey et al., 2006, 165 citations), but also associates with the primary cilium of polarized renal epithelia.

Brown, P.S., E. Wang, B. Aroeti, S. J. Chapin, K. E. Mostov and **K. Dunn**. 2000. Definition of distinct compartments in polarized MDCK cells for membrane-volume sorting, polarized sorting and apical recycling. *Traffic*. 1:124-140. PMID:11208093

Wang, E., P. Brown, B. Aroeti, S. J. Chapin, K. E. Mostov and **K. Dunn**. 2000. Apical and basolateral endocytic pathways of MDCK cells converge in acidic common endosomes distinct from a nearly-neutral apical recycling endosome. *Traffic*. 1:480-493. PMID:11208134

Wang, E., Pennington, J., Goldenring, J., Hunziker, W. and **K. Dunn**. 2001. Brefeldin A rapidly disrupts plasma membrane polarity by blocking polar sorting in common endosomes of MDCK cells. *J. Cell Sci.* 114:3309-3321. PMID:11591819

Babbey, C., Akhtar, N., Wang, E., Chen, C.-H., Grant, B. and **K. Dunn**. 2006. Rab10 mediates transport from sorting endosomes to common endosomes of polarized Madin-Darby canine kidney cells. *Molecular Biology of the Cell*. 17:3156-3175. PMID:16641372

3. Quantitative multiphoton fluorescence microscopy of living organisms. Starting in around 2001, I began to develop methods of intravital multiphoton microscopy of the mouse and rat kidney. This work resulted in the publication of a paper that included the first multiphoton fluorescence images collected from the kidney of living rodents, demonstrating how intravital multiphoton microscopy could be used to characterize a variety of different renal functions (Dunn et al., 2002, 297 citations). This work also provided the foundation for a successful proposal for funding from the NIDDK for the Indiana OBrien Center for Advance Renal Microscopy, continuously funded since 2002 and dedicated to the development and dissemination of intravital microscopy methods for studying kidney physiology. In collaboration with various investigators in the IU Nephrology Division, we have subsequently used these techniques to measure apoptosis *in vivo*, to quantify organic anion transport and to characterize endotoxin handling and signaling in the rodent kidney. In the past few years, my laboratory has developed novel methods of intravital microscopy of the liver, resulting in the first studies dissecting organic anion and bile salt transport at the level of individual hepatocytes *in vivo* (Ryan et al., 2018). We recently developed methods for measuring fluorescence resonance energy transfer *in vivo*, which we applied to measure kinase activity in the liver *in vivo* (Day et al., 2016, 19 citations) and methods for measuring microvascular flow in complex 2D networks, which we applied to measures of microvascular flow in the mouse liver (Clendenon et al., 2019)

Dunn, K., R. Sandoval, K. Kelly, P. Dagher, G. Tanner, S. Atkinson, R. Bacallao and B. Molitoris. 2002. Functional studies of the kidney of living animals using multicolor 2-photon microscopy. *Am. J. Physiol. (Cell)*. 282:C905-C916. PMID:12176747

Day, R., Tao, W. and **K. Dunn**. 2016. A simple approach to measuring FRET in fluorescent biosensors in living animals using two-photon microscopy. *Nature Protocols*. 11:2066-2080, PMID:27685098

Ryan, J., Morgan, R., Chen, Y., Volak, L., Dunn, R. and **K. Dunn**. 2018. Intravital multiphoton microscopy with fluorescent bile salts in rats as an *in vivo* biomarker for hepatobiliary transport inhibition. 2018. *Drug Metab. Disp.* 46:704-718. PMID:29467212

Clendenon, S., Fu, X., Von Hoene, R., Clendenon, J., Sluka, J., Winfree, S., Mang, H., Martinez, M., Filson, A., Klaunig, J., Glazier, J. and **K. Dunn**. 2019. A simple automated method for continuous fieldwise measurement of microvascular dynamics. *Microvascular Res.* 123: 7-13. PMID: 30502365

4. Methods of quantitative microscopy. Throughout my career, I have been active in developing novel tools for quantitative microscopy. During my postdoctoral training, I developed a method for high-resolution measurement of endosome acidification in living cells, based upon confocal emission ratio microscopy. This approach was applied to demonstrate the absence of an effect of a mutant form of CFTR on endosome acidification, and an additional 4 publications during my postdoctoral training. I developed this technique specifically to address regulation of endosome pH in polarized cells, and subsequently applied the approach in work conducted in my own laboratory, providing the first measurements of endosome pH in polarized epithelial cells (Wang et al., 2000, 114 citations). I published an additional 10 papers in the then-developing field of 3D

microscopy (6 as primary author), including one demonstrating the effects of spherical and chromatic aberration on confocal microscopy, two presenting a quantitative analysis of the effects of scattering and spherical aberration on multiphoton microscopy and one presenting a quantitative analysis of the photophysical benefits of spinning-disk confocal microscopy (Wang et al., 2005, 157 citations). Finally, I have been active in developing methods of digital image analysis and quantitative microscopy. Since 2001, I have published 8 papers in this field (3 as primary author), including one describing the Voxx volume visualization software that we developed (Clendenon et al. 2002, 131 citations, over 6000 downloads), two describing the novel software that we developed to correct motion artifacts that occur in intravital microscopy (Dunn et al., 2014), and two describing approaches for significance testing of microscopic colocalization (Dunn et al. 2011, 1015 citations).

Clendenon, J., C. Phillips, R. Sandoval, S. Fang and **K. Dunn**. 2002. Voxx, A PC-based near real-time volume rendering system for biological microscopy. *Amer. J. Phys. Cell.* 282:C213-C218. PMID:11742814

Wang, E., Babbe, C. and **K. Dunn**. 2005. Performance comparison between high-speed Yokogawa spinning disk confocal and single-point scanning confocal systems. *Journal of Microscopy*. 218:148-159. PMID:15857376

Dunn, K.W., Kamocka, M. and J. McDonald. 2011. A practical guide to evaluating colocalization in biological microscopy. *American Journal of Physiology (Cell)*. 300:C723-742. PMID:21209361

Dunn, K., Lorenz, K., Salama, P. and E. Delp. 2014. IMART software for correction of motion artifacts in images collected in intravital microscopy. *Intravital*. 3:44-53. PMID:26090271

Publications - A complete list of my publications may be found online in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/kenneth.dunn.1/bibliography/41158808/public/?sort=date&direction=asc>.

BIOGRAPHICAL SKETCH

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

NAME: Norbert Hajos

ERA COMMONS USER NAME (credential, e.g., agency login): NHAJOS

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Eotvos Lorand University, Budapest, HU	M.Sc.	07/1994	Biology
Semmelweis University, Budapest, HU	Ph.D.	07/1998	Medical Sciences
UCLA, School of Medicine, CA (with Istvan Mody)	Research associate	1996-1998	Neurophysiology
Institute of Experimental Medicine, Budapest, HU (with Tamas F. Freund)	Postdoctoral	05/2005	Neuroanatomy & Neurophysiology
Hungarian Academy of Sciences	Doctor of Science	02/2012	Neurobiology

A. Personal Statement

During my undergraduate studies, I became astonished by the complexity of the brain. Therefore, I joined the lab of Prof. Tamas Freund, a leader in the field of cortical circuit studies to acquire a deeper insight into brain structures. After 3 years in Prof. Freund's lab, I became fascinated with neuroscience and decided to stay with his research group and finish my graduate studies. My research under his supervision led to the discovery of a novel circuit element in cortical networks, the dis-inhibitory interneurons. During this time, I learned that knowledge of the *anatomical structure* provides a solid base for predicting how neural networks may work, however, uncovering their operational principles can only be achieved by studying *neural function*. Therefore, I joined Prof. Istvan Mody's lab at UCLA, where I acquired the fundamentals of *in vitro* electrophysiological techniques. During these years, I provided the first evidence that epileptic seizures induced a long-lasting enhancement of inhibitory synaptic transmission. Our findings showed that synaptic inhibition was enhanced, rather than reduced in the epileptic brain, and inhibitory synapses were not rigid, but had plastic properties. After returning to Hungary from the US, my research focused on understanding the principles of neural circuit operation both at the micro- and mesoscale levels by combining neuroanatomical techniques with electrophysiological methods. After establishing my independent research group, we aimed to understand the circuit operation underlying amygdala function. In spite of numerous investigations concentrating on the amygdala, there was surprisingly little known about the local cell types and their connectivity within this brain region. Taking advantage of transgenic mouse lines and viral techniques, we revealed both the wiring and operational principles of basolateral amygdala microcircuits, the prerequisite for building theories of the amygdalar functions. However, this goal cannot be achieved without understanding how the amygdala directly affects other brain structures. Therefore, we have recently extended our investigations to the pathways connecting the basolateral amygdala with the prefrontal cortex as well as that connecting the midbrain dopaminergic neurons and central amygdala. As abnormal function of both pathways has been implicated in maladaptive behaviors, including individuals who regularly use psychoactive drugs, one current interest of our research group has been to move in the direction of translational experiments using preclinical models of cannabis use. To successfully accomplish this plan, we will build on collaborations with two world-leading researchers in the cannabis field, Profs Ken Mackie and Istvan Katona, with whom I explored the effects of CB1 cannabinoid receptor activation on inhibitory synaptic transmission two decades ago. I am convinced that returning to studying the effects of cannabis at the cortical and subcortical circuit levels and joining forces with

these two experts and additional colleagues in our research center will result in significant outcomes, which can pave the way for my lab to become engaged in drug addiction research for a long term.

In addition to running a lab, over the past 15 years I have gained significant experience in mentoring young scientists and knowledge in diverse grant reviewing processes. First, I reviewed grant proposals submitted to different founding agencies, like The Wellcome Trust Foundation, Hungarian Academy of Sciences, Hungarian, Polish, Dutch, Swiss and French National Science Foundations. Second, I served as a panel member in both the Hungarian and Polish National Science Foundations as well as on the Council of Bolyai Scholarship at the Hungarian Academy of Sciences. Third, I was in charge of establishing the criteria for evaluation of applications submitted to the Biology section of the Council of Bolyai Scholarship. Fourth, I chaired the Neuroscience panel at the Hungarian National Science Foundation for 5 years, where I had the opportunity to establish the criteria for both the evaluation of grant proposals and operation of the panel by recruiting colleagues from abroad. These broad-ranging experiences at the different levels of the grant review process will help me to organize the board for the assessment of proposals submitted to the Pilot Project Core, create principles for the reviewing process, and help to oversee the mentoring of successful and unsuccessful applicants to our pilot project core.

B. Positions and Honors

2021 – present	Professor and Linda and Jack Gill Chair of Neuroscience, Dept. of Psychology & Brain Sciences, Indiana University, Bloomington, IN
2020	Professor, Dept. of Neurobiology, Helsinki University, Helsinki, Finland
2012 – present	Scientific advisor and group leader, Institute of Experimental Medicine, Budapest, HU
2009 – 2012	Research group leader, Institute of Experimental Medicine, Budapest, HU
2005 – 2011	International Senior Research Fellow sponsored by The Wellcome Trust (UK), Institute of Experimental Medicine, Budapest, HU

Other experiences, Service, Professional Membership

2021 - present	Frontiers in Neural Circuits, Reviewing Editor
2019 –	Member of the Advisory Board for Bolyai Scholarship, Hungarian Academy of Sciences
2019 – 2021	Member of the Presidium of the Hungarian Academy of Sciences
2020	Polish Scientific Research Fund, Review Panel Member
2019 – 2020	Clinical Neuroscience, Hungary, Advisory Board Member
2018 – 2020	Vice President of the Neurobiology Committee at the Hungarian Academy of Sciences
2015 – 2018	Member of Neurobiology Committee at the Hungarian Academy of Sciences
2015 – 2019	Hungarian Scientific Research Fund, Chair of the Neuroscience Panel
2013 – 2014	Hungarian Scientific Research Fund, Review Panel Member
2013 – present	European Journal of Neuroscience, Reviewing Editor
1998 – 2005	American Physiological Society, Member
1998 – present	Society for Neurosciences, Member
1998 – present	Federation of European Neuroscience Societies, Member1
1993 – present	Hungarian Neuroscience Society, Member

Honors

2012 – 2017	'Momentum' Programme of the Hungarian Academy of Sciences, Budapest, HU
2005 – 2011	Wellcome Trust International Senior Research Fellowship, The Wellcome Trust, UK
2004	NATO Science Fellowship
2004	Krieg Cortical Kudos of the Cajal Club, Cortical Explorer Award, USA
2003 – 2005	Bolyai Scholarship, Hungarian Academy of Sciences, Budapest, HU
2002	Award of the Hungarian Academy of Sciences for young scientists
1999 – 2002	Bolyai Scholarship, Hungarian Academy of Sciences, Budapest, HU
1998	Award of the Hungarian Electron Microscopic Society
1998	Award of "ifj. Farkas Zsolt emlékére" Foundation, HU
1994	Student Researcher Award of the Hungarian Student Researcher Conference, HU
1993	Student Researcher Award of Eotvos Lorand University, HU

C. Contributions to Science

1. Interneuronal circuits in the hippocampus

As a graduate student, I discovered the presence of a novel, hitherto unrecognized inhibitory cell type in the dentate gyrus and hippocampus that innervate *selectively* other GABAergic cells and avoid excitatory principal neurons. These inhibitory interneurons were named as interneuron-selective interneurons. Based on our neuroanatomical results, we predicted that these inhibitory cells should function as dis-inhibitory neurons, allowing a temporal elevation of principal neuron spiking probability by reducing their inhibitory inputs. Our hypothesis was fully supported by subsequent functional studies. As a postdoctoral researcher, I described the properties of synaptic communication among GABAergic cells. The most prominent discovery, however, was to recognize that pathological neural activities, like seizures lead to a long-lasting increase in the efficacy of synaptic transmission at inhibitory synapses in the dentate gyrus. This plasticity was accompanied with the increased number of GABA-A receptors. In addition to showing that a larger number of postsynaptic receptors underlies the enhanced synaptic signaling, our study was among the first to demonstrate that synaptic inhibition is strengthened, and not weakened as a result of epileptiform activities.

- a. **Hájos N.**, Acsády L. and Freund T.F. Target selectivity and neurochemical characteristics of VIP-immunoreactive interneurons in the rat dentate gyrus. *Eur. J. Neurosci.* 8: 1415-1431. (1996)
- b. Gulyás A.I., **Hájos N.** and Freund T.F. Interneurons containing calretinin are specialized to control other interneurons in the rat hippocampus. *J. Neurosci.* 16: 3397-3411. (1996)
- c. **Hájos N** and Mody I Synaptic communication among hippocampal interneurons: Properties of spontaneous IPSCs in morphologically identified cells. *J Neurosci.* 17: 8427-8442. (1997)
- d. Nusser Z, **Hájos N**, Somogyi P, Mody I. Increased number of synaptic GABA(A) receptors underlies potentiation at hippocampal inhibitory synapses. *Nature* 395: 172-7. (1998)

2. CB1 cannabinoid receptor function in cortical circuits

CB1 receptor-mediated signaling is a widespread communication type in the CNS. I was the first to show that activation of CB1 receptors at GABAergic synapses resulted in a reduction in synaptic inhibition in several cortical areas, including the hippocampus, basolateral amygdala and neocortex. These studies were performed in a collaboration with Profs Ken Mackie and Istvan Katona. In addition, we demonstrated that oscillatory activities at the gamma frequency range (oscillations that are often linked to cognitive processes) could be significantly suppressed by cannabinoids. Finally, we provided the first evidence that CB1 receptor-mediated signaling is dependent on nitric oxide synthesis, when cholinergic receptors are active. Thus, synaptic transmission can be effectively controlled by a cascade of gaseous and lipophilic molecules. This unexpected finding has been strengthened by subsequent studies obtained in several brain regions.

- a. **Hájos N**, Katona I, Naiem SS, Mackie K, Ledent C, Mody I, Freund TF. Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur J Neurosci.* 12(9): 3239-49. (2000)
- b. Katona I, Rancz EA, Acsády L, Ledent C, Mackie K, **Hájos N** and Freund TF. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci.* 2123: 9506-9518. (2001) PMCID: PMC6763903
- c. Bodor A.L., Katona I., Nyíri G., Mackie K., Ledent C., **Hájos N.** and Freund T.F. Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. *J Neurosci.* 25:6845-56. (2005) PMCID: PMC6725346
- d. Makara J.K., Katona I., Nyíri G., Németh B., Ledent C., Watanabe M., de Vente J., Freund T.F. and **Hájos N.** Involvement of nitric oxide in depolarization-induced suppression of inhibition in hippocampal pyramidal cells during activation of cholinergic receptors. *J Neurosci.* 27:10211-22. (2007) PMCID: PMC6672656

3. Circuit mechanisms underlying oscillatory activities in the hippocampus

Rhythmic activities at different frequencies characterize distinct computational modes in cortical networks, yet the mechanisms of oscillogenesis are largely unknown. I have invented a novel type of slice chambers that ensures the necessary oxygen level, which is critical for maintaining oscillatory activities in slice preparations resembling those observed in intact brain. This technical improvement allowed us to reveal the circuit mechanisms underlying the generation of two types of mutually exclusive forms of hippocampal oscillations, the gamma oscillations and sharp wave-ripples. By combining *in vitro* electrophysiology with morphological identification of neuron types, we identified a microcircuit composed of pyramidal neurons and fast spiking basket cells in the CA3 region of the hippocampus that generates these two forms of rhythmic activities under different conditions. Thus, the operation of only two cell types in cortical microcircuits comprised of numerous neuron classes is necessary and sufficient for the generation of intrinsic oscillations within the hippocampal networks. Our data placed fast spiking basket cells into the key position for generating oscillations at high frequencies.

- a. **Hájos N.**, J. Pálhalmi, E.O. Mann, B. Németh, O. Paulsen and T.F. Freund (2004) Spike timing of distinct types of GABAergic interneurons during hippocampal gamma oscillations *in vitro*. *J. Neurosci.* 24:9127-37. PMCID: PMC6730063
- b. **Hájos N**, Ellender TJ, Zemankovics R, Mann EO, Exley R, Cragg SJ, Freund TF, Paulsen O. Maintaining network activity in submerged hippocampal slices: importance of oxygen supply. *Eur J Neurosci.* 29:319-27. (2009) PMCID: PMC2695157
- c. Gulyás AI, Szabó GG, Ulbert I, Holderith N, Monyer H, Erdélyi F, Szabó G, Freund TF, **Hájos N.** Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. *J Neurosci.* 30:15134-45. (2010) PMCID: PMC3044880
- d. **Hájos N**, Karlócai MR, Németh B, Ulbert I, Monyer H, Szabó G, Erdélyi F, Freund TF and Gulyás AI. Input-output features of anatomically identified CA3 neurons during hippocampal sharp wave/ripple oscillation *in vitro*. *J Neurosci.* 33:11677-91. (2013) PMCID: PMC3724544.

4. Microcircuit organization in the basolateral amygdala

Basolateral amygdala is a key structure within the distributed brain networks controlling emotional states and cognitive processes. Combining neuroanatomical techniques and *in vitro* electrophysiology with optogenetics we uncovered some of the key principles in the microcircuit organization within the basal amygdala. For example, we determined that the two basket cell types form independent inhibitory circuits, as they innervate their own kind without targeting the other basket cell type. On top of these surprising findings, we revealed that excitatory neurons efficiently drive the spiking of fast spiking basket cells via their local axon collaterals, while the regular spiking basket cells are only weakly excited. Many factors, including the number of AMPA receptors at excitatory synapses, underlie the differences observed in the recruitments of the two basket cell types by feedback excitation within amygdalar circuits. These results clearly show that the function of the two basket cell types in circuit operation should be distinct. In addition, we recently determined the number of GABAergic cells in the mouse basolateral amygdala and the ratio of distinct inhibitory cell types. These critical network parameters provide a solid base for modelling studies and future investigations aiming to reveal changes in GABAergic networks under pathological conditions.

- a. Veres JM, Nagy GA, **Hájos N.** Perisomatic GABAergic synapses of basket cells effectively control principal neuron activity in amygdala networks. *Elife.* 6. pii: e20721. doi: 10.7554/eLife.20721. (2017) PMCID: PMC5218536
- b. András T, Veres J, Rovira-Esteban L, Kozma R, Vikár A, Gregori E, **Hájos N.** Differential Excitatory Control of Two Parallel Basket Cell Networks in Amygdala Microcircuits. *Plos Biology* e2001421. doi: 10.1371/journal.pbio.2001421. (2017) PMCID: PMC5443504
- c. Rhomberg T, Rovira-Esteban L, Vikár A, Paradiso E, Kremser C, Nagy-Pál P, Papp OI,

Tasan R, Erdélyi F, Szabó G, Ferraguti F, **Hájos N**. VIP-immunoreactive interneurons within circuits of the mouse basolateral amygdala. *J Neurosci* 38:6983-7003. (2018) PMCID: PMC6070667

- d. Vereczki VK, Müller K, Krizsán É, Máté Z, Fekete Z, Rovira-Esteban L, Veres JM, Erdélyi F, **Hájos N**. Total Number and Ratio of GABAergic Neuron Types in the Mouse Lateral and Basal Amygdala. *J Neurosci*. 41:4575-4595. (2021) PMCID: PMC260245

D. Additional Information: Research Support and/or Scholastic Performance

My lab at the IUB was launched with a help a startup packaged provided by the Gill Center, the IU Vice President for Research, and College of Art and Science, IUB in 2021.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Cecilia J. Hillard, PhD

ERA COMMONS USER NAME (credential, e.g., agency login): CHILLARD

POSITION TITLE: Professor of Pharmacology; Director of the Neuroscience Research Center

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
University of Virginia, Charlottesville, VA	B.S.	01/1977	Chemistry
Medical College of Wisconsin	Ph.D.	05/1983	Pharmacology
Medical College of Wisconsin	Post-Doc	07/1985	Biochem Pharmacology

A. Personal Statement

Role and Research: I am a neuropharmacologist with strong interests in the pharmacology of the cannabinoids and biochemistry of the endocannabinoid system. I am particularly interested in the role of the endocannabinoid system in the acute and chronic effects of stress and inflammation on the brain. My research covers the spectrum from biochemical studies to examination of circulating endocannabinoids in human subjects.

I have collaborated with Drs. Mackie and Bradshaw for many years and am very supportive of this P30 proposal. The combination of cores, particularly the lipid measurement and imaging cores, will be extremely beneficial for my research and for those of many others in the cannabinoid research field.

Ongoing and recent research funding I would like to highlight include:**R01 HL154579****Drobyski and Hillard (MPI)****08/01/20-05/31/24**

Mechanistic inflammatory pathways in graft versus host disease

R01 MH121454**Liu and Hillard (MPI)****09/11/19-08/31/24**

Circuit-specific actions of endocannabinoids in stress and mood-disorders

R21 DA051168**Hillard (PI)****04/01/21-03/31/23**

Studies of Cannabidiol in Neurodevelopment

R21 DA049109**Lisdahl and Hillard (MPI)****09/30/19-11/31/22**

Examining the impact of circulating endocannabinoid levels on neurocognition, mood and early cannabis use in youth enrolled in the ABCD study

R01 MH128982

Larson and deRoon-Cassini (MPI); Role: Co-investigator

9/2021-7/2026

Risk and Resilience in Urban Black American Acute Trauma Survivors

R01 NS112194

Pan (PI), Role: Co-Investigator

08/15/19-05/31/23

Neuropathic pain-induced depression: The role of mPFC endocannabinoids

I01 RX0022747

Dean (PI), Role: Co-investigator

04/01/19-03/31/23

VA Rehab R&D Merit Review

Endogenous cannabinoid signaling in the development of chronic neuropathic pain

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2016-present	Associate Dean for Research, Medical College of Wisconsin
2015	co-Interim Senior Associate Dean for Research, Medical College of Wisconsin
2014	Interim Chair, Department of Pharmacology, Medical College of Wisconsin
2010-present	Director, Neuroscience Research Center, Medical College of Wisconsin
2003-present	Professor of Pharmacology and Toxicology, Medical College of Wisconsin
1996-2010	Director, Neuroscience Doctoral Program, Medical College of Wisconsin
1993-2003	Associate Professor of Pharmacology and Toxicology, Medical College of Wisconsin
1985-1993	Assistant Professor of Pharmacology and Toxicology, Medical College of Wisconsin

Honors

2022	Elected vice chair of the 2023 and chair of the 2025 Cannabinoid Function in the CNS GRC
2021	Named the G. Frederick Kasten, Jr. Endowed Chair in Parkinson's Research at the Medical College of Wisconsin
2017	Lifetime Achievement Award from the International Cannabinoid Research Society
2011	Mechoulam Award from the International Cannabinoid Research Society
2011	Distinguished Service Award, Medical College of Wisconsin
2010	Mentor of the year, Graduate School of Biomedical Sciences, Medical College of Wisconsin
2006	Dr. Kit Allen Women's Health Research Award
2002	Medical College of Wisconsin Society of Teaching Scholars
1986	NIH FIRST Award

C. Contributions to Science

1. Endocannabinoid biochemistry. Our laboratory has had a longstanding interest in elucidating the biochemistry of the endocannabinoids. We began these studies in collaboration with Dr. William Campbell, an expert in the biochemistry of arachidonates in the vasculature. We have carried out many studies in this area. Our more important contributions are elucidation of the kinetics and subcellular distribution of fatty acid amide hydrolase (paper a) and demonstration that anandamide is accumulated by primary neurons via a protein-mediated, diffusion driven process (paper b). We have recently reported that a lipid binding protein, sterol carrier protein 2, can bind anandamide and its presence in cells increases anandamide accumulation (paper c) and have embarked on a program, continued in the project described herein, with Dr. Chris Cunningham to identify novel inhibitors of the SCP-2 carrier (paper d).

- a. **Hillard CJ**, Wilkison DM, Edgemon WS, and Campbell WB. Characterization of the kinetics and subcellular distribution of arachidonylethanolamide (anandamide) hydrolase of rat brain. *Biochim. Biophys. Acta* 1257: 249-256, 1995.
- b. **Hillard CJ**, Edgemon WS, Jarrahian A, and Campbell WB. Accumulation of N-arachidonylethanolamide

(anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J. Neurochem.* 69:631-638, 1997.

- c. Liedhegner ES, Vogt CD, Sem DS, Cunningham CW, and **Hillard CJ**. Sterol carrier protein-2: Binding protein for endocannabinoids. *Mol Neurobiol* 50(1):149-58, 2014 PMC4450258
- d. **Hillard CJ**, Huang H, Vogt CD, Rodrigues BE, Neumann TS, Sem DS, Schroeder F, Cunningham CW. Endocannabinoid transport proteins: discovery of tools to study sterol carrier protein-2. *Meth Enzymol* 593:99-121, 2017 PMC6904209

2. The relationships among endocannabinoid signaling, glucocorticoids and the HPA axis. In the early 2000's, we began a series of studies exploring the interactions between the endocannabinoid system (ECS) and the regulation of stress responsivity. In paper a, we demonstrated that CB1 receptor agonists inhibit, while CB1 receptor antagonists increase, restraint stress-induced corticosterone release in male mice. We put forward the hypothesis that the ECS acts as a "gate" on activation of the HPA axis by stress and in other studies demonstrated that the ECS in the amygdala must be suppressed in order for the HPA axis to be fully activated by stress. In another series of studies, including paper b, we demonstrated that the ECS is activated by corticosterone and is required for corticosterone-mediated, long-loop feedback inhibition of the HPA axis in several brain regions. These and other studies led us to hypothesize that corticosterone uses the ECS as a mechanism to modulate synaptic activity in brain. Studies carried out in collaboration with Dr. John Mantsch have extended this hypothesis to study the role of the ECS, particularly in the prelimbic cortex, in stress-potentiated relapse to cocaine seeking behavior (paper c).

- a. Patel S, Roelke CT, Rademacher DJ, Cullinan WE, and **Hillard CJ**. Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis. *Endocrinology* 145:5431-5438, 2004. (News and Views Article about this paper: Tasker, J. Endogenous cannabinoids take the edge off neuroendocrine responses to stress. *Endocrinology* 145: 5429-5430, 2004).
- b. Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, Lee TT, Karatsoreos IN, Mackie K, Viau V, Pickel VM, McEwen BS, Liu QS, Gorzalka BB, and **Hillard CJ**. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. *J. Neurosci.* 31: 10506-10515, 2011. PMC3179266
- c. McReynolds JR, Doncheck EM, Li Y, Vranjkovic O, Graf EN, Ogasawara D, Cravatt BF, Baker DA, Liu QS, **Hillard CJ**, Mantsch JR. Stress promotes drug seeking through glucocorticoid-dependent endocannabinoid mobilization in the prelimbic cortex. *Biol. Psychiatry* 84: 85-94, 2018 PMC5889367
- d. Roberts CJ, Hillard CJ. Peripherally restricted cannabinoid type 1 receptor (CB1R) antagonist, AM6545, potentiates stress-induced hypothalamic-pituitary-adrenal axis activation via a non-CB1R mechanism. *Endocrine* 72:297-300, 2021. PMC8528513

3. Role of changes in endocannabinoids in the sequelae of chronic stress. It is well known that chronic stress exposure results in adaptations in the brain. Given that the ECS regulates synaptic plasticity and is regulated by stress, we have carried out a series of studies demonstrating two important roles for the ECS in the regulation of chronic stress. First, our studies demonstrate that chronic, unpredictable stress results in a downregulation of the ECS, which results in changes in synaptic plasticity and behavior that are the result of reduced CB1 receptor expression (paper a). Second, repeated exposure of rodents to the same stress results in habituation to the stress, and our data suggest that this is also mediated by changes in the ECS, likely at the level of changes in the synthesis or degradation of the endocannabinoids (papers b and c). In a more recent collaboration with several other laboratories, we contributed to a study that continues to demonstrate an important role for the endocannabinoid system in the effects of chronic stress on the amygdala (paper d). Given that habituation to stress is understood to be an important mechanism for stress resilience, these studies demonstrate the potential for manipulation of the ECS to prevent the negative sequelae of chronic stress.

- a. Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, **Hillard CJ**, and Gorzalka BB. Hypoactive endocannabinoid signaling in the hippocampus following chronic stress. *Neuropsychopharmacol.* 30: 508-515, 2005.
- b. Patel S, Roelke CT, Rademacher, DJ and **Hillard CJ**. Inhibition of restraint stress-induced neural and behavioral activation by endogenous cannabinoid signaling. *Eur. J. Neurosci.* 21:1057-1069, 2005

- c. Hill MN, Kumar SA, Filipski SB, Iverson M, Stuhr KL, Keith JM, Cravatt BF, **Hillard CJ**, Chattarji S and McEwen BS: Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar structure. Mol Psychiatry 18: 1125-35, 2013. PMC4148304
- d. Yasmin F, Colangeli R, Morena M, Filipski S, van Der Stelt M, Pittman QJ, **Hillard CJ**, Teskey GC, McEwen BS, Hill MN, Chattarji S. Stress-induced modulation of endocannabinoid signaling leads to delayed strengthening of synaptic connectivity in the amygdala. Proc Natl Acad Sci 117(1):650-655, 2020 PMC6955336

4. CB2 cannabinoid receptor studies. The CB2 cannabinoid receptor has received far less attention than the CB1 receptor, but its prominent role in the regulation of the activation of immune cells makes it a very enticing target for the development of therapeutics that affect neuroinflammation. Our laboratory has carried out a series of studies examining the role of the CB2 receptor in microglia, particularly its role in regulating microglial proliferation and the regulation of its expression by inflammatory stimuli (paper a). Through collaborations with other laboratories, we have determined that CB2 receptor expression is up-regulated in many brain pathologies that are associated with inflammation, including multiple sclerosis, Alzheimer's disease and viral encephalitis and contributes to protective effects in a Parkinson's Disease model (paper c). Recent studies from the laboratory of Alex Straiker utilizing a transgenic CB2 reporter mouse model developed in my laboratory identified an important role of CB2 receptors in the regulation of corneal wound healing (paper b). We have also examined the CB2 receptor in peripheral inflammation and have discovered that loss of T cell CB2R results in very significant worsening of the inflammation associated with graft-versus-host-disease (GVHD, paper d).

- a. Carrier EJ, Kearn CS, Barkmeier AJ, Breese NM, Yang W, Nithipatikom K, Pfister SL, Campbell WB, and **Hillard CJ**. Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. Molec. Pharmacol. 65:999-1007, 2004
- b. Murataeva N, Miller S, Dhopeshwarkar A, Leishman E, Daily L, Taylor X, Morton B, Lashmet M, Bradshaw H, **Hillard CJ**, Romero J, Straiker A. Cannabinoid CB2R receptors are upregulated with corneal injury and regulate the course of corneal wound healing. Exp Eye Res 182:74-84, 2019 PMC6504573
- c. Yu H, Liu X, Chen B, Vickstrom CR, Friedman V, Kelly TJ, Bai X, Zhao L, Hillard CJ, Liu QS. The neuroprotective effects of the CB2 agonist GW842166x in the 6-OHDA mouse model of Parkinson's Disease. Cells 10(12):3548, 2021 PMC8700250
- d. Yuan CY, Zhou V, Sauber G, Stollenwerk TM, Komorowski R, Lopez A, Tolon RM, Romero J, Hillard CJ, Drobyski WR. Signaling through the type 2 cannabinoid receptor regulates the severity of acute and chronic graft versus host disease. Blood 137:1241-1255, 2021 PMC7933769

5. Role of the endocannabinoid system in post-traumatic stress disorder. Post-traumatic stress disorder (PTSD) is a chronic, debilitating disease that occurs in 20-30% of individuals who have a physical or psychological traumatic event. The symptoms of PTSD include sleep disturbances, anxiety, inability to extinguish fearful memories and depressed mood. Given that these psychological processes are known to be modulated by the endocannabinoid system, we have collaborated with several groups to explore the hypotheses that dysregulated endocannabinoid signaling is a consequence and contributor to the development of PTSD. We have collaborated with Kevin Crombie to demonstrate that circulating endocannabinoids link exercise to reduced feelings of threat (paper d); and that circulating endocannabinoids are less likely to be elevated by exercise in individuals with PTSD than healthy controls (paper a). In papers b and c, we have collaborated with the Akirav group in Israel to examine these questions in a preclinical model of PTSD and have found that, indeed, elevation of endocannabinoid signaling has beneficial effects.

- a. Crombie KM, Brellenthin AG, **Hillard CJ**, Kolty KF. Psychobiological responses to aerobic exercise in individuals with posttraumatic stress disorder. J Trauma Stress 31:134-145, 2018
- b. Segev A, Korem N, Mizrachi Zer-Aviv T, Abush H, Lange R, Sauber G, **Hillard CJ**, Akirav I. Role of endocannabinoids in the hippocampus and amygdala in emotional memory and plasticity. Neuropsychopharmacol 43(10): 2017-2027, 2018 PMC6098035
- c. Fidelman S, Mizrachi Zer-Aviv T, Lange R, **Hillard CJ**, Akirav I. Chronic treatment with URB197 ameliorates post-stress symptoms in a rat model of PTSD. Eur Neuropsychopharmacol 28: 630-642, 2018
- d. Crombie KM, Sartin-Tarm A, Sellnow K, Ahrenholtz R, Lee S, Matalamaki M, Almassi NE, Hillard CJ, Kolty KF Adams TG, Cisler JM. Exercise-induced increases in anandamide and BDNF during

extinction consolidation contribute to reduced threat following reinstatement: Preliminary evidence from a randomized controlled trial. Psychoneuroendocrinol 132:105355, 2021 PMC8487992

I have published 254 papers and have an H index of 77. The URL for my complete bibliography is:
<http://www.ncbi.nlm.nih.gov/sites/myncbi/cecilia.hillard.1/bibliography/40588598/public/?sort=date&direction=asc>

BIOGRAPHICAL SKETCH

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NAME: Hohmann, Andrea Grace

ERA COMMONS USER NAME (credential, e.g., agency login): AHOHMANN

POSITION TITLE: Linda and Jack Gill Chair of Neuroscience and Professor

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Brown University (Providence, RI)	Sc.B.	05/1988	Psychology
Brown University (Providence, RI)	Sc.M.	05/1992	Experimental Psych
Brown University (Providence, RI)	Ph.D.	09/1996	Experimental Psych
NIMH, NIH (Bethesda, MD)	Postdoctoral	09/1998	Neuroanatomy
NIDCR, NIH (Bethesda, MD)	Postdoctoral	06/1999	Pain, Neuroanatomy

A. Personal Statement

I am a Linda and Jack Gill Chair of Neuroscience and Professor of Psychological and Brain Sciences who has been studying the therapeutic potential of the endocannabinoid signaling system for over 25 years. My research goal is to identify novel therapeutic interventions for treating pain that lack abuse liability and adverse side-effects (i.e. physical dependence, memory/motor impairment). My lab has focused on harnessing the therapeutic potential of the endocannabinoid signaling system to suppress neuropathic pain while minimizing unwanted side effects (i.e. addiction, reward, psychoactivity). The endocannabinoid system consists of cannabinoid receptors (CB₁ and CB₂), endogenous ligands (endocannabinoids) and the enzymes catalyzing endocannabinoid synthesis and degradation. My laboratory combines approaches from behavioral pharmacology and drug abuse (conditioned place preference, drug self-administration, behavioral pharmacology), neurophysiology, neuroanatomy, biochemistry and molecular biology. My graduate work first demonstrated that cannabinoids suppress activity in nociceptive neurons. My postdoctoral work mapped locations and phenotypes of cells expressing cannabinoid receptors/mRNA. I localized cannabinoid receptor mRNA to dorsal root ganglia (DRG) and demonstrated axonal transport of cannabinoid receptors to periphery. My lab showed that endocannabinoids mediate stress-induced analgesia and validated, with the Piomelli lab, an enzyme (i.e. monoacylglycerol lipase) implicated in endocannabinoid deactivation as a previously unrecognized therapeutic target for pain and stress disorders. My lab showed that CB₂ receptor activation suppressed the processing of pathological pain, and validated CB₂ receptors as an analgesic target, that was particularly efficacious for neuropathic pain but was not associated with tolerance, physical dependence or abuse liability (i.e. was not self-administered in otherwise naïve rats). We validated the therapeutic potential of CB₁ positive allosteric modulation and peripheral cannabinoid mechanisms as a therapeutic strategy to avoid side effects of THC at CB₁, including tolerance, reward and physical dependence. We showed that small molecule CB₂ agonists suppressed opioid tolerance and physical dependence. We developed rodent models of therapeutic self-medication in neuropathic pain and used these models to quantify abuse liability/motivation to self-administer both narcotic and non-narcotic (e.g. cannabinoid CB₂ agonists) analgesics. My lab disrupted protein-protein interactions downstream of NMDA receptors to suppress both pronociceptive signaling cascades and opioid reward without the adverse effects of NMDAR antagonists; assays were transferred to industry and resulted in funding of Phase 1 (R43) and 2 (R44) SBIR proposals to evolve small molecule therapeutics for nNOS-PSD95 and nNOS-NOS1AP protein-protein interactions. My lab has been supported by R01, R21, CEBRA, R43 and R44 grants. I also serve as the co-Director of a NIDA T32 training grant (with Ken Mackie). I am well-positioned to continue my productive collaborations with my IUB colleagues (see joint

publications with Mackie, Bradshaw, Katona and Lu) to elucidate mechanisms underlying addiction and potential treatments using neuroanatomical, lipidomic and computational approaches.

Ongoing and recently completed projects that I would like to highlight include:

R01 DA047858-01 Hohmann-PI; Mackie K-Dual PI 04/01/19-03/31/24

NIH/NIDA CB2 cannabinoid mechanisms for blocking opioid tolerance and dependence

The major goal of this project is to identify the cell types responsible for CB2 suppression of opioid tolerance and physical dependence using a conditional deletion approach. Double label immunohistochemical approaches are used with a subset of the markers proposed for use in present proposal.

R01 DA041229-01A1 Hohmann-PI; Mackie K-Dual PI

NIH/NIDA Role of CB2 in Analgesic Mechanisms

04/15/16-01/31/21

The major goal of this project is to identify the cell types responsible for CB2 analgesic efficacy by conditionally deleting CB2 receptors from neurons, astrocytes and microglia to elucidate therapeutic targets for CIPN. Double label immunohistochemical approaches are used with the markers proposed for use in present proposal.

R01 CA200417-01A1 Hohmann-PI; Courtney-Dual PI; Lai-co-I

NIH/NCI NOS1AP as a novel target for treating pathological pain

01/08/16-12/31/22

The major goal of this project is to validate NOS1AP as a therapeutic target for chemotherapy-induced peripheral neuropathic pain using Alphascreen biochemical assays, primary neuronal culture and in vivo assays of therapeutic efficacy and side effect profiles. Peptide inhibitors developed in Courtney lab are used.

R43 CA241513 Pesce, G (PI); Hohmann (MPI)

09/25/19-04/31/21

NIH/NCI Therapeutic antibodies for treating chemotherapy-induced peripheral neuropathic pain

The major goal of this project is to validate antibody therapeutics or management of chemotherapy-induced peripheral neuropathic pain (CIPN). This is the first antibody agonist therapy for any GPCR. The Hohmann lab performs all in vivo studies.

R44 CA241513-02 Schwimmer (PI); Hohmann (MPI)

05/05/2021-04/30/23

NIH/NCI Therapeutic antibodies for treating chemotherapy-induced peripheral neuropathic pain

The major goal of this project is to validate antibody therapeutics or management of paclitaxel-induced peripheral neuropathic pain (CIPN). This is the first antibody agonist therapy for any GPCR. The Hohmann lab performs all in vivo studies in Year 2-3 of the project and all immunohistochemical and double label studies.

R01 DA030604-09 Silverman, R. (PI); Hohmann (Subcontract PI)

05/01/18-04/30/21

NIH/NIDA New Inactivators of GABA Aminotransferase for Addiction and Epilepsy

The major goal of this project is to validate inhibitors of GABA-AT as a therapeutic strategy for blocking opioid reward and addiction using drug-self-administration and conditioned place preference approaches. The Hohmann performs the in vivo studies

R44 NS098885-02 (Florio: PI; Lai Co-PI; Hohmann Subcontract PI) NIH/NINDS 08/01/18-01/31/22

NIH/NINDS Development of novel small molecule analgesics modulating the nNOS-NOS1AP protein-protein interaction.

The major goal for this SBIR Phase II program is to advance two chemical series through hit-to-lead then early lead optimization studies with the ultimate goal of developing a drug candidate for the treatment of chronic pain. The Hohmann lab performs the in vivo studies.

Indiana Addiction Grand Challenges Hohmann PI; Rebec, Crystal co-PIs

10/15/18-06/31/22

Accelerating solutions to the opioid epidemic by repurposing an cannabinoid CB2 agonist.

This project repurposes a former failed drug clinical candidate as a therapeutic strategy to suppress opioid reward (using conditioned place preference, in vivo drug self-administration and in vivo voltammetry) and opioid-induced respiratory depression (using whole body plethysmography).

T32 DA 024628-01A1 Mackie, K (PD); Hohmann, A. (PD)

07/01/2008-06/30/2025

NIH/NIDA Integrative Predoctoral Training in Drug Abuse Research at Indiana University"

This project supports integrative predoctoral training in the neuroscience of drug abuse at Indiana University.

R21DA042584-01 Hohmann PI; Rebec Dual PI

08/15/16-08/31/20

NIH/NIDA A Novel Mechanism for Decreasing Opioid Reward

This grant tests the hypothesis that disruption of PSD95-nNOS protein-protein interactions will suppress opioid reward using conditioned place preference, drug self-administration and in vivo voltammetry.

3P01 DA09158-01 Makriyannis-PD

08/2022-07/30/27

NIH/NIDA Endocannabinoid Active Sites as Therapeutic Targets. Project 3 (Hohmann-PI): In vivo pharmacology of cannabinoid receptor probes

The major goal of this program project grant is to develop functionally selective cannabinoid agonists with unique signaling profiles and allosteric modulators and characterize efficacy and side effect profiles (tolerance, dependence, antinociception) models.

Citations:

1. **Hohmann, A.G.**, Suplita II, R.L., Bolton, N.M., Neely, M.H., Fegley, D., Mangieri, R., Krey, J.F., Walker, J.M., Holmes, P.V., Crystal, J.D., Duranti, A., Tontini, M., Tarzia G. and Piomelli, D. (2005) An endocannabinoid mechanism for stress-induced analgesia. *Nature* 435: 1108-1112.
2. Gregg, L.C, Jung, K.M., Spradley, J.M., Nylas, R., Suplita II, R.L., Zimmer, A., Watanabe, M., Mackie, K., Katona, I., Piomelli, D. and **Hohmann, A.G.** (2012) Activation of type-5 metabotropic glutamate receptors and diacylglycerol lipase- α initiates 2-arachidonoylglycerol formation and endocannabinoid-mediated analgesia. *The Journal of Neuroscience* 32(28) 9457-9468. PMCID: PMC3652685
3. Gutierrez, T., Crystal, J.D., Zvonok, A.M., Makriyannis, A. and **Hohmann, A.G.** (2011) Self-medication of a cannabinoid CB₂ agonist in an animal model of neuropathic pain. *PAIN* 152: 1976-87. PMCID: 3157548
4. Gutierrez, T., Oliva, I., Crystal, J.D. and **Hohmann, A.G.** (2021) Peripheral nerve injury promotes morphine seeking behavior in extinction. *Experimental Neurology* 338: 113601. NIHMSID16622981

B. Positions, Scientific Appointments, and Honors

Positions

- 2015- Co-Director NIDA Training Grant T32DA024628-01A1 (G. Rebec, A. Hohmann, P. Finn).
2010- Linda and Jack Gill Chair of Neuroscience and Professor, Psychological and Brain Sciences, Indiana University, Bloomington, IN
2009-10 Professor of Psychology and Neuroscience, University of Georgia, Athens, GA
2005-09 Associate Professor, Neurosci & Behav Program, Dept Psychology, Univ of Georgia, Athens, GA
1999-05 Assistant Professor, Neurosci & Behavior Program, Dept Psychology, Univ of Georgia, Athens, GA
1998-99 Staff Research Fellow (Laboratory of M. A. Ruda, PhD), Pain and Neurosensory Mechanisms, NIDCR, NIH, Bethesda, MD
1996-98 Pharmacology Research Associate (PRAT) Fellow (Laboratory of Miles Herkenham), NIGMS, Section on Functional Neuroanatomy, NIMH, Bethesda, MD

Other Experience

- 2019- Full member of NIH study section: Molecular Neuropharmacology and Signaling (SCS)
2013 Elected Chair, Gordon Research Conference on Cannabinoid Function in the CNS
2007-10 Full member of NIH study section: Somatosensory and Chemosensory Systems (SCS)
2003-10 Director, Neuroscience, Cognition and Behavior Group, Institute for Behavioral Research
2002- NIH study section member: SCS; MNPS (standing); MCDN IRG, SSS-P; IFCN-A; ZRGs, CEBRA
2002-10 President, University of Georgia Chapter of the Society for Neuroscience

Honors

- 2020 Finalist: *Ziskind-Somerfeld Research Award, Honorable Mention, Biol Psychiatry* for Slivicki et al. (2018) *Biological Psychiatry* 84: 722-733.
2018 Fellow, *American Association for the Advancement of Science*.
2015 Science Advance in Pain Research (recognition of Deng et al. (2015) *Biol Psychiatry* 77: 475-487)
2011 Ester Fride Award for Major Contributions to Cannabinoid Basic Research, IACM
2011 Presidential Life Science Professor, Lilly Foundation and Indiana University
2010 William A. Owens Award for Outstanding Research, *University of Georgia Research Foundation*
2007 Young Investigator Award, *International Cannabinoid Research Society*
2006 Creative Research Medal, University of Georgia
1999 Scientific Director's Postdoctoral Travel Award for outstanding achievement, NIDCR, Bethesda, MD
1998 Prize for best paper presentation, *International Cannabinoid Research Society*
1996 NRSA Predoctoral Fellowship, National Institute on Drug Abuse (NIDA) (1F31DA05725-01)
1995 Prize for best paper presentation, *International Cannabinoid Research Society*
1995 Prize for outstanding graduate research, *Sigma Xi*, Brown University, Providence, RI
1995-96 Dissertation Fellowship, Brown University, Providence, RI
1991-92 University Fellowship, Brown University, Providence, RI

C. Contributions to Science 115 peer-reviewed papers/16 chapters; H-Index 56; i-10 Index: 104; 12,876 citations

1. Cannabinoids suppress nociceptive processing and neuroanatomical basis of antinociception

When I began my research on cannabinoids, it was not known whether antinociceptive effects of cannabinoids reflected suppression of pain or artifacts of motor impairment/psychoactivity. My work used electrophysiological methods to provide the first demonstration that cannabinoids suppress activity in nociceptive neurons. I mapped locations and phenotypes of cells expressing cannabinoid receptors/mRNA at central and peripheral sites and contributed to the initial characterization of CB₁ receptor knockout (KO) mice. I used receptor binding and autoradiography, *in situ* hybridization and double label studies to localize cannabinoid receptors in DRG, and nociceptive circuits and compare distributions with mu opioid receptors.

- a. **Hohmann, A.G.**, Martin, W.J., Tsou, K. and Walker, J.M. (1995) Inhibition of noxious stimulus-evoked activity of lumbar dorsal horn neurons by the cannabinoid WIN 55,212-2. *Life Sciences* 56: 2111-2118.
- b. **Hohmann, A.G.** and Herkenham, M. (1999) Localization of central cannabinoid CB₁ receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: A double-label *in situ* hybridization study. *Neuroscience*, 90: 923-931.
- c. Zimmer, A., Zimmer, A.M., **Hohmann, A.G.**, Herkenham, M. and Bonner, T.I. (1999) Increased mortality, hypoactivity and hypoalgesia in cannabinoid CB₁ receptor knockout mice. *Proceedings of the National Academy of Sciences U.S.A.* 96: 5780-5785.
- d. **Hohmann, A.G.**, Briley E.M. and Herkenham, M. (1999) Pre- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. *Brain Research* 822: 17-25.

2. Peripheral cannabinoid mechanisms of pain suppression

My lab used behavioral, immunohistochemical and electrophysiological methods to establish that activation of cannabinoid receptors outside the central nervous system suppresses pathological pain *in vivo*. My lab also collaborated with the Piomelli lab to perform *in vivo* validation of the first peripherally restricted inhibitor of the anandamide degrading enzyme fatty-acid amide hydrolase (URB937). We established efficacy of peripheral endocannabinoid mechanisms for suppressing the processing of nociceptive information. We also performed comparative analysis of receptor mechanisms underlying anti-allodynic effects produced from targeting either FAAH or MGL outside the CNS.

- a. Nackley, A.G., Suplita, R.L II and **Hohmann, A.G.** (2003) A peripheral cannabinoid mechanism suppresses spinal Fos protein-expression and pain behavior in a rat model of inflammation. *Neuroscience* 117: 659-70.
- b. Spradley, J.M., Guindon, J. and **Hohmann, A.G.** (2010) Inhibitors of monoacylglycerol lipase, fatty-acid amide hydrolase and endocannabinoid transport differentially suppress capsaicin-evoked behavioral sensitization through peripheral endocannabinoid mechanisms. *Pharmacological Research* 62: 249-58. PMCID: PMC2900457
- c. Guindon, J., Guijarro, A., Piomelli, D. and **Hohmann, A.G.** (2011) Peripheral antinociceptive effects of inhibitors of monoacylglycerol lipase in a rat model of inflammatory pain: A comparative analysis. *British Journal of Pharmacology* 163: 1464-78. PMCID: PMC3165956
- d. Clapper, J.R., Moreno-Sanz, G., Russo, R., Vacondio F., Duranti, A., Tontini, A., Sanchini, A., Sciolino, N.R., Spradley, J.M., **Hohmann, A.G.**, Calignano, A., Mor, M., Tarzia, G. and **Piomelli, D.** (2010) Anandamide signaling suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nature Neuroscience* 13: 1265-1270. PMCID: PMC3260554

3. Cannabinoid CB₂ mechanisms that suppress pain without tolerance or abuse liability

My laboratory was the first to establish that activation of cannabinoid CB₂ receptors suppresses pain processing of pathological pain. Our studies used behavioral, electrophysiological and immunohistochemical methods. We also showed that rats in a neuropathic pain state would self-medicate with a cannabinoid CB₂ agonist whereas control animals did not reliably self-administer the drug in the absence of pathological pain. We showed that preemptive and prophylactic dosing strategies with cannabinoids suppressed chemotherapy-induced neuropathic pain and suppressed development of opioid tolerance and physical dependence.

- a. Nackley, A.G., Zvonok, A.M., Makriyannis, A., and **Hohmann, A.G.** (2004) Activation of cannabinoid CB₂ receptors suppresses C-fiber responses and windup in spinal wide dynamic range neurons in the absence and presence of inflammation. *Journal of Neurophysiology* 92: 3562-3574.
- b. Deng, L., Guindon, J., Cornett, B.L., Makriyannis, A., Mackie K. and **Hohmann A.G.** (2015) Chronic cannabinoid CB₂ activation reverses paclitaxel neuropathy without tolerance or CB₁-dependent withdrawal *Biological Psychiatry* 77: 475-487. PMCID: PMC4209205
- c. Deng, L., Cornett, B.L., Mackie, K. and **Hohmann, A.G.** (2015) CB₁ Knockout mice unveil sustained CB₂-mediated anti-allodynic effects of the mixed CB₁/CB₂ agonist CP55,940 in a mouse model of paclitaxel-induced neuropathic pain. *Mol Pharmacol* 88: 64-74. PMCID: PMC25904556

- d. Lin, X., Dhopeshwarkar, A., Hubregtse, M., Mackie, K. and **Hohmann, A.G.** (2018) The slowly signaling G protein-biased CB₂ cannabinoid receptor agonist LY2828360 suppresses neuropathic pain with sustained efficacy and attenuates morphine tolerance and dependence. *Molecular Pharmacology*, 93: 49-62. PMID: 29192123. PMCID: PMC5749492.

4. 2-arachidonoylglycerol (2-AG) suppresses pain and therapeutic potential of endocannabinoids

My lab was the first to show that endocannabinoids are mobilized on demand by stress to suppress pain (i.e. stress-induced analgesia). These collaborative studies (with the Piomelli lab) identified the first inhibitor of 2-AG deactivation (URB602) and established that inhibition of the enzyme monoacylglycerol lipase produces antinociception. We identified biochemical mechanisms responsible for 2-AG formation *in vivo*. These studies identified the DGL isoform responsible for 2-AG formation *in vivo*, defined the molecular architecture of retrograde 2-AG signaling in the PAG and showed that virally-mediated RNA silencing of DGLα but not DGLβ mRNAs suppressed both 2-AG production and stress antinociception. We identified sites of action of endocannabinoids in suppressing pain using site specific injections (of antagonists and enzyme inhibitors) at spinal and supraspinal sites. We showed that brain permeant and impermeant inhibitors of endocannabinoid deactivation suppress neuropathic pain with efficacy superior to conventional treatments (morphine, amitriptiline, gabapentin) and showed that cisplatin alters endocannabinoid signaling.

- a. **Hohmann, A.G.**, Suplita II, R.L., Bolton, N.M., Neely, M.H., Fegley, D., Mangieri, R., Krey, J.F., Walker, J.M., Holmes, P.V., Crystal, J.D., Duranti, A., Tontini, M., Tarzia G. and **Piomelli, D.** (2005) An endocannabinoid mechanism for stress-induced analgesia. *Nature* 435: 1108-1112.
- b. Clapper, J.R., Moreno-Sanz, G., Russo, R., Vacondio F., Duranti, A., Tontini, A., Sanchini, A., Sciolino, N.R., Spradley, J.M., **Hohmann, A.G.**, Calignano, A., Mor, M., Tarzia, G. and **Piomelli, D.** (2010) Anandamide signaling suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nature Neuroscience* 13: 1265-1270. PMCID: PMC3260554.
- c. Gregg, L.C., Jung, K.M., Spradley, J.M., Nyilas, R., Suplita II, R.L., Zimmer, A., Watanabe, M., Mackie, K., Katona, I., **Piomelli, D.** and **Hohmann, A.G.** (2012) Activation of type-5 metabotropic glutamate receptors and diacylglycerol lipase- α initiates 2-arachidonoylglycerol formation and endocannabinoid-mediated analgesia. *The Journal of Neuroscience* 32(28) 9457-9468. PMCID: PMC3652685
- d. Slivicki, R.A., Saberi, A.A., Iyer V., Vemuri, V.K., Makriyannis, A. and **Hohmann, A.G.** (2018) Brain permeant and impermeant inhibitors of fatty-acid amide hydrolase synergize with the opioid analgesic morphine to suppress chemotherapy-induced neuropathic nociception without enhancing effects of morphine on gastrointestinal transit. *JPET* 367: 551-563. PMCID: PMC6246979

5. Cannabinoid suppression of neuropathic pain

My lab was the first to establish that CB₂ agonists and CB₁ positive allosteric modulators (PAMs) suppress chemotherapy-induced peripheral neuropathy (CIPN) and did not produce physical dependence. We showed that tolerance did not develop to the observed anti-allodynic efficacy and that challenge with either CB₁ or CB₂ antagonists did not precipitate signs of physical dependence or withdrawal. My lab used i.v. drug self-administration to study self-medication of analgesics that differ in abuse liability. My preclinical studies translated into a published double-blind placebo controlled crossover pilot clinical trial for CIPN with Mary Lynch's group. We collaborated with the Ferris lab at Northeastern University to show that inhaled cannabis decouples the raphe nucleus and suppresses CIPN by using a functional brain imaging approach.

- a. Slivicki, R., Xu, Z., Kulkarni, P., Pertwee, R., Mackie, K., Thakur, G. and **Hohmann, A.G.** (2017) Positive allosteric modulation of cannabinoid receptor type 1 suppresses pathological pain without producing tolerance or dependence. *Biological Psychiatry* 10: 722733. PMCID PMC:5758437.
- b. Slivicki, R., Iyer, V., Mali, S., Garai, S., Thakur, G.A., Crystal., J.D. and **Hohmann, A.G.** (2020) Positive allosteric modulation of CB₁ cannabinoid receptor signaling enhances morphine antinociception and attenuates morphine tolerance without enhancing morphine-induced dependence or reward. *Frontiers in Molecular Neuroscience* 13: 54. PMCID: PMC7199816
- c. Gutierrez, T., Crystal, J.D., Zvonok, A.M., Makriyannis, A. and **Hohmann, A.G.** (2011) Self-medication of a cannabinoid CB₂ agonist in an animal model of neuropathic pain. *PAIN* 152: 1976-87. PMCID: 3157548
- d. Lynch, M.E., Rittenberg, P. and **Hohmann, A.G.** (2014) A double blind placebo controlled crossover pilot trial with extension using an oral mucosal cannabinoid extract for treatment of chemotherapy induced neuropathic pain. *Journal of Pain and Symptom Management* 47: 166-73.

Complete List of Published Work in MyBibliography

<http://www.ncbi.nlm.nih.gov/sites/myncbi/andrea.hohmann.1/bibliography/41139383/public/?sort=date&direction=ascending>

BIOGRAPHICAL SKETCH

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

NAME: Jui-Yen Huang

eRA COMMONS USER NAME (credential, e.g., agency login): JUIYENHUANG

POSITION TITLE: Associate Scientist

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
National Cheng-Kung University, Tainan, Taiwan	B.S.	06/2003	Nursing
National Cheng-Kung University, Tainan, Taiwan	M.S.	06/2004	Physiology
National Cheng-Kung University, Tainan, Taiwan	Ph.D.	08/2010	Neuroscience
Baylor College of Medicine, Houston, TX, USA	Postdoctoral	04/2015	Neuroscience
Indiana University, Bloomington, IN, USA	Postdoctoral	07/2017	Neuroscience

A. Personal Statement

My role in this project is that of Associate Scientist and I will contribute 50% professional effort to the project. I am highly motivated to pursue an academic, translational-research career. Neuroscience became particularly fascinating for me when, during my training as a nurse, my patients and I were faced with limited medical treatments for neurological disorders. This was due simply to how little we knew about the brain. During my Ph.D. training, my research focused on exploring the role of fibroblast growth factor 9 (FGF9) in neurodegenerative disease models. To address this, I established and mastered multiple experimental techniques and systems, including primary neuronal/glial cultures, stereotaxic drug and lentivirus production and delivery, and general molecular biology approaches. Along with this training, I also acquired a broad background in free radical biology, basic neuroscience, and neurodegenerative disease. During my postdoctoral training, my research focused on the role of mGluR5 and the endocannabinoid system during cortical brain circuitry assembly. Concurrently, I also developed a research project on the role of FGF-FGF receptors in brain circuitry assembly and maintenance. To best address these projects, I mastered several advanced neuroscience techniques, including *in-utero* electroporation and whole-cell patch clamp electrophysiological recording and carried out extensive studies using conditional transgenics in mice. In 2017 I was promoted to an Assistant Research Scientist to further develop my personal research career. In 2018 I established a Patch-Seq technique that combines whole-cell patch-clamp recording, immunohistochemistry, and single-cell RNA-sequencing to comprehensively profile single neurons from mouse brain slices. In the meantime, I also attended a Neuroimaging Course held by Max-Planck Florida Institute to advance my knowledge in applying state-of-art two-photon Fluorescence Lifetime Imaging Microscopy (2P-FLIM) imaging techniques. Capitalizing on this training, in 2019 I established the cranial window installation technique in the postnatal 8th-9th (P8-P9) neonatal mouse to monitor neuronal calcium dynamics longitudinally (P11-P30) as circuits develop. In 2020 I published a study where we utilized ScaleS (brain clearing methodology) combined with 2P imaging to visualize brain circuitry abnormalities during development of an FGFR mutant mouse. In addition to my research training and experience, I have also trained many undergraduates, PhD students, research associates, and visiting scientists for various surgical and imaging techniques and data analysis. I have developed supervisory, leadership, and administrative skills during my time as an assistant scientist in the Gill Center at IU Bloomington. I also enjoy collaboration with scientists and have published several collaborative papers. Because of my background and experience, I feel I am well prepared to carry out the imaging experiments proposed in this application and look forward to training visiting researchers in these techniques.

B. Positions and Honors

Position and Employment

2022-Present	Associate Scientist in the Gill Center and the Department of Psychological and Brain Science, Indiana University Bloomington, Bloomington, IN, USA
2017-2022	Assistant Scientist in the Gill Center and the Department of Psychological and Brain Science, Indiana University Bloomington, Bloomington, IN, USA

Other Experience and Professional Memberships

2009-Present	The Chinese Society of Cell and Molecular Biology
2008-Present	Society for Neuroscience

Honors

2018	Travel Award, Provost's Travel Award for Women in Science
2015	Travel Award, Keystone Symposia Scholarship
2011	Poster Award, 19 th Symposium on Recent Advances in Cellular and Molecular Biology
2010	Excellent Research Award in Institute of Basic Medical Sciences, National Cheng Kung University
2009	Poster Award, 17 th Symposium on Recent Advances in Cellular and Molecular Biology

C. Contributions to Science

1. **The role FGF9 in Parkinson's disease.** My early publications directly addressed the role of fibroblast growth factor 9 (FGF9) in the MPP⁺- induced rat Parkinsonian model. In this research topic, I acquired the first experimental evidence that FGF9 downregulation caused dopaminergic neuronal death in an MPP⁺- induced-Parkinsonian model. I also demonstrated that FGF9 overexpression protected dopaminergic neurons from MPP⁺-induced dopaminergic neuronal death via upregulation of antioxidant enzyme expression *in vivo*.
 - a. **Huang JY**, Hong YT, Chuang JI. Fibroblast growth factor 9 prevents MPP+-induced death of dopaminergic neurons and is involved in melatonin neuroprotection *in vivo* and *in vitro*. *J Neurochem.* 2009;109(5):1400-12. Epub 2009/05/30. doi: 10.1111/j.1471-4159.2009.06061.x. PubMed PMID: 19476551. Not US government supported.
 - b. **Huang JY**, Chuang JI. Fibroblast growth factor 9 upregulates heme oxygenase-1 and gamma-glutamylcysteine synthetase expression to protect neurons from 1-methyl-4-phenylpyridinium toxicity. *Free Radic Biol Med.* 2010;49(6):1099-108. Epub 2010/07/10. doi: 10.1016/j.freeradbiomed.2010.06.026. PubMed PMID: 20615462. Not US government supported.
 - c. Chuang JI, **Huang JY**, Tsai SJ, Sun HS, Yang SH, Chuang PC, Huang BM, Ching CH. FGF9-induced changes in cellular redox status and HO-1 upregulation are FGFR-dependent and proceed through both ERK and AKT to induce CREB and Nrf2 activation. *Free Radic Biol Med.* 2015;89:274-86. Epub 2015/10/02. doi: 10.1016/j.freeradbiomed.2015.08.011. PubMed PMID: 26424114. Not US government supported.
2. **The role of growth factors in cortical glutamatergic neurons to acquire precise brain wiring.** To further satisfy my interest in how brain circuitry is altered upon neurological insult, my more research has focused on elucidating the molecular mechanisms underlying mGluR5 signaling in regulating cortical sensory circuit formation. I found that functional synaptic calcium-permeable AMPARs are increased in thalamocortical synapses in mGluR5 deficient mouse. This aberrant glutamate transmission leads to dysregulation of several growth factors and their receptors including, *Ngf*, *Fgf7*, *Fgf9*, *Fgf10*, *Fgf22*, *TrkA*, *Fgfr1-3*. Based on these observations, I have uncovered an unexpected role of NGF-TrkA signaling in regulating the dendritic patterning of cortical glutamatergic neurons *in vitro* and *in vivo*. In addition to

exploring the function of NGF-TrkA, I also discovered a novel function of FGF-FGFR signaling on dendritic morphogenesis and axonal wiring *in vivo*.

- a. **Huang JY**, Miskus ML, Lu HC. FGF-FGFR Mediates the Activity-Dependent Dendritogenesis of Layer IV Neurons during Barrel Formation. *J Neurosci*. 2017;37(50):12094-105. Epub 2017/11/04. doi: 10.1523/JNEUROSCI.1174-17.2017. PMCID: PMC5729188.
 - b. **Huang JY**, Lu HC. mGluR5 Tunes NGF/TrkA Signaling to Orient Spiny Stellate Neuron Dendrites Toward Thalamocortical Axons During Whisker-Barrel Map Formation. *Cereb Cortex*. 2018;28(6):1991-2006. Epub 2017/04/30. doi: 10.1093/cercor/bhx105. PMCID: PMC6018836.
 - c. **Huang JY**, Krebs BB, Miskus ML, Russell ML, Duffy EP, Graf JM, Lu HC. Enhanced FGFR3 activity in postmitotic principal neurons during brain development results in cortical dysplasia and axonal tract abnormality. *Sci Rep*. 2020;10(1):18508. Epub 2020/10/30. doi: 10.1038/s41598-020-75537-0. PMCID: PMC7595096.
3. ***Fgfs expression is susceptible to environmental stimuli.*** Dysregulation of *Fgf-Fgfr* signaling occurs in several neurological disorders; however, the underlying mechanisms are unknown. Using a toxin-based Parkinson's disease animal model, I demonstrate that FGF9 expression is suppressed by oxidative stress at the transcriptional level and consequently decreases protein expression. During cortical development, the patterns of *Fgfs-Fgfrs* expression are dynamic. I found that *Fgf9* and *Fgf10* levels are susceptible to glutamate transmission, which may integrate with neural activity to regulate precise synaptic coupling.
- a. **Huang JY**, Hong YT, Chuang JI. Fibroblast growth factor 9 prevents MPP+-induced death of dopaminergic neurons and is involved in melatonin neuroprotection *in vivo* and *in vitro*. *J Neurochem*. 2009;109(5):1400-12. Epub 2009/05/30. doi: 10.1111/j.1471-4159.2009.06061.x. PubMed PMID: 19476551.
 - b. **Huang JY**, Lu HC. mGluR5 Tunes NGF/TrkA Signaling to Orient Spiny Stellate Neuron Dendrites Toward Thalamocortical Axons During Whisker-Barrel Map Formation. *Cereb Cortex*. 2018;28(6):1991-2006. Epub 2017/04/30. doi: 10.1093/cercor/bhx105. PMCID: PMC6018836.
 - c. **Huang JY**, Miskus ML, Lu HC. FGF-FGFR Mediates the Activity-Dependent Dendritogenesis of Layer IV Neurons during Barrel Formation. *J Neurosci*. 2017;37(50):12094-105. Epub 2017/11/04. doi: 10.1523/JNEUROSCI.1174-17.2017. PMCID: PMC5729188.
4. ***Role of endocannabinoids in cortical circuit development.*** In addition to their well-known roles in modulating synaptic transmission and neuronal excitability, the role of endogenous cannabinoids in the developing brain are just beginning to be understood. Building on my experience with mGluR5 signaling in cortical development (mGluR5 activation in cortex often leads to production of the endocannabinoid, 2-AG) and working with Dr. Chiaki Itami, we showed that endogenous cannabinoids have an unexpected role in the activity-dependent refinement of thalamocortical axons. Importantly, exogenous cannabinoids such as THC interfere with this process.
- a. Ballester-Rosado, CJ, Sun, H, **Huang, JY**, Lu HC. mGluR5 Exerts Cell-Autonomous Influences on the Functional and Anatomical Development of Layer IV Cortical Neurons in the Mouse Primary Somatosensory Cortex. *J Neurosci*. 2016 Aug 24;36(34):8802-14. doi: 10.1523/JNEUROSCI.1224-16.2016. PMCID: PMC4995298.
 - b. Itami C, **Huang JY**, Yamasaki M, Watanabe M, Lu HC, Kimura F. Developmental Switch in Spike Timing-Dependent Plasticity and Cannabinoid-Dependent Reorganization of the Thalamocortical Projection in the Barrel Cortex. *J Neurosci*. 2016 Jun 29;36(26):7039-54. doi: 10.1523/JNEUROSCI.4280-15.2016. PMCID: PMC4926245.
 - c. Itami C, Uesaka N, **Huang JY**, Lu HC, Sakimura K, Kano M, Kimura F. Endocannabinoid-dependent formation of columnar axonal projection in the mouse cerebral cortex. *Proc Natl Acad Sci U S A*.

2022;119(37):e2122700119. Epub 20220906. doi: 10.1073/pnas.2122700119. PubMed PMID: 36067295. PMC journal in process

5. **Impact of prenatal opioid exposure in cortical circuit development.** As problematic opioid use and addiction have increased over the past two decades, there has been a dramatic increase in neonates undergoing opioid withdrawal syndrome. In collaboration with Dr. Brady's group at the IU School of Medicine, we found that opioid exposure produced substantial impairments in offspring physical growth, activity in an open field, and sensorimotor milestone acquisition.
- a. Grecco GG, Mork BE, **Huang JY**, Metzger CE, Haggerty DL, Reeves KC, Gao Y, Hoffman H, Katner SN, Masters AR, Morris CW, Newell EA, Engleman EA, Baucum AJ, Kim J, Yamamoto BK, Allen MR, Wu YC, Lu HC, Sheets PL, Atwood BK. Prenatal methadone exposure disrupts behavioral development and alters motor neuron intrinsic properties and local circuitry. *Elife.* 2021;10. Epub 20210316. doi: 10.7554/eLife.66230. PubMed PMID: 33724184; PMCID: PMC7993998.

Complete list of publications from PubMed (13 publications, citation 420, h-index ~10):

<https://www.ncbi.nlm.nih.gov/myncbi/1hKgjBNBgowAa/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: HURD, Yasmin L

ERA COMMONS USER NAME (credential, e.g., agency login): Yasmin_Hurd

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
State University of New York at Binghamton, NY Karolinska Institute, Stockholm, Sweden	BA PhD	06/1982 06/1989	Biochem & Behav Neuropsychopharm

A. Personal Statement

I am Director of the Addiction Institute at the Icahn School of Medicine at Mount Sinai as well as a neuroscientist with over twenty-five years of research experience studying the neurobiology of drug addiction. My multidisciplinary research investigates the neuropathophysiology underlying substance use disorders and related psychiatric illnesses with a focus on the neurobiology of heroin abuse as well as the developmental consequences of cannabis exposure. Our cannabis studies have predominantly focused on the effects of prenatal and adolescent THC exposure which have contributed significantly to expanding knowledge about the protracted effects of developmental THC exposure on adult brain and behavior, and even to demonstrating novel aspects regarding their cross-generational influences. The research strategies routinely conducted in my laboratory relates to molecular and epigenetic studies of postmortem specimens as well as behavioral investigations in our rodent models using a wide battery of behavioral paradigms. Moreover, my group conducts complementary *in vitro* cell culture models to assist in the mechanistic interpretation of molecular findings. We also conduct next generation sequencing to interrogate the genome of the human and rat brain specimens for in-depth bioinformatic analyses with recent studies focused on cell-specific sequencing. Translation is critical for our research, so we normally study both preclinical animal models and human subjects. A key aspect of our current human heroin clinical research focuses on developing cannabidiol (CBD), a non-addictive cannabinoid, as potential treatment for heroin craving and relapse. Thus understanding the cannabinoid system in heroin abuse is of significant importance. Obtaining knowledge regarding protein work to complement our molecular studies is also of high priority and thus would benefit from the enhanced imaging and mass spectroscopy capabilities offered by the Core. Additionally of particular relevance for this application, it is our goal to optimize analysis of rather small-sized specimens from both our animal and human samples so nanoscale strategies to allow cell type and subcellular analyses using mass spectroscopy strategies would be of enormous value to our research program. I attest that I have never published or created research products under another name.

Ongoing and recently completed projects that I would like to highlight include:

DA055434 (Hurd PI) 08/04/22 – 03/31/27

NIH/NIDA

The goal is to expand knowledge about the impact of developmental cannabis/THC exposure on immune-related dysregulation and molecular networks within distinct mesocorticolimbic cell populations causally linked to behavioral disturbances later in life.

R01 DA030359 (Hurd PI)
NIH/NIDA

08/15/16-04/30/2022

Neurodevelopmental effects of cannabis and its epigenetic regulation.
The goals are to study the effects of prenatal and adolescent cannabis exposure on the developing brain and adult brain and behavior.

P01 DA47233 (Hurd PI Project 4; Nestler, Project leader)
NIH/NIDA 12/01/19-11/30/2023
Regulation of gene enhancers in human heroin abuse
Characterize the influence of enhancer regions of the genome, which exert crucial control over gene expression, in heroin addiction through studies of the postmortem human brain and animal heroin self-administration models.

R01 DA048613 (Hurd PI) 08/01/2019 – 06/30/2024
NIH/NIDA
Translating CBD Treatment for Heroin Addiction.
The goals of this project are to study the neurobiological effects of CBD to reduce craving in human opioid users using neuroimaging tools and animal models evaluating the molecular and epigenetic underlying CBD's effects.

UG3 DA050323 (Hurd) 09/30/2019 – 08/31/2024
NIH/NIDA
Cannabidiol in the treatment of opioid use disorder
Conduct pharmacokinetic and pharmacodynamic studies investigating the effects of cannabidiol in healthy controls and in individuals with opioid use disorder.

R01 DA051191 (Hurd PI) 07/15/2021- 03/31/2026
NIH/NIDA
Molecular Neurobiology of Human Opioid Use Disorder
The goal is to study the molecular underpinnings associated with heroin use in mesocorticolimbic brain regions in human heroin users and translational animal models.

Citations:

Ferland., J-M.N., Ellis, R.J., Rompala, G., Landry, J.A., Callens, J.E., Ly A., Frier, M.D., Uzamere, T.O., and **Hurd, Y.L.** Dose mediates the protracted effects of adolescent THC exposure on reward and stress reactivity in males relevant to perturbation of the basolateral amygdala transcriptome. *Molecular Psychiatry*, Epub, Mar 2, 2022.

Ellis, R.J., Bara, A., Vargas, C.A., Frick, A.L., Loh, E., Landry, J., Uzamere, T.O., Callens, J.E., Martin, Q., Rajarajan, P., Brennand, K., Ramakrishnan, A., Shen, L., Szutorisz, H., and **Hurd, Y.L.** Prenatal Δ⁹-tetrahydrocannabinol exposure in males leads to motivational disturbances related to striatal epigenetic dysregulation. *Biological Psychiatry*, S0006-3223(21)01629-2, 2021.

A. Bara, J.N. Ferland, G. Rompala and H. Szutorisz and **Hurd YL**. Cannabis and Synaptic Reprogramming of the Developing Brain. *Nature Reviews Neuroscience*, Jul;22(7):423-438, 2021.

Ferland JN and **Hurd YL**. Deconstructing the neurobiology of cannabis use disorder. *Nature Neuroscience*, 23(5):600-610, 2020.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

- 2017-present Director, Addiction Institute of Mount Sinai, Behavioral Health System.
- 2014-2017 Director, Center for Addictive Disorders, Mount Sinai Health System.
- 2013-present Ward-Coleman Chair in Translational Neuroscience, Mount Sinai.
- 2010- 9/2014 Director of the MD/PhD Program, Mount Sinai.
- 2009-2012 Chief, Center of Excellence in Mood and Motivation, Friedman Brain Institute, Mount Sinai.
- 2008-present Co-Director, Interdisciplinary Training in Drug Abuse Research (T32 post-doctoral training).
- 2006-present Health Systems Specialist, James J Peters VA Medical Center, Bronx, NY, USA.
- 2006-present Professor, Mount Sinai School of Medicine, Depts. Psychiatry, Neuroscience and Pharmacological Sciences, New York, USA.

2004-2005	Assistant Chief Psychiatry Section, Dept. of Clinical Neuroscience, Karolinska Institute, Stockholm.
2002-2005	Professor, Karolinska Institute, Dept. of Clinical Neuroscience, Psychiatry Section, Karolinska Institute, Stockholm, Sweden.
1994-2002	Assistant/Associate Professor ("Docent"), Karolinska Institute, Dept. of Clinical Neuroscience, Psychiatry Section, Karolinska Hospital, Stockholm, Sweden.
1997-2005	Director of graduate studies (studierektor för forskarutbildning), Dept. of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden.
1993- 1997	Research Scientist, Karolinska Institute, Dept. of Clinical Neuroscience, Psychiatry and Psychology Section, Karolinska Hospital, Stockholm, Sweden.
1991-1993	Staff Fellow, National Institute of Mental Health (NIMH), Clinical Brain Disorders Branch, Neuroscience Center at St. Elizabeth's Hospital, Washington, D.C., USA.
1989-1991	Pharmacology Research Associate (PRAT Fellow) of the NIH/National Institute of General Medical Sciences.

Honors

2022	National Academy of Science, Elected member
2021	Sarah Gund Prize Child Mind Institute Distinguished Scientist Award
2020-present	ALBA, Steering Committee member
2020	Mika Salpeter Lifetime Achievement Award, Society for Neuroscience
2020	TEDMed Speaker
2020	Vice Chair, National Academy of Medicine Neuroscience, Behavior, Brain Function & Disorders Interest Group
2019-present	Editorial Board Member, Neuropharmacology
2019-present	National Academies' Forum on Neuroscience and Nervous System Disorders, member
2019-present	Brain & Behavior Research Foundation, Scientific Council member
2019-present	Editorial Board, Biological Psychiatry
2018	Distinguished Woman Scientist of the Year, Yale Medical School
2018	Chair, ACNP Minority Task Force
2017	National Academy of Medicine, Elected member
2017	Lyon-Voorhees Lectureship, University of Colorado
2017-2018	International Scientific Programme Committee for CINP 2018 World Congress
2017	NIH, Center for Scientific Review (CSR) Advisory Council
2016-present	Associate Editor, Journal of American Psychiatry
2016	ACNP, Minority Taskforce vice chair
2016	SfN Public Education and Communication Committee member
2016	NIH WALS Speaker
2016-2017	Chair, Gordon Research Conference: Cannabinoid Function in the CNS
2015-present	Cannabis and Cannabinoid Research journal, editorial board member
2014-2016	Organizing Committee for the Dopamine 2016 Conference
2014-	Molecular Neuropsychiatry, editorial board member
2013-2015	Vice-Chair, Gordon Conference: Cannabinoid Function in the CNS
2012-present	Executive committee, Women in Science and Medicine, Mount Sinai
2012-present	Executive committee, Friedman Brain Institute Mount Sinai
2012-2015	Society for Neuroscience, Young Investigator Award Selection Committee Member

C. Contributions to Science

- After helping to develop the technique of microdialysis to monitor fluctuation of *in vivo* transmitter levels associated with addictive drugs, I subsequently studied downstream molecular alterations at the postsynaptic cell that could maintain the long-term perturbation underlying addiction vulnerability. As such, I was one of the first scientists to study gene expression in the brains of animal that self-administered cocaine in a model relevant to the human condition. Those questions have been a fundamental core of my research efforts throughout my career in trying to consider the translational importance of our studies. I developed a unique postmortem human brain bank collection (primarily heroin abusers and matched controls) and became one of the first investigators to study gene expression in the human brain which at that time was thought impossible due to insurmountable postmortem confounds. In addition, to expanding insights about the human brain,

another important outcome of these studies was the possibility to identify similar molecular disturbances in animal models that could be used to provide mechanistic causal relationships to behavior. Another significant example of my multidisciplinary translational approaches was our developing a combined *in vivo* imaging and chemogenic (DREADD) molecular approach (termed DREAMM) to identify the neuronal network associated with molecular impairments seen in human drug abusers and subjects with major depression.

- (a) **Hurd, Y.L.** and Herkenham, M. Molecular alterations in the neostriatum of human cocaine addicts. *Synapse*, 13, 357-369, 1993.
- (b) **Hurd, Y.L.**, Herman, M.M., Hyde, T.M., Bigelow, L.B., Weinberger, D.R., and Kleinman, J.M. Prodynorphin mRNA expression is increased in the patch vs matrix compartment of the caudate nucleus in suicide subjects. *Molecular Psychiatry*, 2, 495-500, 1997.
- (c) **Hurd, Y.L.** Subjects with major depression or bipolar disorder show reduction of prodynorphin mRNA expression in discrete nuclei of the amygdaloid complex. *Molecular Psychiatry*, 7: 75-81, 2002.
- (d) Michaelides M., SA Anderson, M Ananth, D Smirnov, PK Thanos, JF Neumaier, GJ Wang, ND Volkow, **YL Hurd**. In vivo cell-specific mesocorticolimbic whole-brain circuit dissection in freely-moving animals. *Journal of Clinical Investigations*, 123(12):5342-50, 2013. PMCID: PMC3859392.
2. Studies of the human brain highlighted individual variability in gene and protein expression that were not related to known demographic information. As such, I became interested in understanding potential genetic contributions to the molecular variability evident in our human brain specimens and to drug addiction. In addition to revealing specific molecular neurobiological disturbances in human heroin and cocaine abusers, I was one of the first investigators to demonstrate a significant relationship between individual genetic polymorphisms and gene expression in discrete neuronal populations of drug abusers. Such studies have been validated and provided a strong foundation in the field for molecular genetic studies. We also demonstrated the capacity for analyses of epigenetic modifications in the human brain.
- (a) Drakenberg, K. Nikoshkov, A., Horváth, M.C., Fagergren, P., Gharibyan, A., Saarelainen, K., Rahman, S., Nylander, I., Bakalkin, G., Rajs, J., Keller, E., and **Hurd, Y.L.** Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proc Natl Acad Sci U S A.*, 103:7883-7888, 2006.
- (b) Nikoshkov, A., Drakenberg, K., Wang, X., Horváth, M.C., Keller, E., **Hurd, Y.L.** Opioid Neuropeptide genotypes in relation to heroin abuse: Dopamine tone contributes to reversed mesolimbic proenkephalin expression. *Proc Natl Acad Sci USA.*, 105(2):786-791, 2008.
- (c) Sullivan SE, Whittard JD, Jacobs MM, Ren Y, Mazloom AR, Caputti F, Horvath M, Keller E, Ma'ayan E, Pan Y-X, Chiang LW and **Hurd YL**. ELK1 transcription factor linked to dysregulated striatal mu opioid receptor signaling network and *OPRM1* polymorphism in human heroin abusers. *Biological Psychiatry*, Epub: May 20, 2013. PMCID: PMC4070524.
- (d) Egervari G, Akpoyibo D, Rahman T, Fullard JF, Callens JE, Landry JA, Ly A, Zhou X, Warren N, Hauberg ME, Hoffman G, Ellis R, Ferland JM, Miller ML, Keller E, Zhang B, Roussos P, **Hurd YL**. Chromatin accessibility mapping of the human striatum identifies tyrosine kinase FYN as a promising therapeutic target for heroin use disorder. *Nature Communications*, 11(1):4634, 2020.
3. Another major line of research for which I have been a leader in the field focuses on cannabis. Specifically, I helped to identify cannabis/THC-related alterations in the human fetal brain, replicated in animal models, both prenatal and adolescent development, and in which our research has been able to delineate the long-term impact on epigenetic mechanisms and behavior. We also demonstrated for the first time, the transgenerational effects of THC which has enormous implications.
- (a) Wang, X., Dow-Edwards, D., Andersen, V., Minkoff, H., and **Hurd, Y.L.** *In utero* marijuana exposure associated with abnormal amygdala dopamine D₂ gene expression in the human fetus. *Biological Psychiatry*, 56: 819-825, 2004.
- (b) Ellgren, M., Spano, SM, and **Hurd, Y.L.** Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. *Neuropharmacology*, 32:607-15, 2007.
- (c) DiNieri JA, Wang X, Szutorisz H, Spano SM, Kaur J, Casaccia P, Dow-Edwards D, **Hurd YL**. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. *Biological Psychiatry*, 70:763-9, 2011. PMCID: PMC3186868.
- (d) Miller M.L., B Chadwick, D.L. Dickstein, I Purushothaman, G. Egervari, T. Rahman, C. Tessereau, P.R. Hof, P. Roussos, L. Shen, M.G. Baxter and **Hurd YL**. Adolescent exposure to Δ⁹-tetrahydrocannabinol

alters the transcriptional trajectory and dendritic architecture of prefrontal pyramidal neurons. Molecular Psychiatry, Epub: Oct 3, 2018.

4. As evidenced above, most of the research advances I have pursued in investigating the human brain and our translational animal models in regard to substance abuse relates to heroin and the opioid system. My work has contributed most of the neurobiological knowledge to date related to the molecular neuropathology associated with heroin addiction through studies of the postmortem human brain.
 - (a) Egervari G, Landry J, Callens J, Fullard JF, Roussos P, Keller E and **Hurd YL**. Striatal H3K27 acetylation linked to glutamatergic gene dysregulation in human heroin abusers holds promise as therapeutic target. Biological Psychiatry, Epub, Sep 28, 2016. Apr 1;81(7):585-594, 2017. PMCID: PMC5346335.
 - (b) Egervari G, Jutras-Aswad D, Landry J, Miller ML, Anderson SA, Michaelides M, Jacobs MM, Peter C, Yiannoulos G, Liu X, **Hurd YL**. A Functional 3'UTR Polymorphism (rs2235749) of Prodynorphin Alters microRNA-365 Binding in Ventral Striatonigral Neurons to Influence Novelty Seeking and Positive Reward Traits. Neuropsychopharmacology. 41(10):2512-20, 2016.
 - (c) Kozlenkov A, Jaffe AE, Timashpolsky A, Apontes P, Rudchenko S, Barbu M, Byne W, **Hurd YL**, Horvath S, Dracheva S. DNA Methylation Profiling of Human Prefrontal Cortex Neurons in Heroin Users Shows Significant Difference between Genomic Contexts of Hyper- and Hypomethylation and a Younger Epigenetic Age. Genes (Basel). May 30;8(6), 2017.
 - (d) Kovacs GG, Horvath MC, Majtenyi K, Lutz MI, **Hurd YL**, Keller E. Heroin abuse exaggerates age-related deposition of hyperphosphorylated tau and p62-positive inclusions. Neurobiol Aging. Jul 17 pii: S0197-4580(15)00378-4, 2015.
5. An important scientific contribution highly relevant for the current project are the pioneering preclinical and early phase I and II studies we conducted that showed the potential of CBD to reduce heroin seeking behavior in animals and to reduce cue-induced drug craving in individuals with heroin use disorder. This work has led the field and brought significant attention to CBD with many investigators now studying the effects of CBD as a potential treatment for other substances of abuse and related psychiatric disorders.
 - (a) Ren, Y., Whittard, Higuera-Matas, A., Morris, CV, **Hurd YL**. Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin-seeking and normalizes discrete mesolimbic neuronal disturbances. Journal of Neuroscience, 29:14764-9, 2009.
 - (b) AF Manini, G Yiannoulos, MM Bergamaschi, S Hernandez, R Olmedo, AJ Barnes, G Winkel, R Sinha, D Jutras-Aswad, MA Huestis and **Hurd YL**. Safety and pharmacokinetics of oral cannabidiol when administered concomitantly with intravenous fentanyl in humans. Journal of Addiction Medicine, 9(3):204-10. PMCID: PMC4449284, 2015.
 - (c) **Hurd YL**, Yoon M, Manini AF, Hernandez S, Olmedo R, Ostman M, Jutras-Aswad D. Early Phase in the Development of Cannabidiol as a Treatment for Addiction: Opioid Relapse Takes Initial Center Stage. Neurotherapeutics, Oct;12(4):807-15. PMCID: PMC4604178, 2015.
 - (d) **Hurd YL**, S Spriggs, J Alishayev, G Winkel, K Gurgov, C Kudrich, AM Oprescu and E Salsitz. Cannabidiol Reduces Cue-Induced Craving and Anxiety in Drug-Abstinent Individuals with Heroin Use Disorder: a Double-Blind, Randomized, Placebo-Controlled Trial. American J Psychiatry, 1;176(11):911-922, 2019.

Complete List of Published Work in My Bibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/yasmin.hurd.1/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: Johnson, Clare Therese

eRA COMMONS USER NAME (credential, e.g., agency login): claretheresejohnson

POSITION TITLE: Graduate Research Assistant

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
University of North Carolina, Chapel Hill	BS	05/2018	Psychology
Indiana University, Bloomington	PHD	05/2023	Psychological & Brain Science

A. Personal Statement

I have spent most of my scientific career investigating lipid signaling in the central nervous system, with a particular focus on endocannabinoids and related molecules. As a graduate student in the Bradshaw lab at Indiana University, I have mastered assays to isolate lipids from biological samples and quantify them using HPLC/MS/MS. This skillset has allowed me to explore basic science questions such as the function of the orphan cannabinoid receptor GPR55 and work with a wide range of collaborators assessing the effects of cannabinoids, non-cannabinoid drugs, and behavioral interventions on lipids in a variety of modalities, from brain tissue to breast milk. My ongoing research focuses on sex differences in the effects of drugs of abuse on lipid signaling molecules.

Recently completed projects:

T32DA024628

Mackie (PI), Hohmann (co-PI); Role: Predoctoral Trainee

07/01/2020-06/30/2021

Integrative Predoctoral Training In Drug Abuse Research At Indiana University

Citations:

1. Maciel IS, de Abreu GHD, **Johnson CT**, Bonday R, Bradshaw HB, Mackie K, Lu HC. Perinatal CBD or THC Exposure Results in Lasting Resistance to Fluoxetine in the Forced Swim Test: Reversal by Fatty Acid Amide Hydrolase Inhibition. *Cannabis Cannabinoid Res.* 2021 Jun 28; PMCID: PMC9225394.
2. League AF, Gorman BL, Hermes DJ, **Johnson CT**, Jacobs IR, Yadav-Samudrala BJ, Poklis JL, Niphakis MJ, Cravatt BF, Lichtman AH, Ignatowska-Jankowska BM, Fitting S. Monoacylglycerol Lipase Inhibitor MJN110 Reduces Neuronal Hyperexcitability, Restores Dendritic Arborization Complexity, and Regulates Reward-Related Behavior in Presence of HIV-1 Tat. *Front Neurol.* 2021;12:651272. PMCID: PMC8415271.
3. Sadaka AH, Ozuna AG, Ortiz RJ, Kulkarni P, **Johnson CT**, Bradshaw HB, Cushing BS, Li AL, Hohmann AG, Ferris CF. Cannabidiol has a unique effect on global brain activity: a pharmacological, functional MRI study in awake mice. *J Transl Med.* 2021 May 24;19(1):220. PMCID: PMC8142641.
4. **Johnson CT**, de Abreu GHD. Mackie K, Lu H-C; and Bradshaw HB. Cannabinoids accumulate in mouse breast milk and differentially regulate lipid composition and lipid signaling molecules involved in infant development. *BBA Advances*, Vol 2, 2022, 100054 doi.org/10.1016/j.bbadv.2022.100054 PMC journal in process.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021- Present	Member, American Society of Addiction Medicine
2019- Present	Member, International Cannabinoid Research Society
2018- Present	Graduate Research Assistant, Indiana University
2017	Undergraduate Research Opportunities Program in Science Participant, National University of Singapore
2016- 2017	Undergraduate Research Assistant, University of North Carolina Chapel Hill

Honors

2021	Braude Foundation Award, International Cannabinoid Research Society
2021	Student Travel Award, International Cannabinoid Research Society
2020	Student Travel Award, International Cannabinoid Research Society
2019	Student Travel Award, International Cannabinoid Research Society
2019-2020	Rebec Family Neuroscience Fellowship, Indiana University Bloomington
2018	First Year Fellowship in Neuroscience, Indiana University Bloomington

C. Contribution to Science

1. My graduate research has focused on understanding the effects of drugs on signaling lipids, particularly those involved in the endocannabinoid system. I recently published a literature review exploring the potential of cannabidiol (CBD) to mitigate pro-inflammatory effects of morphine. This paper provides a theoretical framework for further investigation of CBD as an adjuvant treatment with opioids. In addition, I recently published another paper investigating the transfer of phytocannabinoids to breast milk in a mouse model of cannabis consumption during lactation and how it affects the lipid composition of breast milk.
 - a. **Johnson C**, Bradshaw H. Modulatory Potential of Cannabidiol on the Opioid-Induced Inflammatory Response. *Cannabis and Cannabinoid Research*. 2021 June 01; 6(3):211-220. PMCID: PMC8217599
 - b. **Johnson CT**, de Abreu GHD. Mackie K, Lu H-C; and Bradshaw HB. Cannabinoids accumulate in mouse breast milk and differentially regulate lipid composition and lipid signaling molecules involved in infant development. *BBA Advances*, Vol 2, 2022, 100054 doi.org/10.1016/j.bbada.2022.100054 PMC journal in process.

BIOGRAPHICAL SKETCH

NAME: Debra S. Karhson

eRA COMMONS USER NAME (credential, e.g., agency login): DKARHSON

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Drexel University, Philadelphia, PA	B.S.	06/2007	Biomedical Engineering
Tulane University, New Orleans, LA	Ph.D.	08/2014	Neuroscience
Stanford University, Stanford, CA	Postdoctoral training	10/2019	Child Psychiatry and Neurodevelopment Research Training

A. Personal Statement

Impairments in social learning represent the greatest challenge to longevity and sustaining a high quality of life for neurodivergent populations, such as individuals with autism spectrum disorder. While prior research has focused on social neuropeptides (i.e., oxytocin and vasopressin), investigation of the endocannabinoid system is well-suited to offer new insights on social learning. Endocannabinoids (eCBs) are non-traditional neuromodulators that participate in a variety of social cognitive processes, such as motivation, attention, and reinforcement. Moreover, the eCB system maintains the homeostatic balance between excitatory (E) and inhibitory signaling (I), termed the E:I balance, in cortical rhythrogenesis. Therefore, my research examines the role of the endocannabinoid system in the neurobiology and neurophysiological mechanisms of social learning within neurodivergent populations. I use electroencephalography and molecular analysis of biospecimens to close the “bench-to-bedside gap” and hasten the translation of research findings into practical applications. To date, my research efforts in this area are among the first data of its kind in the ASD population to assess the function of the eCB system in ASD pathophysiology. As a new faculty member, I am extending my postdoctoral work through three independent lines of research: (1) determining the endocannabinoid neurobiology of autistic traits between neurodivergent and neurotypical populations; (2) explaining the predictive utility of endocannabinoid biology in the brain dynamics of social learning; and (3) identifying inroads for the development of novel, symptom-specific non-invasive interventions to address autistic features. My long-term goal research is to identify targeted methods to enhance social learning and improve quality of life in neurodivergent individuals.

- a. **Karhson, DS.**, Krasinka, KM., Alhoy Dallaire J., Libove, R.A. Phillips, JM., Chien AS., Garner, JP., Hardan AY, Parker, KJ, “Plasma Anandamide Concentrations Are Lower in Children with Autism Spectrum Disorder.” *Molecular Autism* 9 (March 12, 2018): 18. <https://doi.org/10.1186/s13229-018-0203-y>.
- b. **Karhson, DS.**, Hardan AY, Parker, KJ, “Endocannabinoid Signaling in Social Functioning: An RDoC Perspective” *Translational Psychiatry* (2016) 6, e905; doi:10.1038/tp.2016.169
- c. **Karhson, DS.**, Golob, EJ, “Atypical sensory reactivity influences auditory attentional control in adults with Autism Spectrum Disorders” *Autism Res.* 2016 Jan 18.

B. Positions and Honors

Positions

2003-2004	Co-Op Intern, Department of Neuroscience, Supervised by Drs. Candace Strang and Paul Pfaffinger, Baylor College of Medicine, Houston, TX
2004-2005	Co-Op Intern, Department of Neuroscience, Supervised by Drs. J David Sweatt and Laura Schrader, Baylor College of Medicine, Houston, TX
2005-2006	Co-Op Intern, Department of Oncology, Supervised by Dr. Marc Abrams, Merck and Company, West Point, PA
2006-2007	Laboratory Assistant, Department of Oncology, Supervised by Dr. Frank Rauscher III, Wistar Institute, Philadelphia, PA
2008-2008	Research Technician II, Molecular Physiology & Biophysics, Supervised by Dr. Marta Fiorotto, USDA/ARS Children's Nutrition Research Center, Houston, TX
2008-2014	Graduate Student Researcher, Neuroscience Program, Supervised by Dr. Edward J. Golob, Tulane University, New Orleans, LA
2014-2019	Postdoctoral Fellow, Department of Psychiatry and Behavioral Sciences, Supervised by Drs. Allan Reiss, Karen Parker, and Antonio Hardan, Stanford University, Stanford, CA
2019-2021	Basic Life Science Research Scientist, Department of Psychiatry and Behavioral Sciences, Supervised by Dr. Antonio Hardan, Stanford University, Stanford, CA
2021 - 2022	Assistant Professor of Psychology, University of Texas Permian Basin, Odessa, TX
2022 -	Assistant Professor of Psychology, University of New Orleans, New Orleans, LA

Honors

2009	IBM Corporation Fellow in Computational Science, Tulane Center for Computational Sciences
2012	Jean Harlan Award for Outstanding Graduate Student Presentation – 1st place
2013	Jean Harlan Award for Outstanding Graduate Student Presentation – 1st place
2014	International Meeting for Autism Research Diversity Travel Award, Daughters of Hawaii
2014	NIMH T32 Research Training for Child Psychiatry and Neurodevelopment
2017	Women Who Code Scholarship to Hackbright Academy for Python Programming
2018	Albert and Mary Lasker Foundation, Essay Contest Winner – 2nd place
2019	Carl Storm Underrepresented Minority Fellowship, <i>Gordon Research Conferences</i>
2019	“HyperActive” Trainee Award, 2019 Cannabinoid meeting, <i>Gordon Research Conferences</i>
2020	One of the 1,000 Inspiring Black scientists in America, <i>Cell Mentor</i>
2021	President’s Awards for Excellence Through Diversity, Stanford University
2021	Reimagine Biomedical Research for a Healthier Future Essay Challenge” – Honorable Mention, <i>Health Research Alliance (HRA) and the Public Library of Science (PLOS)</i>
2021	Young International Brain Research Organization Maternity/Parenthood in Neuroscience Grant
2022	Cohort Member of the University of California - San Diego Leading the Advancement of Underrepresented Neuroscientists for Change (LAUNCH) program

C. Contributions to Science

1. Characterized K⁺ channels' role in preclinical models of information processing. My initial contributions to science were dedicated to applying my training in biomedical engineering to support research on the neural mechanisms of learning and memory. I performed two co-operative research opportunities or co-ops in the Neuroscience Department at Baylor College of Medicine. In these co-ops my research efforts focused identifying the role of potassium channels in learning and memory. In my first c-op with Dr. Paul Pfaffinger, I supported graduate and postdoctoral trainee by assisting in experiments that include DNA cloning, Mini/Midi/Maxi prep, western blotting, and cell culture techniques. I also performed an extensive literature review on conservation of structure and function of neuronal potassium channels and created a figure to represent this historical data which was later published in an article by my postdoctoral mentors. In my second co-op in the lab of Dr. J. David Sweatt, I assisted in research examining the role of potassium channel modulation in opioid receptor and kinase activation. I behaviorally characterized transgenic mice lacking potassium channel interacting proteins and analyzed brain-based samples from transgenic mice that had been behaviorally characterized to assess fear conditioning. My data were included in a publication on role

of calsenilin/DREAM/KChIP3 in contextual fear conditioning. In my final co-op, I worked at Merck and Co. under the supervision of Dr. Marc Abrams. Here, I was exposed to biotherapeutic research and how these techniques may be used to research brain disorders that impact learning and memory. I assisted in tissue assessment following exposure to novel therapeutics on the growth of implanted tumor cells to identify candidate biomarkers for targeted development of Phase I clinical trials. I grew and maintained tumor cell lines that were implanted into mice pups and assisted in experiments to test the efficacy of therapeutics on in vivo tumor growth. This industrial research experience reaffirmed my interest in academic research. Prior to starting my graduate training in neuroscience, I participated in an additional research experience to work in the lab of Dr. Marta Fiorotto at the USDA/ARS Children's Nutrition Research Center. Here, I rebuilt and remediated a high-performance liquid chromatography (HPLC) instrument as well as assisted in pediatric animal surgery, maintained mouse pup litters, and used HPLC techniques to examine protein synthesis in skeletal muscles of malnourished mice pups. The resulting data were presented at several muscular dystrophy conferences. These undergraduate experiences strengthened my desire to work with pediatric populations and affirmed my deep interest in a neuroscience career researching neurodevelopmental disorders that affect learning and memory.

- a. Alexander, C., **Karhson, DS.**, Trimmer, J., Sweatt, D., Schrader, L., "The Role of KChIP2 in Learning and Memory and Synaptic Plasticity", Abstract for poster presentation, Society for Neuroscience, Washington, DC 2005
 - b. Alexander JC, McDermott CM, Tunur T, Rands V, Stelly C, **Karhson DS.**, Bowlby MR, An WF, Sweatt JD, Schrader LA., "The role of calsenilin/DREAM/KChIP3 in contextual fear conditioning." *Learning and Memory*. 2009 Feb 17; 16(3): 67-177
 - c. Fiorotto ML, Davis TA, Sosa HA, Estrada IJ, Watson KL, **Karhson DS.**, Age-dependent capacity to accelerate protein synthesis dictates the extent of compensatory growth in skeletal muscle following undernutrition. *The FASEB Journal* 2010; 24: 97–8.
2. Quantified impact of sensory reactivity on attentional control of information processing. To support my interest in autism research, I focused my graduate training on developing experimental skills used in neuroscience experiments with humans. Specifically, I developed expertise in non-invasive neuroimaging techniques and the neuropsychological assessment of neurotypical and non-neurotypical populations. Using these skills, I performed experiments to assess auditory spatial attention gradient in neurotypical controls with electroencephalography (EEG) and repetitive transcranial magnetic stimulation (a form of non-invasive brain stimulation). My results were published in PLOS One and are highly relevant to understanding the neuroanatomical contributions of the right inferior parietal cortex to attentional processing. I then applied my research skills to my specific research interest in autism spectrum disorder (ASD). My dissertation research examined attentional processing to examine neural mechanisms of cognitive information processing in adults with ASD. By combining phenotypic characterization and analysis of event-related potentials collected with EEG during an auditory attention task, I assessed the impact of sensory reactivity on auditory attentional orienting in people with ASD in comparison to age- and IQ-matched neurotypical controls. My results, published in *Autism Research*, suggested enhanced fidelity and perceptual capacity in adults with ASD. Through my graduate work, I realized the untapped research potential of bridging systems-level research with the molecular neuroscience methods I had been exposed to in my undergraduate research. By pursuing a translational research agenda, I would be able to best achieve my long-term career goal of defining the neural mechanisms for the core features of ASD that would lead to the development of feature-specific interventions to improve quality of life in ASD. Therefore, following the completion of my graduate degree in neuroscience, I sought additional research training in neuropsychiatry in neurodevelopment disorders.

- a. **Karhson, DS.**, Golob, E., "Influence of repetitive TMS to right inferior parietal cortex on auditory spatial processing.", Abstract for poster presentation, Organization for Human Brain Mapping, June 6-10, 2010, Barcelona, Spain
- b. **Karhson, DS.**, Mock JR, Golob, EJ, "The role of right inferior parietal cortex in auditory spatial attention: A repetitive transcranial magnetic stimulation study" *PLoS One*. 2015 Dec 4;10(12):e0144221. PMCID: PMC4670170
- c. **Karhson, DS.**, Golob, EJ, "Atypical sensory reactivity influences auditory attentional control in adults with Autism Spectrum Disorders" *Autism Res*. 2016 Jan 18. PMCID: PMC26778164

3. *Biomarker Discovery for Social Information Processing in ASD*. Impairments in social and cognitive functioning in children with ASD are intrinsically related to the changes in information processing. Both, social and cognitive functioning are emergent neural process that can be manipulated by robust molecular neuromodulators. Social behaviors are specifically related to the neuropeptides, oxytocin (OT) and arginine vasopressin (AVP), while both social and cognitive functioning have been associated with the endogenous cannabinoid (or endocannabinoid) system. Therefore, utilizing my experience with HPLC and in collaboration with the Stanford Mass Spectrometry core lab, my initial postdoctoral research has focused on developing liquid chromatography with tandem mass spectrometry (LC-MS/MS) methodology to assess molecular-level neuromodulators of interest (i.e., OT, AVP) in small-volumes of blood and cerebrospinal fluid. I was also able to leverage this technique in a pilot experiment investigating the role of endocannabinoid (eCB) signaling in pediatric ASD pathophysiology. Though eCB signaling dysregulation has been implicated in ASD, to date, it has not been rigorously assessed. Therefore, one of my individual efforts has focused on collection of experimental data on the levels of major eCBs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), in bio-banked samples from children with ASD. The article which details the results from my initial eCB study focusing on AEA group differences in plasma in children with ASD compared to neurotypical controls, was published in Molecular Autism in 2018. While these AEA results have been replicated by two other research groups, no association with specific behavioral profiles has been established. Thus, my short-term goals are clear: 1) replicate and extend what is known about eCB neurobiology in ASD; 2) establish the association between eCB signaling and autistic behaviors; and 3) determine the biology-brain-behavior relationship between eCB signaling and brain dynamics of social learning measured with non-invasive EEG. These short-term goals will aide in achieving my long-term goal of identifying targeted methods to enhance social learning and improve quality of life in neurodivergent individuals.

- a. **Karhson, DS.**, Hardan AY, Parker, KJ, "Endocannabinoid Signaling in Social Functioning: An RDoC Perspective" *Translational Psychiatry* (2016) 6, e905; doi:10.1038/tp.2016.169 PMCID: PMC5048207
- b. Parker, KJ, Oztan, O., Libove, RA, Sumiyoshi, RD, Jackson, LP, **Karhson, DS.**, Summers, J.E., Hinman, KE, Motonaga, KS, Phillips, JM, Carson, DS, Garner, JP, and Hardan AY. "Intranasal oxytocin treatment for social deficits and biomarkers of response in children with autism." *PNAS* (2017). doi: 10.1073/pnas.1705521114. PMCID: PMC5544319
- c. **Karhson, DS.**, Krasinka, KM., Alhoy Dallaire J., Libove, R.A. Phillips, JM., Chien AS., Garner, JP., Hardan AY, Parker, KJ, "Plasma Anandamide Concentrations Are Lower in Children with Autism Spectrum Disorder." *Molecular Autism* 9 (March 12, 2018): 18. PMCID: PMC5848550

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/debra.karhson.1/bibliography/40277534/public/?sort=date&direction=ascending>

D. Additional Information: Research Support

BIOGRAPHICAL SKETCH

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

NAME: Istvan Katona

eRA COMMONS USER NAME (credential, e.g., agency login): Katona

POSITION TITLE: Professor, Naus Family Chair of Addiction Sciences

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Lorand Eotvos University, Hungary	Diploma	09/1992	06/1997	Biology
Semmelweis University, Hungary	Ph.D.	09/1997	09/2000	Neuroscience
Heidelberg University, Heidelberg, Germany	Postdoctoral research	09/2000	09/2002	Neuroscience
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary	Postdoctoral research	01/2003	12/2006	Neuroscience

A. Personal Statement

My major research interest is focused on understanding the molecular and cellular plasticity processes associated with substance use disorders with the goal of developing novel approaches to treating substance use disorders based on this understanding. My specific research area is primarily related to the signaling pathways controlling endocannabinoid and dopaminergic signaling under physiological conditions. Building on this knowledge, our lab aspires to determine how altered dopaminergic and endocannabinoid signaling contributes to pathophysiological processes such as addiction. My laboratory employs a combination of molecular biology, neuroanatomical and electrophysiological techniques, including STORM super-resolution imaging, confocal and electron microscopy, *in situ* hybridization, paired patch-clamp recordings, and behavior. I am a lead author or co-author on 66 studies that have received more than 15,000 citations, and I have an h-index of 47. In terms of synergistic activities, I organized 5 symposia at international neuroscience conferences, gave 100+ invited presentations at conferences and research institutes, been editorial board member at 5 neuroscience journals, served as Chair at the Gordon Research Conference on Cannabinoid Signaling, and as a Program Committee Member for the 8th Federation of European Neuroscience Societies Congress. I led as Head, the Neuroscience Study Section for the Hungarian Science Funding Agency, reviewed for 60+ scientific journals and for 15 international grant agencies. I have 29 years of research experience, worked as Head of Department at the Institute of Experimental Medicine in Budapest for 9 years and then as Professor and the endowed Naus Family Chair for Addiction Sciences in the Department of Psychological and Brain Sciences, Indiana University, Bloomington since 2020. I am actively involved in the training of graduate students (total of 10) and postdoctoral fellows (10), several of whom received national prizes, fellowship awards, joined prestigious international research laboratories and became independent group leaders.

Ongoing projects that I would like to highlight in the context of the current P30 application include:

R21 DA056825 (PI: Katona)**8/2022-7/2024**

Title: Novel tool development for quantitative PharmacostORM super-resolution imaging of the nanoscale distribution of D₃ dopamine receptors.

The major objective of this grant is to develop specific and sensitive fluorescent small molecule ligands for D3 dopamine receptors and to visualize their nanoscale distribution in brain areas associated with substance use disorders.

R01 DA056825 (PI: Cheer, Co-I: Katona)

10/2018-6/2023

Title: *Neurodevelopmental Effects of THC on The VTA Dopamine System and Behavior*

The major objective of this grant is to better understand how prenatal THC exposure alters the maturation of the synaptic inputs of VTA dopaminergic neurons at the nanoscale molecular and cellular levels and how these changes lead to behavioral abnormalities. My responsibility is the correlated confocal and STORM super-resolution investigation of prenatal THC-induced molecular changes in a cell- and synapse-type-specific manner.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments:

2007-2010: Senior Research Fellow, Head of Laboratory of Molecular Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

2011-2020: Head of Department of Molecular and Developmental Neurobiology, Hungarian Academy of Sciences, Budapest, Hungary

2020- : Professor, Naus Family Chair of Addiction Sciences, Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA

Honors:

1997	Richter-Gedeon Research Award
2000-2002	European Molecular Biology Organization Long-Term Fellowship
2003-2010	János Bolyai Fellow, Hungarian Academy of Sciences
2007	Central European Talent Award
2009	International Association for Cannabinoid Medicines Award
2009	European Research Council Starting Grant Winner
2010	The Wellcome Trust International Senior Research Fellowship
2013	Elected Lifetime Member of the Academia Europea (European Academy of Sciences)
2016	Hungarian Academy of Sciences' Academy Prize for outstanding lifetime achievements
2016	Elected Lifetime Member of the European Molecular Biology Organization (EMBO)
2017	NIH Neuroscience Seminar Series Lecture, Bethesda, MD
2019	Chair of Gordon Research Conference on Cannabinoid functions in the CNS, Barcelona, Spain
2020	Naus Family Chair of Addiction Sciences, Indiana University, Bloomington, Indiana, USA

C. Contributions to Science

1. Molecular architecture and function of endocannabinoid signaling:

My laboratory was involved in delineation of the precise molecular organization of the endocannabinoid system in the brain and contributed to the characterization of the specific physiological functions of the several different forms of endocannabinoid signaling. I am working on this field since 1998, and my first major contribution was the direct electron microscopic demonstration that CB1 cannabinoid receptors are presynaptically located on axon terminals in 1999. Subsequently, we uncovered the postsynaptic compartmentalization of DGL-alpha, the synthesizing enzyme of 2-arachidonoylglycerol (2-AG), which is the most abundant endocannabinoid throughout the brain. These findings outlined the molecular organization of the core retrograde signaling pathway of chemical synapses. Unexpectedly, we have later found that NAPE-PLD, the enzyme producing a second endocannabinoid anandamide is presynaptically located in axon terminals. More recently, our team also revealed cell-type-specific quantitative differences in synaptic endocannabinoid signaling, which results in distinct

thresholds for endocannabinoid mobilization. Our past work unraveled the molecular composition and mechanisms of retrograde synaptic signaling and has made significant contributions to our understanding of its physiological and pathophysiological significance. Our more recent work identified the first *in vivo* physiological and pathological function of ABHD4, an enzyme involved in endocannabinoid biosynthesis.

Katona I, Urbán GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K and Freund TF (2006) Molecular composition of the endocannabinoid system at glutamatergic synapses. *The Journal of Neuroscience*, 24:5268-5237. PMCID: PMC1698282

Nyilas R, Dudok B, Urbán GM, Mackie K, Watanabe M, Cravatt BF, Freund TF and **Katona I** (2008) Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. *The Journal of Neuroscience*, 28: 1058-1063. PMCID: PMC6671412.

Katona I and Freund TF (2012) Multiple functions of endocannabinoid signaling in the brain. *Annual Review of Neuroscience*, 35:529-558. PMCID: PMC4273654

László Z, Lele Z, Zöldi M, Miczán V, Mógor F, Simon GM, Mackie K, Kacskovics I, Cravatt BF and **Katona I** (2020) ABHD4-mediated developmental anoikis safeguards the embryonic brain. *Nature Communications*, 11:4363. PMCID: PMC7459116

2. Endocannabinoid signaling in brain disorders:

Research in our laboratory over the years was extended into the pathological implications of impaired endocannabinoid signaling in brain disorders. We put forward the idea that the endocannabinoid-mobilizing molecular machinery protects the brain from excess activity as a “synaptic circuit-breaker”. For example, we found using quantitative molecular analysis that several components of the synaptic circuit-breaker (the retrograde 2-AG signaling pathway) are strongly down-regulated in the hippocampal formation of epileptic patients, which may further aggravate the progression of epileptic activity and neuronal damage. Additionally, we found a pathological alteration in the precise nanoscale integration of DGL- α into the perisynaptic machinery in a mouse model of the Fragile X syndrome, implicating that an impaired synaptic circuit-breaker activity may contribute to the epileptic phenotype of Fragile X patients. In light of the increasing legal availability of medical cannabis preparations and the pivotal function of endocannabinoid signaling in the regulation of brain disorders, our attention turned towards the important, but still largely unresolved question of how the psychoactive cannabinoid compound Δ9-THC affects endocannabinoid signaling on distinct cellular components. In more recent work, we studied how molecular perturbations contribute to substance use disorders by altering endocannabinoid control of dopaminergic signaling.

Katona I and Freund TF (2008) Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nature Medicine*, 14:923-930.

Mátyás F, Urbán GM, Watanabe M, Mackie K, Zimmer A, Freund TF, **Katona I**. (2008) Identification of the sites of 2- arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. *Neuropharmacology*. 54:95-107. PMCID: PMC2238033

Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, Ginger M, Frick A, DiPatrizio NV, Mackie K, **Katona I***, Piomelli D* and Manzoni OJ* (2012) Uncoupling of the endocannabinoid signaling complex in a mouse model of fragile X syndrome. *Nature Communications*, 3:1080. PMCID: PMC3657999

*Frau R, *Miczan V, Traccis F, Aroni S, Pongor C, Saba P, Serra V, Sagheddu C, Fanni S, Congiu M, Devoto P, Cheer J, ***Katona I** and *Melis M (2019) Prenatal THC exposure produces a hyperdopaminergic phenotype rescued by pregnenolone. *Nature Neuroscience*, 22:1975-1985. PMCID: PMC6884689

3. Super-resolution STORM imaging, high-resolution confocal and electron microscopy:

We have used high-resolution immunogold electron microscopy and revealed the canonical molecular blueprint for the spatial organization of endocannabinoid signaling in numerous regions such as the spinal cord, the ventral

segmental area, the central amygdala, the hippocampus and the neocortex. These studies were mostly qualitative, analyzed a heterogeneous population of synapses, and were very laborious. However, our findings on the cell-type-specific quantitative molecular changes in CB1 numbers in epilepsy and the perturbed nanoscale DGL- α targeting at excitatory, but not at inhibitory synapses in the Fragile X syndrome model highlighted the critical importance of developing new imaging approaches. Therefore, we have recently developed a new methodology based on the combination of whole-cell patch-clamp recording and STORM super-resolution imaging. This new approach reveals the nanoscale distribution of a given protein in any subcellular domain, cell-type and brain region, and enables the combined measurement of physiological, anatomical and molecular parameters of a physiological process in any physiological or pathophysiological plasticity paradigm. By using this approach, we could identify for the first time the downregulation of CB1 receptors from GABAergic axon terminals induced by chronic $\Delta 9$ -THC exposure, which has important general impact in light of the expanding legal availability of cannabis preparations. We have also written a standalone open-source software called VividSTORM to facilitate correlated confocal and super-resolution imaging in life sciences. Very recently, we developed a new approach that exploits fluorescent small molecules for STORM super-resolution imaging. This PharmacoSTORM approach opens the way for nanoscale pharmacology that is performed on identified cell types and subcellular compartments. With the help of PharmacoSTORM, we discovered that the antipsychotic and antidepressant medicine called cariprazine (VraylarTM) that was originally developed to treat addiction primarily binds to D3 dopamine receptors that are located on the axons of granule cells in the Islands of Calleja. This is important, because this brain region remained under the radar for current efforts to understand the neurobiological mechanisms of psychiatric disorders.

Dudok B, Barna L, Ledri M, Szabó SI, Szabadits E, Pintér B, Woodhams SG, Henstridge CM, Balla GY, Nyilas R, Varga C, Lee SH, Matolcsi M, Cervenak J, Kacskovics I, Watanabe M, Sagheddu C, Melis M, Pistis M, Contact PD/PI: Katona, Istvan Biosketches Page 21 Soltesz I and **Katona I** (2015) Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. *Nature Neuroscience*, 18:75-86. PMCID: PMC4281300

Lee SH, Ledri M, Tóth B, Marchionni I, Henstridge CM, Dudok B, Kenesei K, Barna L, Szabó SI, Renkecz T, Oberoi M, Watanabe M, Limoli CL, Horvai G, Soltesz I and **Katona I** (2015) Multiple Forms of Endocannabinoid and Endovanilloid Signaling Regulate the Tonic Control of GABA Release. *The Journal of Neuroscience*, 35:10039-57. PMCID: PMC4495235

Barna L, Dudok B, Miczán V, Horváth A, László ZI, and **Katona I** (2016) Correlated confocal and super-resolution imaging by VividSTORM. *Nature Protocols*, 11:163-183.

Prokop S, Ábrányi-Balogh P, Barti B, Vámosi M, Zöldi M, Barna L, Urban GM, Tóth A, Dudok B, Egyed A, Deng H, Leggio GM, Hunyady L, van der Stelt M, Keserű GM and **Katona I** (2021) PharmacoSTORM nanoscale pharmacology reveals cariprazine binding on Islands of Calleja granule cells. *Nature Communications*, 12:6505. PMCID: PMC8586358

BIOGRAPHICAL SKETCH

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

NAME: Keisuke KAWATA

ERA COMMONS USER NAME (credential, e.g., agency login): KKAWATA150

POSITION TITLE: Associate Professor of Clinical Neuroscience

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Henderson State University, Arkadelphia, AR	B.S.	12/2010	Kinesiology/Athletic Training
Temple University School of Medicine, Philadelphia, PA	M.S.	05/2013	Molecular Neuroscience
Temple University School of Medicine, Philadelphia, PA	Ph.D.	07/2016	Clinical Neuroscience

A. Personal Statement

I am a clinical neuroscientist and sports medicine practitioner with a strong record of conducting impactful research in the area of concussive and subconcussive neurodegeneration. My research career has been shaped by diverse brain injury-related research experiences, including studies of an *in-vitro*, mouse model, human laboratory studies, a large scale cross-sectional studies, longitudinal studies, and clinical trials. My multimodal approach of using blood biomarkers, head impact kinematic sensors, neuro-ophthalmologic measures, and advanced neuroimaging is substantiated by my clinical skill in concussion/subconcussion management, which was instilled during my practice in 4 professional-level teams as an athletic trainer (NFL, MLB, MLS, ESPN WWS: see section B). The P30 grant application led by Drs. Mackie and Bradshaw proposes the capacity building, and especially lipid mass spectrometry core is of my interest, as our pilot data from a recent study suggesting that endocannabinoid may play a role in enhancement of neural resiliency to repetitive head injury.

Three of my active federal grants (listed below) combined with 9 internal grants obtained in the past 5 years, have produced 44 publications (first author n=7, middle author n=9, senior author n=28) and 12 manuscripts currently under review. Our research indicate that (1) even mild head impacts (e.g., soccer heading) can cause acute impairments in eye movement and balance, elevations in neural-injury blood biomarkers, and changes in axonal microstructure integrity via diffusion MRI; (2) these mild head impacts, if sustained repetitively, can cause cumulative deficits in the brains of high school and college athletes; (3) chronic cannabis use may relate to the prevention of ocular functional impairment and neuroinflammation after repetitive subconcussive head impacts; concussions can induce mental health symptoms (panic, anxiety, depression) that are moderated by sex; and (4) athletes with ADHD may have reduced resiliency to subconcussive head impacts. My clinical research using an array of neurobiological approach will benefit significantly from the proposed grant.

Ongoing projects that I wish to highlight include:

NIH-NINDS R01 (PI: Kawata) 12/2020 – 11/2025
R01NS113950

Title: Subconcussive neurodegenerative progression in adolescent athletes

NIH-NINDS R21 (MPI: Kawata and Newman) 11/2020 – 10/2022
R21NS116548

Title: Neuroimaging and blood biomarkers for subconcussive neural stress on ADHD

Indiana State Department of Health (MPI Kawata and Shin) 07/2021 – 06/2023

ISCBIRF 00055049

Title: The role of gut microbiome in development of persistent post-concussive symptoms and subconcussive neurodegeneration in adolescents

NIH-NINDS R21 (MPI: Datta and Bangirana)

07/2022 – 06/2024

R21NS129234

Title: Blood-biomarkers and risk factors of acute brain injury associated with neurodisability in Ugandan children [BRAIN-Child]

Role: Co-I

Four of our more influential papers are:

1. Nowak MK, Bevilacqua Z, Ejima K, Huijbregtse ME, Chen Z, Mickleborough TD, Newman SD, & **Kawata K** (2020). Neuro-ophthalmologic response to repetitive subconcussive head impacts: a randomized clinical trial. *JAMA Ophthalmology* 138(4):350-357. PMID: 32053162
2. Huijbregtse ME, Bazarian JJ, Shultz SR, & **Kawata K** (2021). The biological significance and clinical utility of emerging blood biomarkers for brain injury. *Neuroscience & Biobehavioral Reviews* 130: 433–447. PMID: 34474049
3. Zuidema TR, Huijbregtse ME, & **Kawata K** (2022). Blood biomarkers may have found a new frontier in spaceflight. *JAMA Neurology* 79(6):632. PMID: 35435924
4. Kercher KA, Steinfeldt JA, Macy JT, Seo DC, & **Kawata K** (2022). Drill intensity and head impact exposure in adolescent football. *Pediatrics* 150(5):e2022057725

B. Positions and Honors

Positions and employment

2022-present	Associate Professor (with tenure) – Department of Kinesiology, School of Public Health, Indiana University, Bloomington, IN
2022-present	Associate Professor – Program in Neuroscience, Division of Clinical and Translational Neuroscience, College of Arts & Sciences, Indiana University, Bloomington, IN
2022-present	Adjunct Associate Professor – Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN
2016-present	Assistant Professor (Tenure-Track) – Department of Kinesiology, School of Public Health, Indiana University, Bloomington, IN
2016-present	Assistant Professor (Tenure-Track) – Program in Neuroscience, Division of Clinical and Translational Neuroscience, College of Arts & Sciences, Indiana University, Bloomington, IN
2019-present	Adjunct Assistant Professor – Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN
2013-2016	Course Administrator – Anatomy & Physiology II, Department of Kinesiology, College of Public Health, Temple University, Philadelphia, PA
2011-2016	Research Assistant – Temple University, Philadelphia, PA
2011 spring	Athletic Trainer – ESPN Wide World of Sports, Orlando, FL
2011 spring	Spring Camp Athletic Trainer – MLB Atlanta Braves, Orlando, FL
2010-2011	Professional Intern Athletic Trainer – NFL Detroit Lions, Allen Park, MI
2009-2010	Professional Intern Athletic Trainer – MLS Sporting Kansas City, Kansas City, MO

Other Experience and Professional Memberships

2019-present	Certification for administering the Adult ADHD Investigator Symptom Rating Scale (AISRS)
2016-present	Member – Great Lakes Athletic Trainers Association
2016-present	Licensed Athletic Trainer – Indiana

2014-present	Member – American College of Sports Medicine (ACSM)
2014-present	Member – Society for Neuroscience
2014-present	Certified Phlebotomist
2012-present	Approved Clinical Instructor (ACI)
2011-present	Certified Performance Enhancement Specialist – NASM
2011-present	Member – National Academy of Sports Medicine (NASM)
2011-present	Member – Eastern Athletic Trainer's Association (EATA)
2010-present	Certified Athletic Trainer – NATA Board of Certification (ATC)
2009-present	Member – National Athletic Trainer's Association (NATA)

Honors and Awards

2021	Indiana University Outstanding Junior Faculty Award
2020	Indiana University School of Public Health Outstanding Early Career Scholar Award
2018	Military Health System Research Symposium: 1st place in Young Investigators Competition (out of 356 competitors)
2014-2016	Predoctoral Research Fellowship, Pennsylvania Athletic Trainers' Society
2013-2016	4 consecutive Dean's Award winner in Annual Temple University Research Presentation
2010	District VI Representative, Collegiate Sports Medicine Foundation
2010	NFL Ethnic Diversity Award

C. Contribution to Science

My work has centered around traumatic brain injury, particularly in investigating the consequence of concussive and subconcussive brain injury. I employ the laboratory human head impact model and clinical cohort studies, as well as clinical trials, coupled with cutting-edge measures such as head impact kinematics, brain-derived blood biomarkers, ocular-motor performance, and advanced neuroimaging. Many of my laboratory's work have been published in authoritative journals, including *JAMA Ophthalmology*, *JAMA Neurology*, *Pediatrics*, *Journal of Neurotrauma*, *Sports Medicine*, *American Journal of Sports Medicine*, and *JAMA Network Open*.

1. Peripheral inspection of brain damage using brain-derived blood biomarker

As the traumatic brain injury research community pursues the discovery of gold-standard diagnostic metrics, blood biomarkers have shown their promising utility in objectively identify altered neural structures and cell metabolism. Our recent paper shows, for the first time, that neural-cell-derived exosomes reflect a course of concussion recovery, which can be a valuable objective means to determine when athletes and military combatants may safely return to sports activity and military duty, respectively. My laboratory also uses a panel of biomarkers (e.g., S100B, NF-L, Tau, GFAP, UCH-L1) to examine effects of repetitive subconcussive head impacts. We found that plasma levels of S100B, NF-L, GFAP, and UCH-L1 positively correlate with a frequency and magnitude of subconcussive head impacts sustained in both college and high school football players. Our multimodal approach using blood biomarkers and neuroimaging (DTI/NODDI) showed significant associations between serum tau and NF-L with reduced axonal microstructural integrity in high school football players.

- a. **Kawata K**, Mitsuhashi M, & Aldret R (2018). A preliminary report on brain-derived extracellular vesicle as unique blood biomarkers for sport-related concussions. *Frontiers in Neurology* 9, 239. PMC5906531
- b. **Kawata K**, Rubin LH, Takahagi M, Lee JH, Sim T, Szwanki V, Bellamy A, Tierney R, & Langford D (2017). Subconcussive impact-dependent increase in plasma S100B levels in collegiate football players. *Journal of Neurotrauma* 34(14): 2254-2260. PMID: 28181857
- c. **Kawata K**, Rubin HL, Wesley L, Lee JH, Sim T, Tierney R, & Langford D (2017). Acute Changes in Plasma Total Tau Levels Are Independent of Subconcussive Head Impacts in College Football Players. *Journal of Neurotrauma* 35(2), 260-266. PMID: 29073820
- d. **Kawata K**, Steinfeldt JA, Huibregtse ME, Nowak MK, Macy JT, Kercher K, Rettke DJ, Shin A, Chen Z, Ejima K, Newman SD, & Cheng H (2020). Association of proteomic blood biomarkers and DTI/NODDI metrics in adolescent football players. *Frontiers in Neurology* 11:581781. PMC7701105

2. Investigating subconcussive effects through clinical trials

While an evidence from clinical studies has begun to emerge, there are a number of extraneous factors that mask subconcussive effects by modulating outcome variables, precluding accurate interpretation of the results. We have been using our controlled soccer heading model to pinpoint the true effects from repetitive subconcussive effects. My laboratory is one of the original teams to develop such an innovative model, which is able to control ball traveling speed, frequency, interval, and ball placement to the head, as well as to measure the magnitude of a head impact. We have been conducting several interventional trials and addressing what extent of damage subconcussive head impacts cause to ocular-motor and vestibular functions and changes in neural-injury blood biomarker expressions.

- a. Nowak MK, Bevilacqua ZW, Ejima K, Huijbregtse ME, Chen Z, Mickleborough TD, Newman SD, & **Kawata K** (2020). Neuro-ophthalmologic response to repetitive subconcussive head impacts: a randomized clinical trial. *JAMA Ophthalmology* 138(4):350-357. PMC7042902
- b. Huijbregtse ME, Nowak MK, Kim JE, Kalbfell RM, Koppeneni A, Ejima K, & **Kawata K** (2020). Does soccer heading cause an increase in plasma S100B? A randomized controlled trial. *PLOS ONE* 15(10): e0239507. PMC7584162
- c. Wirsching A, Chen Z, Bevilacqua ZW, Huijbregtse ME, & **Kawata K** (2018). Association of acute increase in plasma neurofilament light with repetitive subconcussive head impacts: a pilot randomized control trial. *Journal of Neurotrauma* 35:1-6. PMID: 30019617
- d. Huijbregtse ME, Ejima K, Chen Z, Kalbfell RM, Koppeneni A, & **Kawata K** (2020). Acute time-course changes in CCL11, CCL2, and IL-10 levels after controlled subconcussive head impacts: A pilot randomized clinical trial. *Journal of Head Trauma Rehabilitation* 35(5):308-316. PMID: 32881764

3. Sensory perturbation and head impact kinematics following concussion and subconcussive impacts

Impaired vision and balance are two of the chief complaints in individuals with a concussion. My recent publications have provided evidence that repetitive subconcussive head impacts impair the ocular-motor and vestibular systems. Deficits in ocular-motor function are head impact frequency/magnitude dependent, as measured by a sensor-installed mouthguard. This evidence has challenged the concussion research community by highlighting the importance of tracking subconcussive head impacts and sensory parameters, rather than simply obtaining diagnostic measures after a concussion occurs. My pilot work further suggested that as a result of tracking subconcussive head impacts and eye movement parameters, we were able to predict a concussion before it occurred. We have used a rigorous study design with 12-14 data collections throughout a football season while tracking impact exposure and neuro-ophthalmologic function using a portable ocular-motor headset. Additionally, my team is currently conducting a laboratory study in military personnel to assess whether a sleep-deprived brain decreases its resiliency to subsequent subconcussive head impacts.

- a. **Kawata K**, Rubin HL, Lee JH, Sim T, Takahagi M, Szwanki V, Bellamy A, Assari S, Darvish K, Henderer J, Tierney R, & Langford D (2016). Association of football subconcussive head impacts with ocular near point of convergence. *JAMA Ophthalmology* 134(7):763-769. PMID: 27257799
- b. Zonner SW, Ejima K, Fulgar C, Charleston C, Huijbregtse ME, Bevilacqua ZW, & **Kawata K** (2019). Oculomotor response to cumulative subconcussive head impacts in US high school football players: a pilot longitudinal study. *JAMA Ophthalmology* 137(3): 265-270. PMC6439716
- c. Feller CN, Goldenberg M, Asselin PD, Merchant-Borna K, Abar B, Mannix R, **Kawata K**, & Bazarian JJ (2021). Classification accuracy of comprehensive neuro-ophthalmologic measures of sub-acute concussion. *JAMA Network Open* 4(3):e210599. PMC7930925
- d. Kercher KA, Steinfeldt JA, Macy JT, Seo DC, & **Kawata K** (2022). Drill intensity and head impact exposure in adolescent football. *Pediatrics* 150(5):e2022057725

4. Evaluation of comorbid factors to brain injury

Sports and military-related brain trauma is often diffuse in nature, with many known and unknown comorbidities modulating neural response to the injury. My laboratory investigates the contribution of various factors, including psychiatric disorders (anxiety, depression, panic), ADHD, alcohol and substance use, and muscular damage and exercise effects.

- a. Newman SD, Grantz J, McKinney K, Gutierrez A, & **Kawata K** (2020). Association between history of concussion and substance use is mediated by mood disorder. *Journal of Neurotrauma* 37(1): 146-151. PMC7364309

- b. Nowak MK, Ejima K, Quinn PD, Bazarian JJ, Mickleborough TD, Harezlak J, Newman SD, & **Kawata K** (2022). ADHD may associate with reduced tolerance to acute subconcussive head impacts: a pilot Case-control intervention study. *Journal of Attention Disorder* 26(1):125-139. PMID: 33161816
- c. Huibregtse ME, Zonner SW, Ejima K, Bevilacqua ZW, Newman S, Macy J, & **Kawata K** (2019). Association of acute muscle damage and head impact kinematics in high school American football players. *International Journal of Sports Medicine* 41(1): 36-43. PMC6459945
- d. Macy JT, Kercher K, Steinfeldt JA, & **Kawata K** (2021). The public health threat of fewer U.S. adolescents playing football: Review of measures to improve safety and critical analysis of gaps in the literature. *Public Health Reports* 136(5):562-574. PMID: 33602026

5. Concussion effects on academic performance: return-to-learn

Post-concussion management in sports settings has advanced drastically in the past 2 decades, with many protocols in place, such as safe/graded recovery guideline, potential therapeutic interventions (e.g., exercise protocol, vestibular therapy). However, post-concussion management in academic settings is often neglected, resulting in patients with a concussion may largely be expected to “keep up” with their academic responsibilities. My laboratory has been engaging in this topic of return-to-learn, generating novel dataset to highlight (1) academic difficulty after concussion is moderated by age/grade, (2) some factors (e.g., water consumption, music exposure, exercise) influence recovery speed, and (3) teachers/instructors role in facilitating smooth returning to academic setting.

- a. Holmes AA, Chen Z, Yahng L, Fletcher D, & **Kawata K** (2020). Return to Learn: Academic Effects of Concussion in High School and College Student-Athletes. *Frontiers in Pediatrics* 8:57. PMC7065268
- b. Bevilacqua ZW, Cothran D, Rettke DJ, Nelson-Laird T, Koceja DM, & **Kawata K** (2021). Return-to-Learn: Educator Perspectives on Concussion Management in the College Classroom. *BMJ Open* 20;11(4):e044487. PMC8061863
- c. Bevilacqua ZW, Kerby M, Fletcher D, Chen Z, Merritt R, Huibregtse ME, & **Kawata K** (2019). Preliminary evidence-based recommendations for Return to Learn: a novel pilot study tracking concussion recovery in college students. *Concussion* 4(2): CNC63. PMC6787519
- d. Bevilacqua ZW, Cothran D, Rettke DJ, Nelson-Laird T, Koceja DM, & **Kawata K** (2022). Return to learn: preferences of college educators when receiving concussion medical notes. *Neurotrauma Reports* 3(1): 185–189. PMC9080999

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/1bGhZ7XZQrk5k/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: KEPECS, ADAM

eRA COMMONS USER NAME (agency login): KEPECSA

POSITION TITLE: Professor

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)	Completion Date MM/YYYY	FIELD OF STUDY
Eotvos Lorand University, Budapest	BS	07/1997	Mathematics & Computer Science
Brandeis University, Waltham, MA	PHD	05/2002	Neuroscience
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY	Postdoctoral Fellow	10/2007	

A. Personal Statement

I recently came to Washington University as a professor of neuroscience and psychiatry, with the aspiration to bridge these fields. My lab seeks to reverse engineer the computational and neurobiological processes underlying cognition and decision-making and apply these insights to biological psychiatry. We are engaged in interdisciplinary research at the frontiers of quantitative behavior, neural circuit and computational neuroscience. We have pioneered the study of decision confidence in rodents, established a cross-species theoretical and behavioral framework and identified the key neural mechanisms underlying it in orbitofrontal cortex. We have also discovered principles for how different genetic (Pv, Som, VIP) and connectivity-based (OFC→striatum) neuron types are behaviorally recruited in cortex and identified a cortex-wide disinhibitory circuit motif controlled by VIP interneurons. By identifying the neural processes underlying specific behavioral capacities in rodent models, we also seek insights into what goes awry in the brain during mental illness to link animal studies to psychiatry.

Ongoing and recently completed projects that I would like to highlight include:

R01 MH097061 Kepecs (PI) 09/01/16-08/31/21	Behavioral and neural algorithms for decision confidence
We are developing a new computational approach to neural populations to infer the behavioral algorithm directly from spiking data, in the context of confidence-guided time investment. This proposal does not include any cell-type or circuit-specific aims.	
RF1 MH120034-02 Kepecs (co-PI) 8/2/2020-7/31/22	Generating a formal set of collaborative standards for sharing behavioral data and task designs to enable reproducibility in neuroscience
We are developing an archival data format for behavioral neuroscience, including a formal task specification language, in order to improve sharing, rigor and reproducibility of behavioral neuroscience experiments.	
R01 DA038209 9/15/2015 – 12/31/2020	Functions of distinct orbitofrontal cell-types and pathways in decision making
In this previous project we sought a mechanistic, circuit-level understanding of OFC representations, with a revised set of goals after budget cuts focusing only on the OFC→VS projection.	

Citations:

1. Hirokawa, J., Vaughan, A., Masset, P., Ott, T. & Kepcs A. (2019) Frontal cortex neuron types categorically encode single decision variables. *Nature* 576(7787):446-451 PMID: [31801999](#)
2. Masset, Ott, Lak, Hirokawa and Kepcs (2020) Behavior- and Modality-General Representation of Confidence in Orbitofrontal Cortex. *Cell* 182(1):112-126.e18. doi: 10.1016/j.cell.2020.05.022. PubMed PMID: [32504542](#); PubMed Central PMCID: [PMC8083070](#).
3. Lak A, Hueske E, Hirokawa J, Masset P, Ott T, Urai AE, Donner TH, Carandini M, Tonegawa S, Uchida N, Kepcs A. Reinforcement biases subsequent perceptual decisions when confidence is low, a widespread behavioral phenomenon. *ELife*. 2020 Apr 14;9:e49834. PMID: [32286227](#); PubMed Central PMCID: [PMC7213979](#).
4. Lak A, Nomoto K, Keramati M, Sakagami M & Kepcs A (2017) Midbrain Dopamine Neurons Signal Belief in Choice Accuracy during a Perceptual Decision. *Curr Biol*. 27(6):821-832. PubMed PMID: [28285994](#); PubMed Central PMCID: [PMC5819757](#)

B. Positions, Scientific Appointments, and Honors

Positions and Employment

- 2021 – Robert J. Terry Professor of Neuroscience and Professor of Psychiatry, Washington University in St. Louis, MO
- 2020 – 2021 Professor of Neuroscience and Psychiatry, Washington University in St. Louis, MO
- 2018 – 2019 Chair of Neuroscience Program, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- 2016 – 2019 Professor, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- 2013 – 2016 Associate Professor, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- 2010 – 2019 Adjunct Assistant Professor, SUNY Stony Brook University, Stony Brook, NY
- 2007 – 2013 Assistant Professor, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Other Experience and Professional Memberships:

Selected Honors

- 2014 James and Cathleen Stone Faculty Award, CSHL
- 2010 Eppendorf and Science prize for Neurobiology, Finalist
- 2010 Kavli Frontiers of Science Fellow
- 2009 John Merck Fund Fellowship
- 2009 Klingenstein Fellow
- 2009 Alfred P. Sloan Research Fellow
- 2008 Whitehall Fellow
- 2002,8,13 Faculty of 1000 Notable Paper (4 papers)
- 1998 Center for Advanced Studies in the Space Life Sciences Summer Research Fellowship, Marine Biological Laboratory
- 1998 Soros Foundation Supplementary Grant for Graduate Studies, Brandeis University
- 1997-2001 Alfred P. Sloan Predoctoral Fellowship for Theoretical Neuroscience, Brandeis University
- 1996 American Society for Pharmacology and Experimental Therapeutics Summer Fellowship, Texas Tech University, Health Science Center

Teaching Experience

- 2021– Co-director, System Neuroscience Course, Washington University School of Medicine
- 2007–2019 Instructor, System Neuroscience Course, WSBS, CSHL, NY
- 2018 London Computational Psychiatry Course, UK

- 2008–2017 Lead instructor and Organize, Systems Neuroscience Course
Watson School of Biological Sciences, CSHL, NY
- 2008 Lecturer, Champalimaud Neuroscience Programme, Portugal
- 2007–2009 Lecturer, Scientific Reasoning and Logic, WSBS, CSHL, NY

C. Contribution to Science

1. **Neurobiology of confidence:** As humans, we are aware of our subjective sense of confidence and deploy it to optimize our decision making and learning. Yet the inherently subjective nature of confidence has limited investigations by neurobiologists. I initiated the neurobiological study of this key cognitive computation, “confidence”, in animals, previously considered a metacognitive capacity unique to humans. We developed a cross-species behavioral framework and computational approach, creating a bridge between human self-reported confidence and statistical decision confidence. I was the first to identify neural correlates of confidence in animals. My lab went on to show that orbitofrontal cortex neurons represent abstract confidence, irrespective of sensory modality and inform multiple confidence-guided behavior [3], and OFC specifically required for confidence reports but not sensory decisions. These advances place confidence on a solid footing and pave the way for a mechanistic understanding of how the brain implements confidence-based algorithms to guide behavior. Recently we identified a hitherto not suspected structured and connectivity-defined cell-type specific organization in OFC, with different neuron types carrying different decision variables, like ‘confidence’ and ‘value’.
 - a. Kepecs A, Uchida N, Zariwala HA, Mainen ZF. Neural correlates, computation and behavioural impact of decision confidence. *Nature*. 2008 Sep 11;455(7210):227-31. PubMed PMID: [18690210](#).
 - b. Lak A, Costa GM, Romberg E, Koulakov AA, Mainen ZF, Kepecs A. Orbitofrontal cortex is required for optimal waiting based on decision confidence. *Neuron*. 2014 Oct 1;84(1):190-201. PubMed PMID: [25242219](#); PubMed Central PMCID: [PMC4364549](#).
 - c. Hirokawa, J., Vaughan, A., Masset, P., Ott, T. & Kepecs A. (2019) Frontal cortex neuron types categorically encode single decision variables. *Nature* 576(7787):446-451 PMID: [31801999](#)
 - d. Masset, Ott, Lak, Hirokawa and Kepecs (2020) Behavior- and Modality-General Representation of Confidence in Orbitofrontal Cortex. *Cell* 182(1):112-126.e18. doi: 10.1016/j.cell.2020.05.022. PubMed PMID: [32504542](#); PubMed Central PMCID: [PMC8083070](#).
2. **Inhibitory cortical circuits during behavior:** We pioneered cell-type-specific electrophysiological recordings in behaving mice and showed that three genetically non-overlapping interneuron classes (PV, SOM, VIP ~75% of interneurons) define functional classes with uniform recruitment, , implying that cortical response heterogeneity may arise in part due to cell-type diversity. We discovery that VIP inhibitory interneuron specialize in disinhibition (long hypothesized but not known) and identified a canonical circuit for disinhibition in two cortical areas. We also showed that VIP neurons have a non-canonical behavioral correlate in sensory, they are uniformly recruited by reward and punishment. This shows that behavioral response heterogeneity may be accounted for by cell type diversity and lead to an entirely new framework to think about cortical inhibition: different interneuron types encode behaviorally relevant variables and serve to control information flow at the behavioral timescales (Fishell and Kepecs, 2014, *Nature*).
 - a. Kvitsiani, D., Ranade, S., Hangya, B., Tanaguchi, H., Huang, J.Z., Kepecs, A. (2013) Distinct behavioural and network correlates of two interneuron types in prefrontal cortex. *Nature*, 20; 498(7454):363-6. Pub Med PMID: [23708967](#); PubMed Central PMCID: [PMC4349584](#); doi: 10.1038/nature12176
 - b. Pi H., B. Hangya, D. Kvitsiani, J. I. Sanders, Z. J. Huang and Kepecs A (2013) Cortical interneurons that specialize in disinhibitory control. *Nature* (2013). PubMed PMID: [24097352](#); PubMed Central PMCID: [PMC4017628](#)
 - c. Takada, N, Pi H.J., Sousa V.H., Waters, J., Fishell, G., Kepecs, A, and & Osten, P. (2014) A developmental cell-type switch in cortical interneurons leads to a selective defect in gamma

oscillations. *Nature Communications*. Oct 30;5:533. PubMed PMID: [25354876](#). PubMed Central PMCID: [PMC4220465](#)

- d. Fishell, G. and Kepcs, A., 2020. Interneuron types as attractors and controllers. *Annual review of neuroscience*, 43, pp.1-30. PubMed PMID: [31299170](#); PubMed Central PMCID: [PMC7064158](#).

3. **Computations in neuromodulatory systems:** Neuromodulators can reconfigure circuit operations and our studies have focused on identifying the specific computational variables they signal. Although the forebrain cholinergic system is one of the major neuromodulators, for technical reasons no identified recordings have been done in behaving animals. We discovered that a non-cholinergic basal forebrain population-but not cholinergic neurons-were correlated with trial-to-trial measures of attention. Surprisingly, cholinergic neurons responded to reward and punishment with unusual speed and precision (18 ± 3 ms). These results reveal that the cholinergic system broadcasts a rapid and precisely timed reinforcement signal, supporting fast cortical activation and plasticity. We also studied how midbrain dopaminergic neurons signal reward prediction errors when the stimuli predictive of outcomes are perceptually ambiguous. We formulated a reinforcement learning model with a belief state about the perceptually ambiguous stimulus; this model generates an estimate of the probability of choice correctness, termed decision confidence, and showed that dopamine responses in monkeys performing a perceptually ambiguous decision task comply with the model's predictions. These confidence-dependent dopamine responses emerged prior to monkeys' choice initiation, raising the possibility that dopamine impacts impending decisions, in addition to encoding a post-decision teaching signal.

- a. Hangya, B., Ranade, S., Lorenc, M. & Kepcs A. (2015) Central cholinergic neurons are rapidly recruited by reinforcement feedback. *Cell* Aug 27;162(5):1155-68. PubMed PMID: [26317475](#); PubMed Central PMCID: [PMC4833212](#)
- b. Lak A, Nomoto K, Keramati M, Sakagami M & Kepcs A (2017) Midbrain Dopamine Neurons Signal Belief in Choice Accuracy during a Perceptual Decision. *Curr Biol*. 27(6):821-832. PubMed PMID: [28285994](#); PubMed Central PMCID: [PMC5819757](#)
- c. Laszlovszky T, Dániel Schlingloff, Tamás F. Freund, Attila Gulyás, Kepcs A, Balázs Hangya. (2020) Distinct synchronization, cortical coupling and behavioural function of basal forebrain cholinergic neuron types *Nature Neuro*. 23(8):992-1003. PubMed PMID: [32572235](#); PubMed Central PMCID: [PMC7611978](#).
- d. JF Sturgill, P Hegedus, SJ Li, Q Chevy, A Siebels, M Jing, Y Li, B Hangya, A Kepcs. Basal forebrain acetylcholine signals valence-free reinforcement prediction error *BioRxiv* doi.org/10.1101/2020.02.17.953141

4. **Technology and open source development:** Novel technical approaches have yielded new insights in neuroscience and my laboratory has development molecular, optical and behavioral technologies for systems neuroscience. (1) We developed a receptor complementation strategy enables tropism-free retrograde viral delivery and thereby facilitate efficient retrograde targeting for functional analysis of neural circuits. (2) We have developed the first reconfigurable nanophotonic silicone probe, in collaboration with Michal Lipson (Columbia), an implantable device that with the ability to rapidly switch and route multiple optical beams using a nanoscale switching network. This can be integrated with high-density neural recording technologies, opening the door to implantable probe technologies that are able to simultaneously record and manipulate neurons in freely moving rodents, deep in the brain. (3) We have also developed an array of open-source hardware and software solutions that we have widely shared. We developed the first two-choice decision task and the required interface for head-fixed mice, a low-cost programmable pulse generator for physiology and behavior ("Pulse Pal") and a full behavioral control systems, BPod, that runs all of our behaviors and is presently used by over 80 laboratories world wide – all open sourced.

- a. Sanders J, Kepcs A. (2012) Choice Ball: a response interface for psychometric discrimination in head-fixed mice. *J Neurophysiol*. 2012 Dec;108(12):3416-23. PubMed PMID: [23019000](#); PubMed Central PMCID: [PMC3544881](#)

- b. Sanders J.I. & Kepcs A. (2014) A low cost programmable pulse generator for neurophysiology and behavior. *Front. Neuroeng.* Dec 11;7:43. PubMed PMID: [25566051](#); PubMed Central PMCID: [PMC4263096](#)
 - c. Li, S., Vaughan, A., Sturgill, J.F., Kepcs, A. (2018) A Viral Receptor Complementation Strategy to Overcome CAV-2 Tropism for Efficient Retrograde Targeting of Neurons. *Neuron* 9(5): 905-917. PubMed PMID: [29879392](#)
 - d. Mohanty, A Li, Q. Tadayon MA, Bhatt G, Shim E, Ji X, Cardenas J, Miller SA, Kepcs A, Lipson M (2020) Reconfigurable nanophotonic silicon probes for sub-millisecond deep-brain optical stimulation *Nat Biomed Eng.* 2020 Feb;4(2):223-231. PMID: [32051578](#)
5. **Cross-species computational psychiatry:** We have used a computational behavioral approach to link the feeling of confidence in humans to statistical confidence computations, establishing a bridge across species. The same approach hold great potential to overcome classic limitations in translating psychiatric research. Our recent work built on this approach to study hallucinations in mice. Using analogous behavioral tasks in mice and humans, we showed that hallucination-like percepts—high-confidence false alarms—predicted spontaneous hallucination-proneness in people. This opened a new avenue for circuit-based mechanistic in mice. Our initial work in mice revealed that elevated dopamine in sensory striatum mediates hallucination-like perception by biasing perception toward expectations.
- a. Sanders, J.I., Hangya, B. & Kepcs A. (2016) Signatures of a statistical computation in the human sense of confidence *Neuron* 90(3):499-506. PubMed PMID: [27151640](#); PubMed Central PMCID: [PMC5350614](#)
 - b. Redish, A.D., Kepcs, A., Anderson, L.M., Calvin, O.L., Grissom, N.M., Haynos, A.F., Heilbronner, S.R., Herman, A.B., Jacob, S., Ma, S. and Vilares, I., 2022. Computational validity: using computation to translate behaviours across species. *Philosophical Transactions of the Royal Society B*, 377(1844), p.20200525.
 - c. Lak A, Hueske E, Hirokawa J, Masset P, Ott T, Urai AE, Donner TH, Carandini M, Tonegawa S, Uchida N, Kepcs A. Reinforcement biases subsequent perceptual decisions when confidence is low, a widespread behavioral phenomenon. *ELife*. 2020 Apr 14;9:e49834. PMID: [32286227](#); PubMed Central PMCID: [PMC7213979](#).
 - d. Schmack, K., Bosc, M, Ott, T., Sturgill J.F., A. Kepcs (2021) Striatal Dopamine Mediates Hallucination-Like Perception in Mice, *Science*, 372 eabf4740. PubMed PMID: [34957854](#); PubMed Central PMCID: PMC8710889.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/adam.kepcos.1/bibliography/41889905/public/?sort=date&direction=asc>

BIOGRAPHICAL SKETCH

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

NAME: Adam Kimbrough

eRA COMMONS USER NAME (credential, e.g., agency login): ADAM_KIMBROUGH

POSITION TITLE: Assistant Professor

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
North Carolina State University, Raleigh, NC	B.S.	12/2007	Biological Sciences
Florida State University, Tallahassee, FL	Ph.D.	05/2015	Neuroscience
The Scripps Research Institute, La Jolla, CA	Postdoc	03/2019	Neuroscience
University of California, San Diego, CA	Proj Scientist	08/2020	Neuroscience
Purdue University, West Lafayette, IN	Assistant Prof	Current	Neuroscience

A. Personal Statement

I have a broad background in behavioral neurobiology and systems neuroscience with a focus on addiction and mental health disorders (particularly alcohol and oxycodone). I received my Ph.D. from Florida State University working under Dr. Thomas A. Houpert to study the neurobiology of olfactory and taste learning. As a postdoctoral researcher, I worked with Dr. Olivier George at The Scripps Research Institute and the University of California, San Diego, to study alcohol, nicotine, cocaine, methamphetamine, oxycodone, and other drugs of abuse, with a particular emphasis on translational behavioral models of addiction and identifying neural networks that are involved in withdrawal. As a postdoctoral researcher I successfully adopted brain clearing techniques and network analysis tools to develop an approach to assess brain-wide neural network function in preclinical models. I am carrying the approaches I developed forward into my own independent research laboratory.

I started my laboratory at Purdue University in September of 2020 with a focus on identifying changes occurring in the brain caused by substance use disorder that result in increased motivation for drug use. The primary substance we study is alcohol, which I am currently funded by a R00 Pathway to Independence. In the last several years I have been extremely productive, publishing 6 manuscripts in 2020 and 2 in 2021 thus far, with more currently in press and under review. Overall, my research encompasses aspects of behavioral, systems, and molecular neuroscience and neuropharmacology. I utilize a wide variety of research techniques, including several cutting-edge approaches, such as single-cell whole-brain imaging and graph theory, to assess brain-wide neuronal activation and functional networks of cognitive states. In my research, I seek to pair cutting-edge technical skills with well-validated and translational models of addiction.

B. Research and/or Professional Experience**Employment**

Undergraduate Researcher, Bioinformatics, North Carolina State University (05-09/2007)

Graduate Student, Neuroscience, Florida State University (08/2008-05/2015)

Adam Kimbrough, Ph.D.

Postdoctoral Researcher, Neuroscience, The Scripps Research Institute (07/2015-03/2019)

Assistant Project Scientist, Psychiatry, University of California, San Diego (04/2019-08/2020)

Assistant Professor, Basic Medical Sciences, Purdue University (09/2020-Present)

Honors

- 2006-2007 Deans List, North Carolina State University
2008-2010 Neuroscience Fellowship, Florida State University
2010-2014 Institutional Training Grant Recipient, National Institutes of Health (NIDCD T32-000044)
2010 Travel Award, Florida State University, Congress of Graduate Students
2010 Travel Award, Florida State University, Biological Sciences
2011 Faculty 1000 Award (which places the work in a library of the top 2% of published articles in biology and medicine) for Kimbrough et al., Systemic 5-bromo-2-deoxyuridine induces conditioned flavor aversion and c-Fos in the visceral neuraxis, *Learning and Memory*, 2011, 18:292-295.
2015-2018 Institutional Training Grant Recipient, National Institutes of Health (NIAAA T32-AA007456),
2017 Articles of Public Interest Feature for Kimbrough et al., Intermittent access to ethanol drinking facilitates the transition to excessive drinking after chronic intermittent ethanol vapor exposure, *Alcoholism: Clinical and Experimental Research*, 2017, 41:1502-1509.
2018 Larry H. Parsons Travel Award in Alcohol and Addiction Research, The Scripps Research Institute
2018 Society of Fellows Travel Award, The Scripps Research Institute
2018-2023 Pathway to Independence Award, National Institutes of Health (NIAAA K99/R00-AA027301)
2019 University of California San Diego Psychiatry Department 14th Annual Lewis L. Judd Symposium Best Poster Award
2019 Junior Investigator Award: Research Society on Alcoholism
2019 Memorial Award: Research Society on Alcoholism

Society Memberships:

- Society for Neuroscience (2009-Present)
Society for the Study of Ingestive Behavior (2010-2015)
Research Society on Alcoholism (2016-Present)

Symposium Chair and Organizer:

Research Society on Alcoholism 2019 "BRAIN WIDE NEURAL ACTIVATION IN ANIMAL MODELS OF ALCOHOL ABUSE AND DEPENDENCE"

Ad hoc review for:

Appetite; Alcohol; Addiction Biology; Cognitive, Affective, and Behavioral Neuroscience; Scientific Reports; Journal of Neuroscience; Neuropharmacology; Pharmacology Biochemistry and Behavior; Psychopharmacology

C. Contribution to Science

Complete List of Published Work in **MyBibliography**:

<https://www.ncbi.nlm.nih.gov/myncbi/adam.kimbrough.1/bibliography/public/>

1. Toxicity of 5-bromo-2-deoxyuridine (BrdU) in animal studies

5-Bromo-2-deoxyuridine (BrdU) is used to mark cells that divide during the S phase of the cell cycle in a permanent manner by incorporating itself into DNA in place of thymidine. BrdU is commonly used in

Adam Kimbrough, Ph.D.

mammalian studies of adult neurogenesis because of these properties. Previous studies assumed that BrdU had no aversive or toxic effects in rats or mice at doses that are commonly used in neurogenesis studies. However, I found that when a normally rewarding flavor was paired with intraperitoneal injections of BrdU (at standard experimental doses), conditioned flavor aversion occurred in rats. This effect was so strong that BrdU pairing resembled the effects of pairing the flavor with lithium chloride, the standard agent that is used in conditioned taste aversion studies. This study provided evidence that BrdU can be aversive/toxic at doses that are commonly used to study adult neurogenesis. Often studies that examine adult neurogenesis examine effects on behaviors and as such need to consider any potential aversions that may be produced by BrdU. This finding is critically important when assessing previous and future research using BrdU. The publication for this work received a Faculty 1000 award in recognition of its importance and contribution to the field.

Kimbrough A., Kwon B.S., Eckel L.A., Houpt T.A. (2011) Systemic 5-bromo-2-deoxyuridine induces conditioned flavor aversion and c-Fos in the visceral neuraxis. Learning and Memory 18, 292-295.

2. Alcohol dependence and withdrawal behavior

I have characterized the ways in which alcohol dependence and withdrawal are produced and cause withdrawal symptoms. I examined the way in which previous drinking experience (either binge-like or constant) affects the transition to alcohol dependence and compulsivity in a rat model of alcohol dependence. I found that 5 months of prior binge-like drinking led to a more rapid transition to alcohol dependence compared with 5 months of non-binge-like drinking. However, both groups of rats that had prior alcohol experience had much higher levels of compulsivity for alcohol once dependent compared with dependent rats with no prior alcohol experience and nondependent rats. Thus, prior binge-like drinking resulted in a faster transition to alcohol dependence, but any prior alcohol experience led to a significant increase in compulsivity to drink alcohol.

In another study, I characterized irritability-like behavior in rats that were alcohol-dependent, nondependent alcohol drinkers, or alcohol-naïve. When rats were made dependent on alcohol, they exhibited a dramatic increase in aggressive irritability-like behavior compared with nondependent rats and naïve rats at both 8 h of withdrawal and 2 weeks of protracted abstinence. I found that the increase in irritability-like behavior was at least partially driven by corticotropin-releasing factor-1 (CRF₁) receptors, in which a CRF₁ antagonist that was injected systemically before testing reduced the number of aggressive responses in dependent rats. Irritability-like behavior has been traditionally difficult to characterize in drug addiction because of the lack of suitable and valid tests to examine this behavior. This study marked one of the first assessments of this type of behavior in alcohol addiction, which may also be applicable to other drugs of abuse.

Kimbrough A., Kim S., Cole M., Brennan M., George O. (2017) Intermittent access to ethanol drinking facilitates the transition to excessive drinking after chronic intermittent ethanol vapor exposure. *Alcoholism: Clinical and Experimental Research*, 41(8), 1502-1509.

Kimbrough A., de Guglielmo G., Kononoff J., Kallupi M., Zorrilla E.P., George O. (2017) CRF₁ receptor-dependent increases in irritability-like behavior during abstinence from chronic intermittent ethanol vapor exposure. *Alcoholism: Clinical and Experimental Research*, 41(11), 1886-1895.

Kononoff J., Kallupi M., **Kimbrough A.**, Conlisk D., de Guglielmo G., George O. (2018) Systemic and intra-habenular activation of the orphan G protein-coupled receptor GPR139 decreases compulsive-like alcohol drinking and hyperalgesia in alcohol-dependent rats. *eNeuro*, 5(3), ENEURO.0153-18.2018

3. Behavioral modeling of oxycodone and caffeine self-administration

Adam Kimbrough, Ph.D.

Oxycodone is a major societal problem and responsible for a large number of overdose deaths. To date the available preclinical behavioral models to explore oxycodone use disorder have been limited. I have examined sex differences in oxycodone self-administration and characterized different aspects of oxycodone withdrawal to establish translational validity of a preclinical model of oxycodone self-administration. We found in our rat model of oxycodone self-administration that females take more oxycodone than males. Further we found that females showed lower levels of brain oxycodone even though there were no sex differences in plasma oxycodone levels. This suggests that the mechanism for increased oxycodone intake in female rats may be due to differences in brain oxycodone.

Caffeine is one of the most widely used psychoactive compounds worldwide, however there is controversy as to whether or not caffeine can be addictive. There have been limited preclinical studies due to the inability to establish a valid model of voluntary intake of caffeine. We established a voluntary model of caffeine self-administration in rats and found individual differences in caffeine preference that resembled preference patterns seen in the human population. Further, we found that rats did not show signs of withdrawal from caffeine, but the rats that had a high preference for caffeine showed compulsive-like caffeine intake.

Kimbrough A.*, Kononoff, J.*., Simpson, S.*., Kallupi M., Sedighim, S., Palomino, K., Conlisk, D., Momper, J.D., de Guglielmo, G., George O. (2020) Oxycodone self-administration and withdrawal behaviors in male and female Wistar rats. *Psychopharmacology*, 237(5), 1545-1555.

Lee, C.H., George, O., **Kimbrough, A.** (2020) Chronic voluntary caffeine intake in male Wistar rats reveals individual differences in addiction-like behavior. *Pharmacology, Biochemistry & Behavior*, 191.

4. Neural networks associated with alcohol and drugs of abuse

I have examined neural activation and functional networks that are associated with alcohol, psychostimulant, and oxycodone withdrawal/abstinence. We found that abstinence from alcohol dependence led to a massive increase in coactivation among brain regions compared with controls. The structural organization of the brain was altered, such that the number of modules (groups of brain regions with similar function/coactivation) was reduced in an alcohol-abstinent state compared with controls. This finding mirrors data from humans that showed a decrease in modularity and cognitive dysfunction associated with various diseases. We also characterized the specific hub brain regions, or regions with the most intra/intermodule connectivity, of the neural network and found that several novel brain regions within an extended amygdala module may drive neural activation during abstinence. These regions include the tuberal nucleus, intercalated amygdala, posterior cortical amygdala, parasubthalamic nucleus, and others, and several will be examined further in future research. Finally, we found that the modular structure of the brain during alcohol abstinence very closely resembled the postulated three-stage hypothesis of addiction.

Similarly, in psychostimulants (nicotine, cocaine, and methamphetamine) we found that withdrawal from dependence produced increases in coactivation among brain regions and an overall decrease in modularity compared to controls. Withdrawal from methamphetamine and cocaine were found to produce similar brain network profiles, whereas nicotine withdrawal more closely resembled control brains, suggesting that the more subtle changes in network function in nicotine addiction may result in large behavior changes. Furthermore, the primary similarity across all network profiles of withdrawal is a reduction in modularity and increase in coactivation, indicating that across drugs brain activity may be vastly different at the individual brain region level, warranting further focus on individual drugs, beyond generalizations to addiction overall.

In oxycodone dependent rats we examined neural activity occurring in intoxicated and withdrawal states and how depletion of the microbiome affected these activity states. We found that both intoxicated and withdrawal states lead to changes to neuronal ensemble activity compared to controls and that depletion of the microbiome greatly influenced these changes.

Adam Kimbrough, Ph.D.

Kimbrough A., Lurie D.J., Collazo A., Kreifeldt M., Sidhu H., Macedo G.C., D'Esposito M., Contet C., George O. (2020) Brain-wide functional architecture remodeling by alcohol dependence and abstinence. *Proceedings of the National Academy of Sciences* 4(117).

Kimbrough A., Smith L.C., Kallupi M., Simpson S., Collazo A., George O. Dysmodularity of whole-brain functional architecture as a final common pathway in psychostimulant addiction. *bioRxiv* 743799; doi:<https://doi.org/10.1101/743799>

Simpson S., **Kimbrough A.**, Boomhower, B., McLellan, R., Hughes, M., Shankar, K., de Guglielmo, G., George, O. (2020) Depletion of the Microbiome Alters the Recruitment of Neuronal Ensembles of Oxycodone Intoxication and Withdrawal. *eNEURO* 7(3), ENEURO.0312-19.2020.

Smith L.C., **Kimbrough A.**, (2020) Leveraging Neural Networks in Preclinical Alcohol Research. *Brain Sciences*.

D. Research Support

Ongoing

NIAAA K99/R00-AA027301 (9/21/2018-8/31/23) \$1,033,308 Total Cost

Role: *Principal Investigator*

The purpose of this study is to investigate brain-wide neural activation associated with alcohol abstinence using unbiased single-cell whole-brain imaging. Brain-wide network function will be assessed using computational-based methods, such as hierarchical clustering and graph theory. These methods will enable the identification of key brain regions that may be responsible for neural activity that is associated with abstinence-related behavior. The key brain regions that are identified will be further examined for functional significance to alcohol-related behavior.

BIOGRAPHICAL SKETCH

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

NAME: Lu, Hui-Chen

eRA COMMONS USER NAME (credential, e.g., agency login): HL690781

POSITION TITLE: Linda and Jack Gill Professor

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
National Taiwan University	B.S.	1987-1991	Zoology
Baylor College of Medicine	Ph.D.	1992-1997	Developmental Biology
Baylor College of Medicine	Postdoc	1997-1998	Developmental Biology
Baylor College of Medicine	Postdoc	1998-2003	Neuroscience

A. Personal Statement

Trained as a developmental biologist during my Ph.D. thesis and then as a neuroscientist during my post-doc, I acquired expertise not only in molecular biology/biochemistry/surgical skills but also in anatomy/histology and synaptic electrophysiology. For the past nineteen years, my laboratory's research has focused on how neural circuits are established and how dysfunctional neuronal circuits may underlie various neurological disorders. Merging these interests, we have also explored factors that allow established neural circuits to maintain their health through development and how environmental factors such as sensory experience and exogenous cannabinoids can impact neural circuits and result in lasting behavioral changes. We have identified potential cellular mechanisms by which endogenous cannabinoid (eCB) signaling refines thalamocortical and corticothalamic axonal tracts and axonal arborizations. By thorough behavioral analysis, we revealed a role of GPR55 in motor function and how prenatal manipulation of eCB levels has a lasting impact on behavior. Many of our cannabinoid studies were conducted as collaborations with Drs. Ken Mackie and Heather Bradshaw. Together, we have published numerous papers together. Currently, Dr. Mackie and I are MPIs on a NIDA-R01 (DA053746) exploring the impact of adolescent phytocannabinoids on neural circuit maturation.

As a PI for NIH and private foundation grants, I have a demonstrated record of initiating, executing, and completing substantial research projects incorporating a combination of molecular, biochemical, electrophysiological, behavioral, and genetic approaches. The acquisition of two dual-PI R21 grants with Dr. Ken Mackie enabled us to study how endogenous cannabinoid signaling instructs neural circuit development. These studies enabled us to identify potential cellular mechanisms by which endogenous cannabinoid (eCB) signaling refines thalamocortical and corticothalamic axonal tracts and axonal arborizations. Recently, by thorough behavioral analysis, we revealed how prenatal manipulation of eCB levels has a lasting impact on behavior. Several future studies have been proposed in one recent R01 application aiming to understand the impacts of perinatal cannabis exposure. The proposal has received favorable reviews with scores of 12%. Our investigations on the formation of neural circuits also led us to wonder how the established neural connections are maintained. We were fortunate to acquire research grants from Fidelity and Belfer Foundations. These funds enabled us to discover that NAD synthesizing enzymes NMNATs can also moonlight as molecular chaperones to reduce protein stress. The exciting data on NMNATs enabled us to acquire an R01 from NINDS to elucidate how the neuroprotection offered by NMNAT2 maintains neuronal health. This R01 (NS086794) has been renewed recently for an additional five years. Overall, our studies are highly collaborative, bringing together scientists from various disciplines, including Dr. David Bennett at Rush University for human studies and Dr. Fumitaka Kimura at Osaka Univ. After working at Baylor College of Medicine for 23 years, I moved to

the Gill Center at IU Bloomington 2015 as an endowed Gill Chair. The Gill Center is a collaborative group of neuroscientists with expertise in cannabinoids, pain, and drug discovery, including Drs. Ken Mackie, Andrea Hohmann, Richard DiMarchi, Norbert Hájos, etc., which provides an excellent environment for conducting the studies we propose here. As the director for the Gill center since 2017, I have expanded the scope of our annual Gill symposium, the largest neuroscience event in Indiana, with additional awardees and travel fellows as well as the event durations. I also was able to recruit the sixth Gill chair Dr. Norbert Hájos. I am assisted by two exceptional administrative assistants who have been with me since the start of my directorship. Following are some of my earlier studies and impactful reviews

1. Jane E. Lauckner, Jill B. Jensen, Huei-Ying Chen, **Hui-Chen Lu**, Bertil Hille, and Ken Mackie (2008) "GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current", *Proc Natl Acad Sci U S A*, 105: 2699-2704 (cited by 690). PMCID: PMC2268199
2. Chia-Shan Wu, Christopher P. Jew, **Hui-Chen Lu** (2011) "Lasting impacts of prenatal cannabis exposure and the role of endogenous cannabinoids in the developing brain", *Future Neurology*, 6:459-480. (cited by 136) PMCID: PMC3252200
3. **Hui-Chen Lu** and Ken Mackie (2016) "An introduction to the endogenous cannabinoid system", *Biol Psychiatry*, 79:516-25. PMCID: PMC4789136 (cited by 774)
4. Gregory G. Grecco, Briana Mork, Jui-Yen Huang, Corinne E Metzger, David L Haggerty, Kaitlin C Reeves, Yong Gao, Hunter Hoffman, Simon N Katner, Andrea R Masters, Cameron W Morris, Erin A Newell, Eric A Engleman, Anthony J Baicum , Jiuen Kim, Bryan K Yamamoto, Matthew R Allen, Yu-Chien Wu, **Hui-Chen Lu**, Patrick L. Sheets, Brady K. Atwood (2021) "Prenatal methadone exposure disrupts behavioral development and alters motor neuron intrinsic properties and local circuitry". *Elife*. doi: 10.7554/eLife.66230. PMCID: PMC7992998. (Altmetric Score 33)

Ongoing funded projects that I would like to highlight include:

Agency: NIH-NINDS, Title: Molecular and genetic studies of NMNAT2 in neuroprotection, Type: R01 NS086794 , Role: PI, Period: 09/15/14-05/31/26

Agency: NIH-NIDA, Title: Mechanisms and treatment of adolescent phytocannabinoid impairment of prefrontal cortex function. Type: R01 DA053746, Role: PI (MPI with Dr. Mackie) Period: 05/01/22-02/28/27

B. Positions, Scientific Appointments and Honors

Positions and Employment:

1991-1992	Research Assistant with Y. Henry Sun, Ph.D., Academia Sinica, Taiwan
1992-1997	Graduate Student with Gregor Eichele, Ph.D., Baylor College of Medicine, Houston, TX
1997-1998	Postdoctoral Fellow with Gregor Eichele, Ph.D., Baylor College of Medicine, Houston, TX
1998-2003	Postdoctoral Fellow with Michael C. Crair, Ph. D., Baylor College of Medicine, Houston, TX
2003-2005	Non-Tenure Track Assistant Professor, Baylor College of Medicine, Houston, TX
2005-2012	Tenure-Track Assistant Professor, Baylor College of Medicine, Houston, TX
2012-2015	Associate Professor (tenured), Baylor College of Medicine, Houston, TX
2015-present	Linda and Jack Gill Chair of Neuroscience and Professor (tenured), Indiana University, Bloomington, IN.
2017-present	Director, Linda and Jack Gill Center for Biomolecular Science, Indiana University

Honors

1996-1997	Markey Charitable Trust Foundation Graduate Student Fellowship
1997	Deborah K. Martin Achievement Award in Biomedical Research
1997-1998	Max-Planck postdoctoral fellowship
1999-2003	NIH NRSA postdoctoral fellowship F32NS11034
2008-2010	NARSAD Young Investigator Award
2008-2016	Associate Member for Faculty of 1000
2010	Travel Award for NIDA Workshop: Informatics for Data and Resource Discovery in Addiction

Service

Editorial board for Scientific Reports. Ad hoc reviewer for eLife, PLOS Biol., Mol. Psychiatry, Proc Natl Acad Sci U S A., Nature Communications, J. Neurosci, Cerebral Cortex, Molecular Neurodegeneration, Eur. J. Neurosci. Neurobiology of Disease, Scientific Report, J. Neurophysiology, Neuroscience Letters, Neuropsychopharmacology, International Journal of Developmental Neuroscience, Brain Research, and Brain Research Bulletin; Ad hoc grant reviews for NIH-DBD, NIH-NDPR, National Science Foundation (NSF), MRC (UK), Taiwan The Thematic Research Program, Indiana CTSI, and Alzheimer's Association (US); PBS/PNS colloquium committee (IUB); PNS executive committee (IUB); Lab Animal Research advisory committee (IUB); committee member for numerous graduate students; MSBII space committee (IUB); endowed chair search committee (IUB); Gill steering committee (IUB).

C. Contributions to Science

The role of endocannabinoid system in developing neural circuits and motor behaviors

Cannabis is the most commonly used illicit substance among pregnant women. Human epidemiological and animal studies have found that prenatal cannabis exposure influences brain development and can have long-lasting impacts on cognitive functions. In collaboration with Drs. Mackie, Harkany, Kimura, etc, we found that the endocannabinoid system is abundantly expressed in the developing nervous system. In later studies, we showed that removing CB1R affects various aspects of brain development, including cortical neuron migration, axonal pathfinding and fasciculation as well as synapse specification. These findings may underlie the observation from human studies that substantial maternal cannabis exposure leads to cognitive deficits in their offspring. These studies also identified a role for the novel cannabinoid receptor, GPR55, in motor behaviors. More recently, we found that CB1R is required for pruning thalamocortical axons that overshoot into superficial cortical layers. Intriguingly, postnatal cannabis exposure disrupts thalamocortical axon arborizations. A central goal of our research is to understand how the endogenous cannabinoid system refines sensory circuits and whether it collaborates with glutamate neurotransmission-dependent mechanisms, especially mGluR5 signaling. In many synapses, mGluR5 activation increases 2-AG, one of the two major endocannabinoids.

1. Chia-Shan Wu, Jie Zhu, Jim Wager-Miller, Shan Wang, Dennis O'Leary, Krisztina Monory, Beat Lutz, Ken Mackie, and **Hui-Chen Lu** (2010) "Requirement of cannabinoid CB₁ receptor in cortical pyramidal neurons for appropriate development of corticothalamic and thalamocortical projections", *European J. Neuroscience*, 32:693-706. PMCID: PMC2714552 (cited by 131)
2. Chia-Shan Wu, Hongmei Chen, Hao Sun, Jie Zhu, Chris P. Jew, Jim Wager-Miller, Alex Straiker, Corinne Spencer, Heather Bradshaw, Ken Mackie, **Hui-Chen Lu** (2013) "GPR55, a G protein coupled receptor for lysophosphatidylinositol, plays a role in motor coordination", *Plos One* 8:e60314. PMCID: PMC3614963 (cited by 105)
3. Chiaki Itami, Naofumi Uesaka, Jui-Yen-Huang, **Hui-Chen Lu**, Kenji Sakimur, Masanobu Kano, and Fumitaka Kimura (2022) "Endocannabinoid-dependent formation of columnar axonal projection in the mouse cerebral cortex", *Proc Natl Acad Sci U S A.* 119(37):e2122700119. doi: 10.1073/pnas.2122700119. PMC journal – in process.
4. Izaque S. Maciel, Gabriel HD de Abreu, Claire T. Johnson, Rida Bonday, Heather B. Bradshaw, Ken Mackie, **Hui-Chen Lu** (2021). Perinatal CBD or THC Exposure Results in Lasting Resistance to Fluoxetine in the Forced Swim Test: Reversal by Fatty Acid Amide Hydrolase Inhibition. *Cannabis Cannabinoid Res.* 2021. PMCID: PMC9225394 (Altmetric Score 68)

Glutamatergic neurotransmission in the formation and plasticity of sensory circuits

Another of my primary research interests is to elucidate the signaling cascades underlying proper neural circuit connections during brain development and to understand how sensory experiences affect neural circuit wiring and cognitive behaviors. These interests, spanning an organism's entire life, are driven by a growing appreciation that mis-wiring of neuronal circuits during early life is likely to be a major cause of neurological disorders, including autism and schizophrenia. When I started my post-doctoral training, the majority of research in this area was conducted in cats or monkeys. I took advantage of the rich opportunity afforded by genetically-modified mice and employed transgenic mice as a model system to explore these questions. These initial studies took advantage of mouse genetics and the beautiful anatomical organization of the whisker-barrel system. My studies provided strong support for the role of ionotropic glutamatergic neurotransmission in shaping developing neural circuits. A major finding from these studies was that reduced cAMP/PKA signaling triggered by glutamatergic receptors altered AMPAR trafficking and subsequently

abnormal cortical map formation. The research in my own laboratory has extended from these earlier studies to the cellular and molecular mechanisms regulated by glutamate transmission in shaping cortical circuits.

1. **Hui-Chen Lu**, Wei-Chi She, Daniel T. Plas, Paul E. Neumann, Roger Janz and Michael C. Crair (2003) "Adenylyl cyclase I regulates AMPA receptor trafficking during mouse cortical 'barrel' map development", *Nature Neuroscience*, 6: 939-947.
2. **Hui-Chen Lu**, Daniel A Butts, Pascal S. Kaeser, Wei-Chi She, Roger Janz and Michael C. Crair (2006) "Role of efficient neurotransmitter release in barrel map development", *Journal of Neuroscience*, 26: 2692-2703.
3. Jui-Yen Huang and **Hui-Chen Lu** (2017) "mGluR5 Tunes NGF/TrkA Signaling to Orient Spiny Stellate Neuron Dendrites Toward Thalamocortical Axons During Whisker-Barrel Map Formation", *Cereb Cortex*. 2017 Apr 27:1-16. PMCID:PMC6018836 (Altmetric Score 4).
4. Jui-Yen Huang, Marisha Lynn Miskus, and **Hui-Chen Lu** (2017) "FGF-FGFR mediates the activity-dependent dendritogenesis of layer IV neurons during barrel formation" *Journal of Neuroscience*, 37:12094-12105. PMCID: PMC5729188

Genetic dissection of the role of mGluR5 in brain development and behaviors

The metabotropic glutamate receptor 5 (mGluR5) is a group 1 metabotropic glutamate receptor that signals via G proteins to activate multiple signaling cascades. mGluR5 mutations have been identified in some ADHD and schizophrenic patients and is postulated to be involved in multiple, clinically relevant maladies.

Pharmacological studies implicate this receptor in synaptic function/plasticity and cognitive behaviors. When I first established my lab, we demonstrated the role of mGluR5 in cortical map formation and functional development/plasticity of sensory pathways using global mGluR5 knockout mice. Next, we employed sophisticated genetic tools (conditional deletion, *in utero* electroporation) to understand the contribution of mGluR5 signaling in specific neuronal populations at specific times to sensory circuit formation, synaptic function/plasticity, and behavior. Thus far, our studies have found distinct roles for mGluR5 in cortical excitatory neurons to instruct dendritic morphogenesis and in regulating the excitatory-inhibitory balance of cortical circuits. Following up on our anatomical and electrophysiological studies, we found distinctive behavioral deficits after deletion of mGluR5 from specific populations of glutamatergic and GABAergic neurons. The implications of these studies are numerous, since excitation-inhibition imbalance is an important contributor to many neurological/psychiatric disorders.

1. Wei-Chi She, Charles Quairiaux, Michael J. Albright, Yu-Chi Wang, Denisse E. Sanchez, Poh-Shing Chang, Egbert Welker, **Hui-Chen Lu** (2009) "Roles of mGluR5 in synaptic function and plasticity of the mouse thalamocortical pathway", *European J. Neuroscience*, 29: 1379–1396. PMCID: PMC2714552
2. Carlos J Ballester Rosado, Michael J Albright, Chia-Shan Wu, Chun-Chieh Liao, Jie Zhu, Shen-Ju Chou, Dennis D O'Leary, Li-Jen Lee, and **Hui-Chen Lu** (2010) "mGluR5 in cortical excitatory neurons exerts both cell autonomous and nonautonomous influences on cortical somatosensory circuit formation". *Journal of Neuroscience*, 30:16896-909. PMCID: PMC3008407
3. Chris P. Jew, Chia-Shan Wu, Hao Sun, Jie Zhu, Jui-Yen Huang, Dinghui Yu, Nicholas J. Justice, **Hui-Chen Lu** (2013) "mGluR5 ablation in cortical glutamatergic neurons increases novelty-induced locomotion", *PLOS ONE*, 8:e70415. PMCID: PMC3734292
4. Carlos J Ballester Rosado, Hao Sun, Jui-Yen Huang, and **Hui-Chen Lu** (2016) " mGluR5 exerts cell-autonomous influences on the functional and anatomical development of layer IV cortical neurons in the mouse primary somatosensory cortex", *Journal of Neuroscience*, 36: 8802-14. PMCID: PMC4995298 (Editor's choice)

NMNAT2, a newly identified neuronal maintenance factor

Proper brain function requires an active maintenance program to sustain neuronal health. Environmental stressors detrimentally impact the nervous system, predisposing it to neuronal dysfunction and degeneration if neuroprotective mechanisms are weakened. We and others have identified that NMNATs (nicotinamide mononucleotide adenylyl transferases) maintain neuronal integrity and facilitate proper neural function throughout life. NMNAT2 is the major NMNAT isoform expressed in the mammalian brain and is extremely labile, with a half-life of less than two hours. We have found that NMNAT2 abundance is significantly reduced in Alzheimer's Disease (AD) brains. Using the FTDP-17 tauopathy animal model, rTg4510 mice, we found that NMNAT2 levels were substantially decreased *prior* to the onset of neurodegeneration. Most importantly,

exogenous (viral) *Nmnat2* expression in rTg4510 hippocampus reduced neurodegeneration and the accumulation of toxic tau species. Furthermore, reducing NMNAT2 function in mice leads to axonal deterioration. Our recent studies found that NMNAT2 can act as molecular chaperone in addition to synthesizing NAD. Depending on the nature of insults or stresses, NMNAT2 uses either its NAD enzymatic activity or chaperone function to protect neurons. These findings indicate that NMNAT2 is a potential target for therapeutic interventions in neurodegeneration. We have successfully conducted a phenotypic screen with primary neurons and identified several NMNAT2 modulators, including caffeine. Currently, we are examining the endogenous role of NMNAT2 and exploring how NMNAT2 protect neurons during aging.

1. Cecilia Ljungberg, Yousuf O. Ali, Jie Zhu, Chia-Shan Wu, Kazuhiro Oka, R. Grace Zhai, **Hui-Chen Lu** (2012) "CREB-activity and *nmnat2* transcription are down-regulated prior to neurodegeneration, while NMNAT2 over-expression is neuroprotective, in a mouse model of human tauopathy", *Human Mol. Genetics*, 21:251-67, PMCID: PMC3276285 (cited by 97)
2. Richard A. Slivicki, Yousuf O. Ali, **Hui-Chen Lu**, Andrea G. Hohmann (2016) "Impact of genetic reduction of NMNAT2 on chemotherapy-induced losses in cell viability In vitro and peripheral neuropathy in vivo", *PLoS One*, 11:e0147620. doi: 10.1371/journal.pone. PMCID: PMC4726514
3. Yousuf O. Ali, Hunter M. Allen, Lei Yu, David Li-Kroger, Dena Bakhshizadehmahmoudi, Asante Hatcher, Christin McCabe, Jishu Xu, Nicole Bjorklund, Giulio Taglialatela, David A. Bennett, Philip L. De Jager, Joshua M. Shulman, Hugo Bellen, **Hui-Chen Lu** (2016) "NMNAT2:HSP90 complex mediates proteostasis in proteinopathies", *PLOS Biol.* 14(6):e1002472. PMCID: PMC4890852. (Altmetric Score 264; 99% of the same age; in the top 50 most-downloaded among all 2016 PLOS Biol. papers.)
4. Yousuf O. Ali, Gillian Bradley, **Hui-Chen Lu** (2017) "Screening with an NMNAT2-MSD platform identifies small molecules that modulate NMNAT2 levels in cortical neurons", *Scientific Reports* 7:43846. PMCID: PMC5358788 (Altmetric Score 509; picked up by >54 news outlets; 99% of the same age).

Animal models of Neurological Disorders

Related to my interests in neural circuit formation and maintenance, we have also collaborated with Huda Zoghbi, Hui Zheng, and Daoyun Ji to understand how neural circuits become defective in disease conditions. For example, we found that deletion of MeCP2 only in GABAergic neurons results in excitatory/inhibitory imbalance and Rett syndrome-like behavioral deficits. The discovery of NF- κ B signaling in Alzheimer's Disease highlights the involvement of neuroinflammation in disease progression. In vivo multiple unit recordings of presymptomatic tauopathy mice uncovers abnormal circuit function. These discoveries promoted us to examine presymptomatic gene expression changes. Our expertise/knowledge in normal circuit formation and function have allowed us to make progress in elucidating disease etiology. Animal models studies will help to elucidate the progression of neurological disorders and perhaps develop novel therapeutic interventions.

1. Hsiao-Tuan Chao, Hongmei Chen, Rodney C. Samaco, Mingshan Xue, Maria Chahrour, Jong Yoo, Jeffrey L. Neul, Shiaoching Gong, **Hui-Chen Lu**, Nathaniel Heintz, Marc Ekker, John L.R. Rubenstein, Jeffrey L. Noebels, Christian Rosenmund, Huda Y. Zoghbi (2010) "Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes", *Nature*, 468(7321):263-9. PMCID: PMC3057962 (cited by 1090)
2. Kihoon Han, J. Lloyd Holder Jr, Christian P. Schaaf, Hui Lu, Hongmei Chen, Hyojin Kang, Jianrong Tang, Zhenyu Wu, Shuang Hao, Sau Wai Cheung, Peng Yu, Hao Sun, Amy M. Breman, Ankita Patel, **Hui-Chen Lu**, Huda Y. Zoghbi (2013) "SHANK3 overexpression causes manic-like behaviour with unique pharmacogenetic properties", *Nature* 503:72–77. PMCID: PMC3923348 (cited by 294)
3. Hong Lian, Li Yang, Allysa Cole, Lu Sun, Angie C.-A. Chiang, Stephanie W. Fowler, David J. Shim, Jennifer Rodriguez-Rivera, Giulio Taglialatela, Joanna L. Jankowsky, **Hui-Chen Lu**, Hui Zheng (2014) "NF κ B-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's Disease", *Neuron* 85:101-15. PMCID: PMC4289109 (cited by 291)
4. Salil Sharma, Ines Khadimallah, Adam Corya Williamson, Yousuf Omar Ali, Xi Rao, Yunlong Liu, **Hui-Chen Lu** (2018) "Presymptomatic change in microRNAs modulates Tau pathology", *Sci Rep.* 8:9251. doi: 10.1038/s41598-018-27527-6. PMCID: PMC6006352. (Altmetric Score 103)

Complete list of publications from PubMed (61 publications, citations 7248, h-index ~39)

<https://www.ncbi.nlm.nih.gov/sites/myncbi/hui-chen.lu.1/bibliography/43127381/public/?sort=date&direction=descending>

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Margaret M. McCarthy

eRA COMMONS USER NAME: MARGARETMCCARTHY

POSITION TITLE: Professor and Chair

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

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Missouri, Columbia, MO	B.A., M.A.	1981,1984	Biology
Rutgers University, Newark NJ	Ph.D.	1989	Behavioral Neuroscience
Rockefeller University, New York, NY	Post Doc	1992	Neurobiology
National Institutes of Health, Rockville, MD	NRC Fellow	1993	Neurogenetics

A. Personal Statement

My research interests are focused on brain development and the origins of sex differences in behavior. My work uses neuroanatomical and molecular genetics approaches to investigate morphological sex differences, gene transcription networks and epigenetic modifications mediating development of select brain regions in male and female rat brains. Cellular endpoints include regulation of proliferation, cell fate decisions, synaptic patterning and dendritic morphology. Behavioral endpoints were previously focused on adult reproductive behaviors but in recent years have shifted to the ethologically relevant social play behavior. Our research has found unusual roles for membrane derived signaling molecules and this has naturally led to the endocannabinoid system, one of the earliest and most ubiquitous of signaling molecules in the brain. We discovered a previously unknown role for endocannabinoids in driving phagocytosis by microglia to control astrocyte cell number and ultimately adolescent social behavior. Current work is exploring the timing of THC exposure on that endpoint as well as identifying which cell types both make and respond to endocannabinoids.

1. Van Ryzin, J. Marquardt A.E., Argue K.J., Vecchiarelli H.A., Ashton S.E., Arambula S.E., Hill M.N., **McCarthy, M.M.** (2019) Microglial phagocytosis of newborn cells is induced by endocannabinoids and sculpts sex differences in juvenile rat social play. *Neuron*, 102: 435-449. PMID: 30827729
2. Nugent BM, Wright CL, Shetty AC, Hodes GE, Lenz KM, Mahurkar A, Russo SJ, Devine SE, **McCarthy MM.** (2015) Brain feminization requires active repression of masculinization via DNA methylation. *Nature Neuroscience*. 18(5):690-7. PubMed PMID: 25821913. PMCID: PMC4519828
3. **M.M. McCarthy** , Nugent BM and Lenz KM (2017) Neuroimmunology and neuroepigenetics in the establishment of sex differences in the brain. *Nature Neuroscience Reviews* 18: 471-484. PMID: 28638119
4. D.L. Krebs-Kraft, M.N. Hill, C.J. Hillard, and **M.M. McCarthy** (2010) Sex difference in cell proliferation in developing rat amygdala mediated by endocannabinoids has implications for social behavior. *Proceedings of the National Academy of Sciences*. (2010) Nov 23;107(47):20535-40. PMCID: PMC2996668.

B. Positions and Honors

1979 - 1983 Research Assistant, University of Missouri, Medical School, Columbia, MO
1984 - 1989 Research Fellow, Institute of Animal Behavior, Rutgers University, Newark, NJ
1985 - 1989 Adjunct Lecturer, Dept. of Biological Sciences, Rutgers University, Newark, NJ
1989 - 1992 Postdoctoral Associate, Rockefeller University, New York, NY
1992 - 1993 National Research Council Fellow, National Institutes of Health, Rockville, MD
1993 - 1998 Assistant Professor, Dept. of Physiology, University of Maryland, Baltimore, MD
1998 - Ad Hoc Member, Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel, EPA - Evaluation of the Endocrine Disrupters Screening and Testing Advisory Committee
1998 - Study Section Member, NIDR - RFA, "Sex and Gender-Related Differences in Pain and Analgesic Response"
1998 - 2002 Associate Professor, Dept. of Physiology, University of Maryland, Baltimore, MD
2002 - Professor, Department of Physiology, University of Maryland, Baltimore, MD
2002 - 2005 Director of Graduate Education and Associate Director - Program in Neuroscience, University of Maryland, Baltimore, MD
2003 - Study Section Member – NIDA Special Emphasis Panel on RFA “Chronic Stress and its Relation to Drug Abuse and Addiction”
2003 - 2006 Study Section Member - NNB, NIH
2004 - Member of the National Advisory Mental Health Council Workgroup: Setting Priorities for the Basic Sciences of Mental Health of the NIMH.
2004 - 2008 Associate Editor - Hormones and Behavior
2005 - 2009 Assistant Dean for Graduate Studies
2005- Member NIMH Special Emphasis Panel SMH1 ERB-L, Research Training in Neuroscience
2007 - 2011 Associate Editor – Journal of Neuroscience
2008 - 2013 Editor – Endocrinology
2009 - 2011 Associate Dean for Graduate Studies
2010 - Chair – Department of Pharmacology
2014- Advisory Board Member, eNeuro
2015 - Associate Editor, Hormones and Behavior
2015 - Researcher of the Year, University of Maryland Baltimore
2016 - Ad Hoc Study Section Member – Cellular and Molecular Biology of Glia, CSR.
2017 - Member Board of Scientific Councilors, National Institutes of Mental Health
2017 - Fellow, American Association for the Advancement of Science
2018 - Chair – Board of Scientific Councilors, National Institutes of Mental Health
2018 – 2020 Member-at-Large, Section V Neuroscience, AAAS.
2019 Director, Program in Neuroscience, University of Maryland Baltimore

C. Contributions to Science

For my complete NCBI bibliography: <https://www.ncbi.nlm.nih.gov/sites/myncbi/1Ncj4nNkWeEkB/bibliography/44096419/public/?sort=date&direction=ascending>. H-index= 80 (Scopus)

1. Neural control of social play behavior. The etiology of play and its functional significance were a topic of intense interest to neuroscientists in the 1980's when the rat was the preferred laboratory animal. This emphasis was lost when the mouse became the model animal of choice due to its superior genetic malleability. We now have the opportunity to return to the paradigm of social play with new platform independent tools. My group has been exploring lay intently over the past 5 years, having discovered a highly novel source of regulation of the developing neural circuitry, endocannabinoids and microglia. Simply put, males have a higher endocannabinoid tone in the neonatal medial amygdala. The increased EDC's motivate microglia to phagoptosis (engulf and kill living cells) that are predominantly precursors to astrocytes, resulting in fewer glia in the male amygdala. For reasons not yet understood, this leads to greater neuronal activation in the medial amygdala and correlates with more robust play. There are many more questions to ask and answer about the neural control of play.

Van Ryzin, J. Marquardt A.E., Argue K.J., Vecchiarelli H.A., Ashton S.E., Arambula S.E., Hill M.N., **McCarthy, M.M.** (2019) Microglial phagocytosis of newborn cells is induced by endocannabinoids and sculpts sex differences in juvenile rat social play. *Neuron*, 102:435-499. PMID: 30827729.

Argue KJ, VanRyzin JW, Falvo DJ, Whitaker AR, Yu SJ, **M.M. McCarthy**. (2017) Activation of both CB1 and CB2 endocannabinoid receptors is critical for masculinization of the developing medial amygdala and juvenile social play behavior. *eNeuro*. PMID 28144625

Argue KJ, **M.M. McCarthy**. (2016) Characterization of juvenile play in rats: importance of sex of self and sex of partner. *Biology of Sex Differences*. 6:16. PMID:26361539.

Argue KJ, **M.M. McCarthy**. (2015). Utilization of same- vs. mixed-sex dyads impacts the observation of sex differences in juvenile social play behavior. *Current Neurobiology* 6(1):17-23. PMID: 26924913

2. Neurogenesis and glial genesis in the developing brain. The discovery of ongoing neurogenesis in the mature brain was one of the most transformative observations in neuroscience. We have focused on postnatal neuro and glial genesis, a time period that receives much less attention, and we have determined that in the hippocampus newborn males show twice the rate of neurogenesis as females. Conversely in the amygdala, females make more new cells, most of which will become neurons, than males. We have further determined that endocannabinoids regulate the sex difference in cell genesis in the amygdala and that this is correlated with the sex difference in juvenile social play behavior, a novel observation about the factors governing this complex behavior.

J.-M. Zhang, A.M. Konkle, S. Zup and **M.M. McCarthy** (2008) Impact of sex and hormones on cell proliferation in the developing hippocampus: A novel source of sex dimorphism? *European Journal of Neuroscience* 27: 791-800. PMCID: PMC2735768

J.M. Bowers, J. Waddell and **M. M. McCarthy** (2010) A developmental sex difference in hippocampal neurogenesis is mediated by endogenous estradiol. *Biology of Sex Differences*, 1:(1):8. PMCID: PMC3016241

D.L. Krebs-Kraft, M.N. Hill, C.J. Hillard, and **M.M. McCarthy** (2010) Sex difference in cell proliferation in developing rat amygdala mediated by endocannabinoids has implications for social behavior. *Proceedings of the National Academy of Sciences*. (2010) Nov 23;107(47):20535-40. PMCID: PMC2996668.

Waddell, J., .M. Bowers, N.S. Edwards, C.L. Jordan and **M.M. McCarthy** (2013) Dysregulation of neonatal hippocampal cell genesis in the androgen insensitive Tfm rat. *Hormones and Behavior*. 64: 144-152. PMCID: PMC3753588

3. Epigenetics and sex differences in the brain. There has been a renaissance in the study of epigenetics with the discovery of its central role in both cancer and the nervous system. Early life experiences can permanently imprint on the brain via epigenetic changes to the DNA and surrounding histones and this can have profound influences on the relative vulnerability of adults to disease and dysfunction. We conducted some of the first studies on how steroid hormones can imprint epigenetically on the developing brain to organize differences between males and females in adult physiology and behavior. We have also contributed importantly to the dissemination of information on approaches and interpretation of epigenetics data by highlighting important exceptions to the canonical views of DNA methylation as purely repressive of transcription. Most recently my laboratory generated a paradigm shift in understanding of sexual differentiation of sexual behavior with the discovery that the feminization program of development requires suppression of the masculinization program via DNA methylation, and that steroid hormones emancipate the male gene expression profile by reducing activity of DNA methylating enzymes.

D.

M.M. McCarthy, A.P. Auger, T.L. Bale, G.J. De Vries, G.A. Dunn, N.G. Forger, E.K. Murray, B.M. Nugent, J.M. Schwarz and M.E. Wilson (2009) Mini-Symposium - The epigenetics of sex differences in the brain. *Journal of Neuroscience*, 29(41):12815–12823. PMCID: PMC2788155

Schwarz, J. M., Nugent, B. M., & **McCarthy, M. M.** (2010). Developmental and hormone-induced epigenetic changes to estrogen and progesterone receptor genes in brain are dynamic across the life span. *Endocrinology*, 151(10), 4871–4881. PMCID: PMC2946142

McCarthy MM and B.M. Nugent (2013) Epigenetic contributions to hormonally mediated sexual differentiation of the brain, *Journal of Neuroendocrinology*, 25(11); 1133-40, PMID: 23919286 PMCID-n/a

Nugent BM, Wright CL, Shetty AC, Hodes GE, Lenz KM, Mahurkar A, Russo SJ, Devine SE, **McCarthy MM**. (2015) Brain feminization requires active repression of masculinization via DNA methylation. *Nature Neuroscience*. 18(5):690-7. PubMed PMID: 25821913. PMCID: PMC4519828

4. Inflammatory and immune mediated sex differences in the brain. That sexual differentiation of the brain is mediated by gonadal steroids has been known since the late 1950's but the mechanism by which steroids alter the developmental trajectory has been mysterious. Emphasis was appropriately placed on neurotransmitters and growth factors as being the primary targets for steroid modulation but no clear pathway was identified. We discovered that a primary target of steroid action is regulation of the cyclooxygenase enzymes which are rate limiting in prostaglandin synthesis. One prostaglandin in particular, PGE2, is both necessary and sufficient for masculinization of the developing brain. We subsequently found that microglia are an important partner with neurons and astrocytes in prostaglandin production and the establishment of a masculine pattern of synaptic density. These studies identified a novel mechanism by which synaptic patterns are established in the developing brain and closely align that pattern with adult copulatory behavior. We then expanded our studies of prostaglandins to the cerebellum where we found an important role in Purkinje neuron maturation.

S.K. Amateau and **M. M. McCarthy** (2002) A novel mechanism of dendritic spine plasticity involving estradiol induction of prostaglandin-E2. *Journal of Neuroscience* 22:8586- 8596.

S.K. Amateau and **M.M. McCarthy** (2004) Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. *Nature Neuroscience* 7:643-650

C.L. Wright, S.R. Burks and **M.M. McCarthy** (2008) Identification of prostaglandin E2 receptors mediating perinatal masculinization of adult sex behavioral and neuroanatomical correlates. *Developmental Neurobiology*, 68:1406-1419. PMCID: PMC2725403

Lenz, K. M., Nugent, B. M., Haliyur, R., & **McCarthy, M. M.** (2013). Microglia are essential to masculinization of brain and behavior. *Journal of Neuroscience*, 33(7), 2761–2772.1268-12.2013 PMCID: PMC3727162

5. Sensitive periods in brain development. The establishment of sex differences in the brain occurs during a narrow sensitive window. Understanding the onset and offset of this particular sensitive window helps inform other periods during which the brain may be particularly sensitive to perturbations that can exert lasting influences. We have explored the impact of steroids on multiple endpoints during the early life period and determined how these impact on behavior in both young and adult animals. We have also conducted one of the most extensive characterizations of steroid levels in the developing brain, including the POA, hypothalamus, cortex and hippocampus and found surprising differences in the amount of steroid and the transient nature of sex differences in levels of estradiol, testosterone and dihydrotestosterone. This has proven a valuable asset for interpreting the impact of exogenous hormone treatment and perturbations in endogenous levels.

J.A. Mong, E. Glaser and **M.M. McCarthy** (1999) Gonadal steroids promote glial differentiation and alter neuronal morphology in the developing hypothalamus. *Journal of Neuroscience*, 19:1464-

1472.

Konkle, A. T. M., & **McCarthy, M. M.** (2011). Developmental Time Course of Estradiol, Testosterone, and Dihydrotestosterone Levels in Discrete Regions of Male and Female Rat Brain. *Endocrinology*, 152(1), 223–235. PMCID: PMC3033055

Bale, T. L., Baram, T. Z., Brown, A. S., Goldstein, J. M., Insel, T. R., **McCarthy, M. M.**, Nestler, E. J. (2010). Early Life Programming and Neurodevelopmental Disorders. *Biological Psychiatry*, 68(4), 314–319. PMCID: PMC3168778

J. M. Bowers, M. R. Pérez Pouchoulén, N.S. Edwards and **M.M. McCarthy** (2013) Foxp2 mediates sex differences in ultrasonic vocalization by rat pups and directs order of maternal retrieval. *Journal of Neuroscience*, 33(8):3276 –3283. PMCID: PMC3727442

BIOGRAPHICAL SKETCH

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

NAME: Mirnics, Karoly

ERA COMMONS USER NAME (credential, e.g., agency login): karolymirnics

POSITION TITLE: Director of Munroe-Meyer Institute, UNMC; Hattie B. Munroe Professor of Psychiatry, Pharmacology and Experimental Neuroscience, Biochemistry and Molecular Biology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Novi Sad, Former Yugoslavia	M.D.	07/1986	Medicine
University of Pittsburgh, Pittsburgh, PA, USA	Postdoctoral	12/1998	Biological Psychiatry
Semmelweis University, Budapest, Hungary	Ph.D.	10/2010	Biological Psychiatry

A. Personal Statement

I have a broad background in molecular neurobiology of brain diseases >30 years of experience in various molecular biology techniques, including generation of transgenic mice, immunohistochemistry and *in situ* hybridization, qPCR, *in situ* proteomics, laser dissection microscopy, microfluidics, transcriptome profiling, advanced cloning techniques, sterol biochemistry and mouse behavior. I have been trained as postdoctoral fellow with Drs. Pat Levitt and David A. Lewis at University of Pittsburgh, established my own research laboratory at University of Pittsburgh in 1998, and moved to Vanderbilt University in 2006. In 2016 we relocated our laboratory to the Munroe-Meyer Institute for Genetics and Rehabilitation at the University of Nebraska Medical Center.

I have been collaborating with Dr. Korade (multi-PI) for 15 years, resulting in >40 co-authored publications, and we run a joint laboratory at UNMC. We have complementary expertise – Dr. Korade is a developmental neurobiologist, while I am a disease-oriented neuroscientist with psychopharmacology background. Our shared interest is primarily focused on effects of the role of sterol biosynthesis during development, and consequences of sterol inhibition in the developing brain – both by genetic mutations and commonly used prescription medications. This proposal grown out of a wealth of findings generated by the R01 MH110636 “*Vulnerability of DHCR7+/- mutation carriers to aripiprazole and trazodone treatment*” (PI: Mirnics; 06/01/17-05/31/22), with no overlap.

Ongoing and recently completed projects that I would like to highlight include:

R01 MH067234-12 (PI: Mirnics)**2017/06 - 2022/05**

Title: Effects of environmental challenges on genetically modified interneuronal subpopulations

Description: We study how different inhibitory brain cell types control various behaviors, focusing on those that show alteration in schizophrenia. Furthermore, we are trying to understand how this process is influenced by two distinct environmental insults: cannabinoid exposure in adolescence and prenatal maternal immune activation during fetal life. We are taking advantage of a novel transgenic mouse technology developed in the previous grant cycle.

R01 MH110636 (PI: Mirnics)**2017/03 - 2022/02**

Title: Vulnerability of DHCR7+/- mutation carriers to aripiprazole and trazodone treatment

Description: This project is testing the vulnerability of the DHCR7+/- gene mutation carriers to aripiprazole and trazodone exposure. We are testing biochemical, gene expression and behavioral consequences of the interaction between the DHCR7+/- gene mutation and treatment, assessing the long-lasting effects.

Our most relevant publications for this proposal include:

1. Korade Z, Heffer M, **Mirnics K** (2021). Medication effects on developmental sterol biosynthesis. *Mol Psychiatry*, DOI: 10.1038/s41380-021-01074-5, PMC8490477 (Online ahead of print).
2. Genaro-Mattos TC, Klingelsmith KB, Allen LB, Anderson A, Tallman KA, Porter NA, Korade Z, **Mirnics K.** (2021). Sterol Biosynthesis Inhibition in Pregnant Women Taking Prescription Medications. *ACS Pharmacol Transl Sci* 4(2):848-857, PMC8033759.
3. Genaro-Mattos TC, Anderson A, Allen LB, Tallman KA, Porter NA, Korade Z, **Mirnics K.** (2020) Maternal cariprazine exposure inhibits embryonic and postnatal brain cholesterol biosynthesis. *Mol Psychiatry*. 25(11):2685-2694, PMC7577905.
4. Genaro-Mattos TC, Anderson A, Allen LB, Korade Z, **Mirnics K.** (2019) Cholesterol Biosynthesis and Uptake in Developing Neurons. *ACS Chem Neurosci*.10(8):3671-3681, PMC7184320.

B. Positions and Honors

Positions:

- 1999 – 2000 Research Assistant Professor, Department of Neurobiology, University of Pittsburgh
2001 – 2006 Assistant Professor, Departments of Psychiatry and Neurobiology, University of Pittsburgh
2006 – 2008 Associate Professor, Department of Psychiatry, Vanderbilt University, Nashville, TN
2009 – 2016 Professor, Department of Psychiatry, Vanderbilt University, Nashville, TN
2009 – 2016 Vice Chair for Research, Department of Psychiatry, Vanderbilt University, Nashville, TN
2011 – 2016 James G. Blakemore Endowed Professor, Dept of Psychiatry, Vanderbilt U, Nashville, TN
2011 – 2020 Research Professor, University of Szeged, School of Medicine, Szeged, Hungary
2012 – 2016 Senior Fellow, Vanderbilt Institute for Integrative Biosystems Research and Education
2012 – 2016 Associate Director of Vanderbilt Kennedy Center, Vanderbilt University, Nashville, TN
2016 - Professor of Psychiatry, Pharmacology and Experimental Neuroscience,
Biochemistry and Molecular Biology, UNMC, Omaha, NE
2016 - Director and Hattie B. Munroe Professor
Munroe-Meyer Institute for Genetics and Rehabilitation, UNMC, Omaha, NE

Honors:

- Current Editorial Boards (selected): Biological Psychiatry, Neurobiology of Disease, Progress in Neurobiology, European Neuropsychopharmacology, Current Genomics, Croatian Medical Journal, ...
2003 – 2005 Counterdrug Technology Assessment Center (Office of the President of the USA) Member
2005 Elected member of the Foreign Scientist Council, Hungarian Academy of Sciences
2005 Elected member of American College of Neuropsychopharmacology (ACNP)
2005 Elected member of European College of Neuropsychopharmacology (ECNP)
2006 NARSAD Daniel X. Freedman Prize runner-up
2010 – NARSAD Council Member
2010 – 2014 Research, Development and Innovation Program Committee of the European Union – Member
2010 – 2014 Chartered Member of Neural Basis of Psychopath, Add and Sleep Dis (NPAS) Study Section
2011 – 2016 James G. Blakemore Endowed Professor, Dept of Psychiatry, Vanderbilt U, Nashville, TN
2011 – 2016 CME Director, ACNP
2014 Honorary Member of Hungarian Neuropsychopharmacology Society (5th in history)
2014 – 2015 Chartered Member of Pathophys Basis of Mental Disord and Addictions (PMDA) Study Section
2014 Elected to Fellow of ACNP
2016 - Named Hattie B. Munroe Professor at UNMC
2017 - 2020 Chartered member, NIH PMDA study section
2018 - Autism Action Partnership “*Help is Hope*” Annual Award
2019 - “*Friend of Scottish Rite*” Annual Award
2018 - 2020 Chair of NIH PMDA study section
2020 - Board of Directors, Special Olympics International
2020 - Chair of Research Committee, Special Olympics International

C. Contributions to Science

C1. Described sterol homeostasis in the developing brain and sterol biosynthesis inhibition by commonly used prescription medications. We described the extent and profile of sterol biosynthesis in developing neurons and astrocytes; ascertained the biochemical changes and toxicity of 7-DHC; established and validated the sterol biosynthesis inhibiting effects of commonly used prescription medications; characterized the disruptions in transgenic mouse models of sterol inhibition; validated our *in vitro* and transgenic mouse findings in human dermal fibroblasts and serum of patients; and discovered a gene-medication interactions. In addition to the publications listed in the personal statement (see the 4 citations above and in Dr. Korade's Biosketch), the following studies are the most relevant for the current proposal:

- a. Genaro-Mattos TC, Anderson A, Allen LB, Korade Z, **Mirnics K.** (2021) Altered Cholesterol Biosynthesis Affects Drug Metabolism. *ACS Omega* 6(8):5490-5498, PMC7931400.
- b. Korade Z, Allen LB, Anderson A, Tallman KA, Genaro-Mattos TC, Porter NA, **Mirnics K.** (2021) Trazodone effects on developing brain. *Transl Psychiatry* 11(1):85, PMC7851398.
- c. Genaro-Mattos TC, Allen LB, Anderson A, Tallman KA, Porter NA, Korade Z, **Mirnics K** (2019). Maternal aripiprazole exposure interacts with 7-dehydrocholesterol reductase mutations and alters embryonic neurodevelopment. *Mol Psychiatry*. 24(4):491-500. PMCID: PMC6477890.
- d. Koczok K, Gurumurthy CB, Balogh I, Korade Z, **Mirnics K** (2019). Subcellular localization of sterol biosynthesis enzymes. *J Mol Histol*. 50(1):63-73. PMC6467513.

C2. Pioneered DNA microarray gene expression profiling of human brain disorders.

We identified and described synaptic and chaperone changes in postmortem tissue of subjects with schizophrenia, discovered RGS4 (for which he holds a patent) as a disease-associated gene and developed a novel microarray platform. Furthermore, in 2000 we pioneered molecular pathway-based transcriptome analysis of brain disorders.

- a. Horvath S, **Mirnics K*** (2015). Schizophrenia as a Disorder of Molecular Pathways. *Biol Psychiatry* 77(1):22-8. PMC4092052.
- b. Arion D, Horvath S, Lewis DA, **Mirnics K*** (2009). Infragranular gene expression disturbances in the prefrontal cortex in schizophrenia: signature of altered neural development? *Neurobiol Dis*. 37:738-746, PMC2823856.
- c. **Mirnics K**, Middleton FA, Lewis DA, Levitt P (2001): Analysis of complex brain disorders with gene expression microarrays: schizophrenia as a disease of the synapse. *Trends Neurosci*. 24:479-486.
- d. **Mirnics K**, Middleton FA, Marquez A, Lewis DA, Levitt P (2000): Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron*. 28:53-67.

C3. Established peripheral dermal fibroblast from patients as a promising *in vitro* model for molecular biology studies.

Dermal fibroblasts are a simple, relevant, and much underutilized model for studying molecular processes of patients with neuropsychiatric and neurodevelopmental disorders. We successfully used this model to define the baseline mRNA and microRNA differences between MDD patients and matched controls. Furthermore, we also used this model to show that dermal fibroblasts from MDD patients respond differently to metabolic challenges than the control fibroblasts. The studies suggest that analyses of dermal fibroblasts might lead to the discovery of promising peripheral biomarkers of neuropsychiatric disorders that could be potentially used to aid the diagnosis and allow mechanistic testing of disturbed molecular pathways.

- a. Kálmán S, Garbett KA, Janka Z, **Mirnics K*** (2016). Human dermal fibroblasts in psychiatry research. *Neuroscience*. 320:105-21. PMC4777687.
- b. Garbett KA, Vereczkei A, Kálmán S, Wang L, Korade Ž, Shelton RC, **Mirnics K.*** (2015) Fibroblasts from patients with major depressive disorder show distinct transcriptional response to metabolic stressors. *Transl Psychiatry*; PMC4354345.
- c. Garbett KA, Vereczkei A, Kalman S, Brown JA, Taylor WD, Faludi G, Korade Z, Shelton RC, **Mirnics K** (2014). Coordinated messenger RNA/MicroRNA changes in fibroblasts of patients with major depression. *Biol Psychiatry* 77(3):256-65, PMC4254393.

- d. Kálmán S, Garbett KA, Vereczkei A, Shelton RC, Korade Z, **Mirnics K.** (2014) Metabolic stress-induced microRNA and mRNA expression profiles of human fibroblasts. *Exp Cell Res.* 320(2):343-53, PMC3902643.

C4. Effects of environmental influences on genetic background, brain development and function.

Our studies have shown that environmental enrichment can reduce amyloid deposition in AD animal models, which is accompanied by a specific, neuroprotective gene expression pattern, and that physical activity correlates with gene expression patterns in the non-human primate brain. Furthermore, using three different models of maternal immune activation we have shown that there is a robust, and potentially sustained immune activation of the fetal brain even in the absence of viral infection.

- a. Garbett KA, Hsiao EY, Kalman S, Patterson PH, **Mirnics K*** (2012). Effects of maternal immune activation on gene expression patterns in the fetal brain. *Transl Psychiatry* 2, e98, PMC3337077.
- b. Mitchell AC, Leak RK, Garbett K, Zigmond MJ, Cameron JL, **Mirnics K*** (2011). Physical activity-associated gene expression signature in nonhuman primate motor cortex. *Obesity (Silver Spring)*. 20:692-698. PMC3872776.
- c. Mitchell AC, Leak RK, Zigmond MJ, Cameron JL, **Mirnics K*** (2012). Gene transcripts associated with BMI in the motor cortex and caudate nucleus of calorie restricted rhesus monkeys. *Genomics*. 99:144-151. PMC3292695.
- d. Lazarov O, Robinson J, Tang YP, Hairston IS, Korade-Mirnics Z, Lee VM, Hersh LB, Sapolsky RM, **Mirnics K***, Sisodia SS (2005). Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell*, 120:701-713, PMID: 15766532.

C5. Built and characterized novel GABA system related transgenic animal models

In 2010 we developed a novel transgenic mouse technology, using BAC-driven, miRNA-mediated silencing in vivo. The next five years were spent understanding how different interneuronal subpopulations control behavior. This was achieved by silencing the critical GABA synthesis gene, *GAD1*, in different subpopulation of interneurons, while simultaneously labeling the targeted cells with fluorescent markers. The results show that the same gene, when knocked down in different subpopulations of cells, gives rise to different (and often opposing) behavioral phenotypes. To date, miRNA-mediated *GAD1* knockdown animals were generated for SST, NPY, CB1R, PARV and CCK-expressing interneurons, and are freely shared with the scientific community.

- a. Brown JA, Ramikie TS, Schmidt MJ, Baldi R, Garbett KA, Everheart MG, Warren LE, Gellert L, Horvath S, Patel S, **Mirnics K*** (2015). Inhibition of parvalbumin-expressing interneurons results in altered attention and increased novelty-seeking behaviors. *Mol Psychiatry*, PMC4516717.
- b. Schmidt MJ, Horvath S, Ebert P, Norris JL, Seeley EH, Brown J, Gellert L, Everheart M, Garbett KA, Grice TW, Caprioli RM, **Mirnics K.*** (2014) Modulation of behavioral networks by selective interneuronal inactivation. *Mol Psychiatry*. 19:580-587. PMC4179403.
- c. Brown JA, Horvath S, Garbett KA, Schmidt MJ, Everheart M, Gellert L, Ebert P, **Mirnics K*** (2014) The role of cannabinoid 1 receptor expressing interneurons in behavior. *Neurobiol Dis.* 63:210-221, PMC3946968.
- d. Garbett KA, Horvath S, Ebert PJ, Schmidt MJ, Lwin K, Mitchell A, Levitt P, **Mirnics K*** (2010). Novel animal models for studying complex brain disorders: BAC-driven miRNA-mediated in vivo silencing of gene expression. *Mol Psychiatry*. 15:987-995, PMC3011211.

Complete List of Published Work:

137 NCBI My Bibliography publications (of 177):

<https://www.ncbi.nlm.nih.gov/myncbi/karoly.mirnics.1/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: Daniel J. Morgan

ERA COMMONS USER NAME (credential, e.g., agency login): MORGANDA

POSITION TITLE: Associate Professor and Vice Chair

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Grinnell College, Grinnell IA	B.A.	1993-1997	Biology
Rutgers University, Piscataway NJ	Ph.D.	1997-2004	Neuroscience
University of Washington, Seattle WA	Postdoctoral	2004-2007	Kinase Signaling
Indiana University, Bloomington IN	Postdoctoral	2007-2010	Cannabinoid Signaling

A. Personal Statement

The primary focus of my laboratory is to understand the role of neuropeptide and endocannabinoid signaling in human health and disease including cannabinoid tolerance, drug addiction, and metabolic homeostasis. Currently my group is funded by NIDA to assess the mechanisms responsible for the pain-relieving effects of cannabinoids (DA044999). A diversity mentoring administrative supplement to this parent award provides support for a pathway program for local minority high school students to become involved in biomedical research. I am enthusiastic about new interactions and collaboration between faculty members at Marshall University and the Cannabis Research Center you are building.

Ongoing and recently completed projects that I would like to highlight include:

Ro1 DA044999, Morgan (PI) NIH/NIDA Mechanisms of cannabinoid tolerance. The goal of this study is to determine whether JNK-mediated Δ ⁹ -THC tolerance is mediated through direct phosphorylation of the CB ₁ receptor. Role: PI	08/01/18-07/31/23
Ro1 DA044999-05S2, Morgan (PI) NIH/NIDA Mechanisms of cannabinoid tolerance. The goal of this diversity mentoring administrative supplement is to support a pathway program for local minority high school students to obtain biomedical research experience. Role: PI	08/01/22-07/31/23
R21 DE028650, Hu (PI) NIH/NIDCR The role of chronic cannabis and its two major psychoactive ingredients in papillomavirus-associated oropharyngeal disease. The goal of this study is to determine whether Δ ⁹ -THC, cannabidiol, or inhaled cannabis enhance papillomavirus virulence in oropharyngeal disease.	04/01/18-03/31/20

Role: Co-investigator

SAP#4100079742, Morgan (PI) 07/01/18-06/30/19

PA State Dept. of Health CURE Tobacco Settlement Funds

Agonist-specific mechanisms of cannabinoid tolerance.

The goal of this project is to provide Bridge funding for the re-submission of 1R01 DA044999.

Role: PI

Seminal Citations:

1. **Morgan DJ**, Davis, BJ, Kearn, CS, Marcus, DA, Cook, AJ, Wager-Miller, J, Straiker, AS, Myoga, MH, Karduck, J, Leishman, E, Sim-Selley LJ, Czyzyk, TA, Bradshaw, HB, Selley, DA Mackie, K. (2014). Mutation of putative GRK phosphorylation sites in the cannabinoid receptor 1 (CB1) confers resistance to cannabinoid tolerance and hypersensitivity to cannabinoids in mice. *Journal of Neuroscience*. **34** (15): 5152-63. (PMCID: PMC3983798).
2. Marcus DJ, Zee, ML, Hughes, A, Yuill, MB, Hohmann, AG, Mackie K, Guindon, J, and **Morgan DJ**. (2015). Tolerance to the antinociceptive effects of chronic morphine requires c-Jun N-terminal kinase. *Molecular Pain*. **11**: 34. (PMCID: PMC4465431).
3. Yuill, MB, Hale, D, Guindon, J, and **Morgan, DJ**. (2017). Functional interactions between opioids and the cannabinoid receptor 2 agonist JWH-133 in inflammatory pain. *Molecular Pain*. **13**: 1-15. (PMCID: PMC5593227).
4. Henderson-Redmond, A.N., Nealon, C.M., Davis, B.J., Yuill, M.B., Sepulveda, D.E., Blanton, H., Zee, M.L., Haskins, C.P., Marcus, D.J., Mackie, K., Guindon, J., and **Morgan, D.J.** (2020). c-Jun N terminal kinase signaling pathways mediate cannabinoid tolerance in an agonist-specific manner. *Neuropharmacology*. **164**: 107847. (PMID: 31758947).

B. Positions and Honors

Positions

1996	Summer Intern, Iowa State University, Ames, IA
1997-2004	Graduate Assistant and Postdoctoral Fellow, Rutgers University, Piscataway, NJ
2004-2007	Senior Fellow, University of Washington School of Medicine, Seattle, WA
2007-2010	Postdoctoral Fellow, Indiana University, Bloomington, IN
2010-2015	Research Scientist, Indiana University, Bloomington, IN
2012-2020	Assistant Professor, Penn State University, Hershey, PA
2020	Associate Professor (with Tenure), Penn State University, Hershey, PA
2020-present	Adjunct Associate Professor, Penn State University, Hershey, PA
2020-2021	Associate Professor (with Tenure) and Associate Vice Chair of Biomedical Sciences, Joan C Edwards School of Medicine at Marshall University, Huntington, WV
2021-present	Associate Professor (with Tenure) and Vice Chair of Biomedical Sciences, Joan C Edwards School of Medicine at Marshall University, Huntington, WV

Honors

1993-1997	Trustee Honor Scholarship-Grinnell College.
1997-1999	NIH IMSD Minority Development Fellowship- Rutgers.
2000	Champions Scholarship-Rutgers
2005-2007	Cardiovascular Pathology Training Fellowship-University of Washington
2006	American Society for Cell Biology Travel Award
2009	NIDA Early Career Investigator Travel Award
2010	Chicago Society for Neuroscience Postdoctoral Poster Award
2011	Julius Axelrod Symposium Award
2013-2015	Satvir Tevethia Junior Faculty Research Scholar Award
2014	Winter Brain Conference Travel Award Fellow
2014	CSHL/NIDA Cellular Biology of Addiction Short Course Travel Award
2017	Jackson Laboratories Genetics of Addiction Short Course Travel Award

Other Experience and Professional Societies

1998-present	Member, Society for Neuroscience
2010-present	Member, American Society for Pharmacology and Experimental Therapeutics (ASPET)
2009-present	Member, International Cannabinoid Research Society
2013-present	Ad hoc reviewer for PLOS One
2014	Ad hoc grant reviewer for Austrian Science Fund.
2014	Ad hoc reviewer for European Journal of Pharmaceutical Science
2014-2016	Ad hoc reviewer, special issue guest editor for Progress in Neuro-Psychopharmacology and Biological Psychiatry
2016	Ad hoc grant reviewer for Health Research Council of New Zealand.
2016-present	Member, ASPET Neuropharmacology Executive Committee
2016	Travel Fellow Committee, Winter Conference on Brain Research
2017-present	Program Committee, Winter Conference on Brain Research
2017-present	Ad hoc reviewer for Neuropharmacology, British Journal of Pharmacology, Scientific Reports, Acta Pharmacologia Sinica, ACS Neuroscience, and Drug and Alcohol Dependence Program Committee and Session Chair, International Cannabinoid Research Society
2018	Ad hoc reviewer for Alcoholism: Clinical and Experimental Research
2018-present	Ad hoc reviewer for Pain, Neuroscience Letters, Pharmacology, Biochemistry, and Behavior, and European Journal of Pharmacology
2019-present	Canadian Institutes of Health College of Reviewers
2019	Panel Member, Congressionally Directed Medical Research Programs, Spinal Cord Injury Research Program (SCIRP)
2019	Penn State Hershey Leadership Academy for Excellence in Academic Medicine
2019-2020	Panel Member, Florida Department of Health Biomedical Research Programs
2020-present	Editorial Board, Pharmacology Research & Perspectives
2020-present	Editorial Board, Frontiers in Molecular Neuroscience
2021-present	Editorial Board, Journal of Pharmacology and Experimental Therapeutics
2021	John Marshall Leaders Fellowship Program
2021-present	Secretary/Treasurer, ASPET Neuropharmacology Executive Committee
2022-present	AAMC Leadership Education and Development (LEAD) Program

C. Contributions to Science

“My Biography” at NCBI:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/daniel.morgan.1/bibliography/45974986/public/?sort=date&direction=ascending>

My graduate work focused on understanding the role of **neuropeptide processing and signaling**. I characterized the distribution of proSAAS transcript and the processing of the proSAAS protein precursor during development. I produced proSAAS knock-out (KO) mice lacking this novel neuropeptide and demonstrated that this neuropeptide is involved in regulating body weight, anxiety-like behaviors, and cocaine response.

- A. **Morgan DJ**, Mzhavia N, Peng B, Pan H, Devi LA and JE Pintar. (2005). Embryonic gene expression and pro-protein processing of proSAAS during rodent development. *Journal of Neurochemistry*. **93**: 1454-63. (PMID: 15935061).
- B. **Morgan, DJ**, Wei, S, Gomes, I, Czyzyk, TA. Mzhavia, N, Pan, H., Devi, LA, Fricker, LD, Pintar, JE. (2010). The propeptide precursor proSAAS is involved in fetal neuropeptide processing and body weight regulation. *Journal of Neurochemistry*. **113**: 1275-1284. (PMCID: PMC3510705).
- C. Berezniuk, I., Sironi, J., Rodriguez, R.M., Zee, M.L., Pintar, J.E., **Morgan, D.J.**, Wetsel, W.C., Fricker, L.D. (2017). ProSAAS-derived peptides are regulated by cocaine, and contribute to the physiological effects of cocaine administration in mice. *Journal of Neurochemistry*. **143**: 268-81. (PMCID: PMC5693316).

- D. Aryal, D., Rodriguez, R.M., Nguyen, N.L., Pease, M.W., **Morgan, D.J.**, Pintar, J.E., Fricker, L.D., Wetsel, W.C., (2022). Mice lacking proSAAS display alterations in emotion, consummatory behavior and circadian entrainment. *Genes Brain Behavior*. In Press. (PMID: 35878875).

My work as a senior postdoctoral fellow, research scientist, and assistant professor focused on understanding the mechanisms responsible for **tolerance to cannabinoid and opioid drugs**. We have demonstrated that mice expressing a desensitization-resistant form of CB₁ are acutely more sensitive to cannabinoids and develop tolerance to cannabinoids more slowly. We are also actively engaged in examining the role of JNKs in tolerance to cannabinoid drugs such as Δ⁹-THC.

- A. Nealon, CM, Henderson-Redmond, AN, Hale, DE, and **Morgan, DJ.** (2019). Tolerance to WIN55,212-2 is significantly delayed in desensitization-resistant S426A/S430A mice. *Neuropharmacology*. **148**:151-159. (PMCID: PMC6535342).
- B. Blanton, H.L., Breslford, J., DeTurk, N., Pruitt, K., Narasimhan, M., **Morgan, D.J.**, and Guindon, J. (2019). Cannabinoids: Current and Future Options to Treat Chronic and Chemotherapy-Induced Neuropathic Pain. *Drugs*. **79** (9): 969-995. (PMID:31127530).
- C. Piscura, M.P., Sepulveda, D.E., Guindon, J., Henderson-Redmond, A.N., **Morgan, D.J.** Cannabinoid tolerance in S426A/S430A x beta-arrestin2 double mutant mice. *Journal of Pharmacology and Experimental Therapeutics*. In Press.

Currently my laboratory is also interested in understanding the sex-specific mechanisms of cannabinoid tolerance. We have found that female mice on a C57Bl6 background display enhanced tolerance for cannabinoids despite showing a reduced initial acute response to cannabinoid agonists.

- A. LaFleur, R.A., Wilson, R.P., **Morgan D.J.**, Henderson-Redmond, A.N. (2018). Sex differences in the development of tolerance to delta-9-tetrahydrocannabinol (Δ⁹-THC) and CP55,940 in a mouse model of inflammatory pain. *Neuroreport*. **29**: 447-52. (PMCID: PMC6112616).
- B. Henderson-Redmond, A.N., Crawford, L.C., Sepulveda, D.E., Hale, D.E., Lesperance, J.J., **Morgan, D.J.** (2021). Sex differences in tolerance to delta-9-tetrahydrocannabinol (Δ⁹-THC) in mice with cisplatin-evoked chronic neuropathic pain. *Frontiers in Biomedical Sciences*. **8**: 684115.
- C. Henderson-Redmond, A.N., Sepulveda, D.E., Ferguson, E.L., Kline, A.M., Piscura, M.K., **Morgan, D.J.** (2021). Sex-specific mechanisms of tolerance for the cannabinoid agonists CP55,940 and delta-9 tetrahydrocannabinol (Δ9-THC). *Psychopharmacology*. **239**(5): 1289-1309.

My laboratory is also interested in how **cannabis substance use disorder** develops and also how the **opioid and endocannabinoid signaling systems modulate alcohol, opiate, and food addiction**. My laboratory has found increased Δ⁹-THC dependence and ethanol consumption in mutant S426A/S430A mutant mice with enhanced endocannabinoid signaling. We have also found increased ethanol drinking in humanized mice expressing the MOR A118G polymorphism.

- A. Henderson-Redmond, AN, Guindon, J, and **Morgan DJ.** (2016). Roles for the endocannabinoid system in ethanol-motivated behavior. *Progress in Neuro-psychopharmacology and Biological Psychiatry*. **65**, 330-9. (PMCID: PMC4679600).
- B. Marcus, D.J., Zee, M.L., Davis, B.J., Haskins, C.P., Andrews, M.J., Amin, R., Henderson-Redmond, A.N., Mackie, K., Czyzyk, T.A., **Morgan D.J.** (2016). Mice expressing a “hyper-sensitive” form of the cannabinoid receptor 1 (CB1) are not obese or diabetic. *PLOS One*. **11**(8): e0160462. (PMCID: PMC4976987).
- C. Marcus, D.J., Henderson-Redmond, A.N., Zee, M.L., Amin, R., Farnsworth, J.C., Andrews, M.J., Davis, B.J., Mackie, K., and **Morgan, D.J.** (2017). Mice expressing a “hyper-sensitive” form of the cannabinoid receptor 1 (CB1) show modestly alcohol preference and consumption. *PLOS One*. **12**(4): e0174826. (PMCID: PMC5398885).

- D. Henderson-Redmond, A.N., Lowe, T., Kline, A.M., Tian, X.B., **Morgan D.J.** (2018). Increased ethanol drinking in "humanized" mice expressing the mu-opioid receptor A118G polymorphism are mediated through sex-specific mechanisms. *Brain Research Bulletin*. **138**: 12-19. (PMCID: PMC5796878).

I have a long-standing interest in the mechanisms of **mu opioid receptor (MOR) signaling and tolerance to MOR agonists**. We have recently shown that JNK signaling is involved in chronic tolerance to the antinociceptive and antiallodynic effects of morphine in a chemotherapy-induced model of neuropathic pain. We also find evidence of unidirectional cross-tolerance and additive antinociceptive effects between morphine and the CB2 selective agonist, JWH133.

- A. Schuller, A., King, M., Zhang, J., Bolan, E., Pan, X., Morgan, D.J., Chang, A., Czick, M., Unterwald, E., Pasternak, G., and Pintar, JE. (1999). Retention of heroin and morphine-6-glucuronide analgesia in MOR-1 exon 1 knockout mice insensitive to morphine: Evidence for a novel MOR-1 related receptor. *Nature Neuroscience*. **2**: 151-156.
- B. Henderson-Redmond, A.N., Yuill, M.B., Lowe, T., Kline, A.M., Zee, M.L., Guindon, J., and **Morgan, DJ.** (2016). Morphine-induced anti-nociception and reward in "humanized" mice expressing the mu opioid receptor A118G polymorphism. *Brain Research Bulletin*. **123**: 5-12. (PMCID: PMC4848164).
- C. Yuill, M.B., Zee, M.L., Marcus, D.J., and **Morgan D.J.** (2016). Tolerance to the anti-nociceptive and hypothermic effects of morphine are mediated by multiple isoforms of c-Jun N-terminal Kinase. *Neuroreport*. **27**: 392-6. (PMCID: PMC4808337).
- D. Nealon, C.M., Patel, C., Worley, B.L., Henderson-Redmond, A.N., **Morgan, D.J.**, and Czyzyk, T.A. (2018). Alterations in nociception and morphine antinociception in mice fed a high-fat diet. *Brain Research Bulletin*. **138**: 64-72. (PMID: 28684345).

BIOGRAPHICAL SKETCH

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

NAME: Nah, Gabriel D.

ERA COMMONS USER NAME (credential, e.g., agency login): GABRIELNAH

POSITION TITLE: Graduate Student

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Lincoln University	BS	05/2019	Biology

A. Personal Statement

I am a fourth-year doctoral student in the Program in Neuroscience and the Department of Psychological and Brain Sciences at Indiana University in Bloomington, Indiana. I have a background in neuroscience. My research focuses on studying the cognitive impairments associated with mild traumatic brain injury (mTBI). My current research projects involve using rodents to study memory impairment associated with mTBI using various behavioral models. During my tenure at Lincoln University, I participated in two NSF summer internships where I oversaw the planning, conducting, and presenting of my research projects. I was in charge of the care, experimentation, and euthanasia of all animals used in the experiments. The data from one of those projects was used in a larger study and published in a peer-reviewed journal. As a graduate student, I am not only managing research projects but also conducting personnel training for lab equipment, animal behavior training, and histology. Furthermore, I have a commitment to advancing diversity and inclusion in science, which is demonstrated by my sustained outreach and involvement in this realm. In summary, I have the necessary qualifications to carry out any proposed research project.

B. Positions, Scientific Appointments, and Honors**Positions**

2021 – Present	NIMH T32 Clinical Translational Science Research Predoctoral Training Program
2021 – 2022	Graduate Student Representative, Program in Neuroscience Executive Committee, Indiana University, Bloomington, IN
2021 – Present	Member, National Neurotrauma Society
2021 – Present	Member, Comparative Cognition Society
2019 – Present	Member, Society for Neuroscience
2018	Student Research Assistant, Children's Hospital of Philadelphia, Philadelphia, PA
2017	Student Research Assistant, Biology Department, Lincoln University, Lincoln University, PA

Honors

2022	Neuroscience Fall Travel Award, Program in Neuroscience, IU
2022	Neuroscience Spring Travel Award, Program in Neuroscience, IU
2021	Neuroscience Fall Travel Award, Program in Neuroscience, IU
2021	Harlan Scholar Research Fellowship, Dept. of Psychological and Brain Sciences, IU
2021	Neuroscience Spring Travel Award, Program in Neuroscience, IU
2021 – Present	Society for Neuroscience, Neuroscience Scholars Program
2019 – 2020	Rebec Family Fellowship Recipient, IU
2018	Lincoln University Science Fair Honorable Mention

2018	Best Presentation, Student Research Day, CIRP, Children's Hospital of Philadelphia
2015 – 2019	Lincoln University Tier II Academic Scholarship
2015 – 2019	Horace Mann Bond Honors Program
2015 – 2017	Academic Dean's Honor List

C. Contributions to Science

My first publication highlighted how traumatic brain injury impedes the expression of parvalbumin neurons in the dentate gyrus of the hippocampus.

- a. Folweiler KA, Xiong G, Best KM, Metheny HE, **Nah GD**, Cohen AS. *Traumatic brain injury diminishes feedforward activation of parvalbumin-expressing interneurons in the dentate gyrus*. eNeuro (2020). <https://doi.org/10.1523/ENEURO.0195-19.2020> PMCID: PMC7675145

BIOGRAPHICAL SKETCH

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

NAME: Patel, Sachin

ERA COMMONS USER NAME (credential, e.g., agency login): PATELS2

POSITION TITLE: Professor, Department of Psychiatry and Behavioral Sciences, Northwestern University Feinberg School of Medicine

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
University of California Santa Barbara	BS	06/1998	Psychology
Medical College of Wisconsin	PHD	06/2004	Pharmacology
Medical College of Wisconsin	MD	06/2006	Medicine
Vanderbilt University School of Medicine	Resident	06/2010	Psychiatry

A. Personal Statement

The goal of our research is to investigate the role of bioactive lipids including endocannabinoids (eCBs) in the pathophysiology of severe psychiatric disorders including alcohol use disorder, depression and post-traumatic stress disorder (PTSD). Our programs focus is on understanding the developmental, molecular, and synaptic adaptations in eCB that occur in animal models of psychiatric disease. By understanding bioactive lipid adaptations that occur during the development of mental illness we hope to uncover novel molecular targets for drug development. We also evaluate the effects of genetic and pharmacological modulation of lipid signaling components in preclinical models of psychiatric disorders. By integrating analyses across multiple levels, from molecules to circuits, we hope to develop integrated models of how specific signaling systems interact with hard-wired neural circuits to sculpt complex behavior, and how these systems are dysregulated in models of psychiatric illness. Over the past 10 years I have trained or am currently training, 10 PhD students. All previous trainees continued their research training in academic postdoctoral positions. I also have trained 6 postdoctoral research fellows and 3 research faculty in the laboratory. Six of my trainees have successfully competed for NIH fellowships or private research fellowships from non-profit organizations such as the Brian and Behavior foundation and I have a strong commitment and track record of successful mentorship of students and fellows.

B. Positions, Scientific Appointments, and Honors**Positions**

- 1999 - 2006 Student, Medical Scientist Training Program, Medical College of Wisconsin
- 2006 - 2010 Resident Physician, Department of Psychiatry, Vanderbilt University Medical Center
- 2010 - 2014 Assistant Professor (tenure-track), Departments of Psychiatry and Molecular Physiology & Biophysics, Vanderbilt University School of Medicine
- 2013 -2018 Director, Division of Addiction Psychiatry, Department of Psychiatry, Vanderbilt University School of Medicine
- 2014 -2018 Associate Professor (tenured), Departments of Psychiatry and Molecular Physiology & Biophysics, Vanderbilt University School of Medicine
- 2018-2021 Director, Division of General Psychiatry, Department of Psychiatry, Vanderbilt University School of Medicine
- 2018-2021 James G. Blakemore Professor of Psychiatry and Behavioral Sciences, Molecular Physiology & Biophysics, and Pharmacology, Vanderbilt University Medical Center

2022-current Lizzie Gilman Professor and Chair, Department of Psychiatry and Behavioral Sciences, Northwestern University Feinberg School of Medicine

Scientific Appointments

- 2001 - Member, International Cannabinoid Research Society
2001 - Member, Society for Neuroscience
2014 - Associate Member, American College of Neuropsychopharmacology
2018- Member, American Society for Clinical Investigation
2018- Member, American College of Neuropsychopharmacology
2018- Member, American Society for Pharmacology and Experimental Therapeutics
2013- Editorial Board *Neuropsychopharmacology*
2019 Frontiers In Neuroscience
 Guest Associate Editor: Endocannabinoid Retrograde Transmission
2021 Alcohol Research: Current Reviews
 Guest Editor: Cannabinoid-Alcohol Interactions
2022- Deputy Editor: Journal of Clinical Investigation

Honors

- 1997 Presidents Undergraduate Research Award, University of California
1998 Howard Hughes Undergraduate Biomedical Research Fellowship, University of California
2001 Medical Student Summer Research Fellowship, Medical College of Wisconsin
2006 Quick Award in Biochemistry, Department of Biochemistry, Medical College of Wisconsin
2006 HOPE Initiative Award for Community Service in Psychiatry, Medical College of Wisconsin
2006 Elliot Newman Award for Best House Staff Research Presentation, Vanderbilt University School of Medicine
2008 Outstanding Resident Award, NIMH/NIH
2009 Resident Research Prize, Department of Psychiatry, Vanderbilt University
2009 Scientific Achievement Award, International Cannabinoid Research Society
2009 Memorial Travel Award, American College of Neuropsychopharmacology (ACNP)
2010 Hollander Award, Department of Psychiatry, Vanderbilt University School of Medicine
2011 Certified by the American Board of Psychiatry and Neurology, ABPN
2013 Presidential Early Career Award for Scientists and Engineers (PECASE)
2015 NARSAD Independent Investigator Award
2015 Young Investigator Award, International Cannabinoid Research Society
2013 James G. Blakemore Chair in Psychiatry Research, Vanderbilt University Medical Center
2016 Member, Molecular Neuropharmacology and Signaling SRG, CSR/NIH
2017 Vice chair, Cannabinoids in the CNS Gordon research Conference
2018 Simons Foundation Autism Research Initiative (SFARI) Explorer Award
2018 Member, American Society for Clinical Investigation
2021 Co-Chair, Cannabinoids in the CNS Gordon research Conference

B. Contributions to Science

Defining eCB signaling as a key regulator of stress response physiology: Our group provided the first experimental data supporting a clear role of endocannabinoids in the regulation of anxiety-related behaviors and central stress responses. We were the first to posit endocannabinoid signaling as a mechanism subserving stress habituation and resiliency to stress-induced psychopathology and have led the field in the investigation of the role of endocannabinoid signaling in stress response physiology at the neurochemical, behavioral and synaptic level. Most recently, we provided compelling genetic evidence that 2-AG deficiency is causally related to the expression of pathological anxiety and depressive behaviors and regulates susceptibility to psychopathology after stress exposure. We also provided the first evidence that

pharmacological augmentation of 2-AG signaling prevents stress-induced behavioral pathology and promote resilience to adverse effects of stress and the role of PFC-BLA eCB signaling collapse in the translation of stress to anxiety-like behavior. In summary, we are on the forefront of basic-translational research on the role of 2-AG signaling in stress adaptation and development and validation of 2-AG-based pharmacological treatment approaches for stress-related neuropsychiatric disorders.

- a. Marcus DJ, Bedse G, Gaulden AD, Ryan JD, Kondev V, Winters ND, Rosas-Vidal LE, Altemus M, Mackie K, Lee FS, Delpire E, **Patel S.** (2019), Endocannabinoid Signaling Collapse Mediates Stress-Induced Amygdalo-Cortical Strengthening. *Neuron* 105(6): 1062-1076. PMID [319848734](#).
- b. Bluett, R.J., Baldi, R., Haymer, A., Hartley, N.D., Marcus, D., Mardam-Bey, R., Shonesy, B.C., Uddin, J., Marnett, L.J., Colbran, R.J., Winder D.G., **Patel, S.** (2017), Endocannabinoid Signaling Modulates Susceptibility to Traumatic Stress Exposure. *Nature Communications*, Mar 28;8:14782. PMCID [28348378](#)
- c. Bedse, G., Hartley, N.D., Neale, E., Gaulden, A.J., Patrick, T., Kingsley, P.J., Md. Uddin, J., Plath, N., Marnett, L.J., and **Patel, S.**, (2017), Functional Redundancy Between Canonical Endocannabinoid Signaling Systems in the Modulation of Anxiety. *Biological Psychiatry*, 82(7):488-499. PMCID: [PMC5585044](#)
- d. Shonesy BC, Bluett RJ, Ramikie TS, Báldi R, Hermanson DJ, Kingsley PJ, Marnett LJ, Winder DG, Colbran RJ, **Patel S.** Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. *Cell Reports*, 2014 Dec 11;9(5):1644-53. PubMed PMID: [25466252](#); PubMed Central PMCID: [PMC4268380](#).

Elucidating COX-2 as a novel eCB metabolic pathway with therapeutic potential: In collaboration with Dr. Marnett, our groups were the first to validate COX-2-mediated metabolism as a novel endocannabinoid inactivation pathway in the brain. In addition, we have validated a novel class of "substrate-Selective COX-2 inhibitors" or "SSCIs", which are able to increase brain endocannabinoid levels via COX-2 inhibition without inhibition of canonical prostaglandin synthase functions of COX-2. Moreover, we have initial data validating the utility of SSCIs in preclinical models of mood and anxiety disorders. Together, our recent work in this area suggests that COX-2 is a third endocannabinoid inactivation pathway in the CNS, and that substrate-selective COX-2 inhibition increased central endocannabinoid signaling and subsequent activity at a variety of target receptors. Our work has opened completely new area of endocannabinoid biology based on COX-2-mediated oxidation metabolism and has revealed COX-2 as a novel molecular target for the development of endocannabinoid-based therapeutics for a potentially broad range of CNS-related disorders.

- a. Hermanson DJ, Gamble-George JC, Marnett LJ, **Patel S.** Substrate-selective COX-2 inhibition as a novel strategy for therapeutic endocannabinoid augmentation. *Trends Pharmacol Sci*. 2014 Jul;35(7):358-67. PubMed PMID: [24845457](#); PubMed Central PMCID: [PMC4074568](#).
- b. Gamble-George, J.C., Halladay, L., Báldi, R., Kocharian, A., Silva, C., Roberts, H., Haymer, A., Marnett, L.J., Holmes, A., **Patel, S.** Cyclooxygenase-2 inhibition reduces stress-induced affective pathology. *eLife*, 2016, 10;5 e15137. PubMed PMID [27162170](#); PubMed Central PMCID: [PMC4862754](#).
- e. Morgan, A., Kondev, V., Bedse, G., Baldi, R., Marcus, D., **Patel, S.**, (2019), Cyclooxygenase-2 inhibition reduces anxiety-like behavior and normalizes enhanced amygdala glutamatergic transmission following chronic oral corticosterone treatment. *Neurobiology of Stress* 10(11):100190. PMCID: [PMC6710599](#).
- f. Morgan, A., Gaulden, A., Altemus, M., Williford, K., Centanni, S., Winder, D.G, and **Patel, S.** (2020) Cyclooxygenase-2 inhibition prevents stress-induced amygdala activation and anxiety-like behavior. *Brain, Behavior and Immunity* 89:513-517. PMCID: [PMC7572634](#)

Mechanisms mediating stress effects on eCB synaptic signaling: A third component of our research focuses on understanding the role of endocannabinoid signaling at the synaptic level globally, with specific interest in stress-related brain regions such as the basolateral and central amygdala, and in the adaptations in synaptic endocannabinoid signaling associated with stress exposure and models of neuropsychiatric

disorders. Our recent work in this area has focused on elucidating the stress-adaptations in short and long-term endocannabinoid plasticity at basolateral GABAergic synapses, and in the cellular mechanisms regulating endocannabiboid signaling at central amygdala glutamate synapses. Ongoing work is aimed at 1) elucidating the synaptic mechanisms by which cannabinoids regulate central amygdala CRF signaling relevant to cannabis and alcohol withdrawal, and 2) defining the cellular and synaptic mechanisms of action of endocannabinoid-based therapeutics such as SSCIs and FAAH inhibitors.

- a. **Patel S***, Kingsley PJ, Mackie K, Marnett LJ, Winder DG. Repeated homotypic stress elevates 2-arachidonoylglycerol levels and enhances short-term endocannabinoid signaling at inhibitory synapses in basolateral amygdala. *Neuropsychopharmacology*. 2009 Dec;34(13):2699-709. PubMed PMID: [19675536](#); PubMed Central PMCID: [PMC2881681](#).
- b. Sumislawski JJ, Ramikie TS, **Patel S***. Reversible gating of endocannabinoid plasticity in the amygdala by chronic stress: a potential role for monoacylglycerol lipase inhibition in the prevention of stress-induced behavioral adaptation. *Neuropsychopharmacology*. 2011 Dec;36(13):2750-61. PubMed PMID: [21849983](#); PubMed Central PMCID: [PMC3230498](#).
- c. Shonesy BC, Wang X, Rose KL, Ramikie TS, Cavener VS, Rentz T, Baucum AJ 2nd, Jalan-Sakrikar N, Mackie K, Winder DG, **Patel S***, Colbran RJ*. CaMKII regulates diacylglycerol lipase- α and striatal endocannabinoid signaling. *Nat Neurosci*. 2013 Apr;16(4):456-63. PubMed PMID: [23502535](#); PubMed Central PMCID: [PMC3636998](#). (*co-senior authorship)
- b. Ramikie TS, Nyilas R, Bluett RJ, Gamble-George JC, Hartley ND, Mackie K, Watanabe M, Katona I, **Patel S**. Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. *Neuron*. 2014 Mar 5;81(5):1111-25. PubMed PMID: [24607231](#); PubMed Central PMCID: [PMC3955008](#).

h-index 50, i10-index 71, RCR mean 3.0 median 2.48; total citations 8173

Complete List of Published Work in My Bibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/sachin.patel.1/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: Porreca, Frank

eRA COMMONS USER NAME (credential, e.g., agency login): FRANKP

POSITION TITLE: Professor of Pharmacology

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Villanova University, Villanova, Pennsylvania	BS	05/1975	General Science Honors
Drexel University, Philadelphia, Pennsylvania	MS	06/1979	Biomedical Engineering
Temple University School of Medicine, Philadelphia, Pennsylvania	PHD	05/1982	Pharmacology
University of Arizona, Tucson, Arizona	Postdoctoral	06/1984	Pharmacology

A. Personal Statement

Several themes have guided my research including (a) the role of descending modulatory circuits in pain and headache; (b) the intersection between pain and reward pathways; (c) the mechanisms by which opioids and stress engage descending modulatory circuits and (d) the discovery of molecules that can act within these circuits to provide initial research tools for mechanistic evaluation and possible advancement to development as therapeutics. Our studies have focused on the motivational aspects of pain and the reward of pain relief that engage the classical mesocorticolimbic reward circuit (i.e., basal ganglia circuit). In addition, we have demonstrated that pain and stress activate "anti-reward" and pronociceptive pathways in the amygdala, in part, through the activity of dynorphin at kappa opioid receptors (KOR)(extended amygdala circuit). This work has collectively served as the basis for the development of a novel KOR antagonist that has now advanced to phase II clinical trials and has been the basis of the NIDA Center of Excellence for Addiction Studies (CEAS) that is aimed at discovery of novel non-addictive pain medications. We have also studied the cognitive deficits induced by chronic pain by focusing on adaptive changes in the frontal cortex and influences on the nucleus accumbens (cortico-striatal circuit). Our studies have also explored mechanisms that may preferentially promote pain in a sexually dimorphic fashion and our work has uncovered a link between stress-activation of kappa opioid receptor expressing cells in the hypothalamus, dysregulation of pituitary prolactin and female-selective sensitization of nociceptors that may help to explain the increased prevalence of pain in women especially in functional pain conditions such as migraine. These studies are a part of investigation of mechanisms of headache pain and have been recently extended to studies of post-traumatic headache.

My research program is nationally and internationally recognized and has been continuously funded by the NIH. I have had a successful record in mentoring junior basic science and clinical faculty and have served as the mentor for doctoral students and post-doctoral fellows. I have directed multiple neuroscience, pain and addiction-related programs at the University including the serving as the Scientific Director of the Comprehensive Pain and Addiction Center and the Director of the Center for Excellence in Addiction Studies.

- a. Navratilova, E., Xie, J.Y., Meske, D., Qu, C., Morimura, K., Okun, A., Arakawa, N., Ossipov, M., Fields, H.L., **Porreca, F.** Endogenous opioid activity in the anterior cingulate cortex is required for relief of pain. *J Neurosci.* 2015 May 6; 35(18):7264-71. PMCID: PMC4420787
- b. Chen Y, Moutal A, Navratilova E, Kopruszinski C, Yue X, Ikegami M, Chow M, Kanazawa I, Bellampalli SS, Xie J, Patwardhan A, Rice K, Fields H, Akopian A, Neugebauer V, Dodick D, Khanna R, **Porreca F.** The prolactin receptor long isoform regulates nociceptor sensitization and opioid-induced

- hyperalgesia selectively in females. *Sci Transl Med*. 2020 Feb 5;12(529). PubMed PMID: 32024801; PubMed Central PMCID: PMC7523341.
- c. Navratilova, E., Ji, G., Phelps, C., Qu, C., Hein, M., Yakhnitsa, V., Neugebauer, V. **Porreca, F.** Kappa opioid signaling in the central nucleus of the amygdala promotes disinhibition and aversiveness of chronic neuropathic pain. *Pain*. 2019 Apr; 160(4):824-832. PMCID: PMC6424634
 - d. Navratilova, E., Xie, J.Y., Okun, A., Qu, C., Eyde, N., Ci, S., Ossipov, M.H., King, T., Fields, H.L., **Porreca, F.** Pain relief produces negative reinforcement through activation of mesolimbic reward-valuation circuitry. *Proc Natl Acad Sci U S A*. 2012 Dec 11;109(50):20709-13. PMCID: PMC3528534

Ongoing and recently completed projects that I would like to highlight include:

R01 NS114888

Porreca/Anderson/Navratilova (MPI)

07/15/2020 – 06/30/2025

Mechanisms and Therapeutic Strategies for Post-traumatic Headache (PTH)

R01 NS109255

Navratilova/Ji, Role: Co-I

08/15/2019 – 06/30/2024

Pronociceptive and Antinociceptive Opioid Mechanisms in the Central Nucleus of the Amygdala

CDMRP-PR180415

Schwedt/Porreca (MPI)

09/01/2019 - 08/31/2023

A Multidisciplinary Translational Approach to Investigate the Mechanisms, Predictors, and Prevention of Persistent Post-Traumatic Headache

R01 NS106902

Porreca/Neugebauer (MPI)

04/01/2018 – 03/31/2023

Stress-Induced Descending Facilitation from Amygdala Kappa Opioid Receptors in Functional Pain

P01 DA041307

Porreca (PI)

05/01/2017 – 04/30/2023 (NCE)

New Modalities for the Treatment of Pain and Drug Abuse

R01NS120395

Porreca; Navratilova; Neugebauer (MPI)

07/01/2021-06/30/2026

A Prolactin-Mediated neuroendocrine Link between Stress-Induced Latent Sensitization and Female-Selective Pain

W81XWH-21-1-0569

Porreca (PI)

08/15/2021-07/31-2024

Preclinical evaluation of Mechanisms and Therapies for Persistent Post-traumatic Headache

P30DA051355

Porreca (PI)

08/15/2021-07/31/2026

The Center of Excellence in Addiction Studies

R43NS124466

Riviere/Porreca (MPI)

07/01/2021 – 12/31/2022

Long acting and peripherally restricted kappa-opioid receptor agonists for acute migraine treatment

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022 -Present	Cosden Professor of Pain and Addiction Studies
2012 - Present	Senior Consultant, Mayo Clinic, Scottsdale, AZ
2006 - 2010	Director, Theme for Medical Neuroscience, University of Arizona, Tucson, AZ
2005 - Present	Member, Arizona Cancer Center, University of Arizona, Tucson, AZ
2005 - Present	Associate Department Head, Department of Pharmacology, University of Arizona, Tucson, AZ
1995 - Present	Professor of Pharmacology and Anesthesiology, University of Arizona, Tucson, AZ
1991 - Present	Professor of Pharmacology, University of Arizona, Tucson, AZ
1988 - 1991	Associate Professor of Pharmacology, University of Arizona, Tucson, AZ
1985 - 1988	Assistant Professor of Pharmacology, University of Arizona College of Medicine, Tucson, AZ
<u>Mentor</u>	Junior faculty (5 all of whom obtained independent NIH funding), post-doctoral fellows (35 almost all are pursuing independent careers in academia or industry), graduate students (30 Ph.D., 8 M.S. students), medical students (3) and undergraduate students (10)
<u>Reviewer</u>	For many scientific journals including Science, Nature, Nature Neuroscience, Science Translational Medicine, Brain, Annals of Neurology, PNAS and others
<u>Editorial Boards</u>	Including past Editor of Life Sciences, Associate Editor (Pharmacology) of Pain
<u>Grant Reviewer</u>	For multiple NIH panels including the former Somatosensory and Chemosensory as well as many ad hoc sessions as well as for the Department of Defense and European agencies
<u>Professional memberships</u>	Society for Neuroscience, Member, International Association for the Study of Pain USASP (formerly American Pain Society), American Society for Pharmacology and Experimental Therapeutics

Honors (selected)

2022	Cosden Endowed Professorship
2000	F. W. Kerr Award, American Pain Society
2017	John J. Bonica Award, Eastern Pain Association
2017	Mary Ellen Jeans Award, Canadian Pain Society
2017	P.D. Wall Award, British Pain Society
2016	Ronald Melzack Award, International Association for the Study of Pain
2015	Fellow, American Headache Society
2014	Sunderland Award, Australian Pain Society
	Invited speaker at >300 International scientific meetings or institutions. Scientific organizer of the Pain Mechanisms and Therapeutics Conference (formerly Spring Pain Conference)
2001	Fellow, American Association for the Advancement of Science

C. Contribution to Science

1. Kappa opioid receptor and negative affect of pain: The human experience of pain is woven from the integration of sensory and affective dimensions. Little is understood about the mechanisms that promote the negative affect, and motivational consequences of pain. We have used preclinical models to demonstrate that chronic pain-induced kappa opioid receptor (KOR) signaling drives the aversive qualities of pain and the motivation to seek relief. We have found that KOR signaling in the amygdala and anterior cingulate cortex promotes pain aversiveness and that KOR-expressing circuits oppose the consequences of activation of mu opioid receptors in these brain regions.
 - a. Navratilova, E., Ji, G., Phelps, C., Qu, C., Hein, M., Yakhnitsa, V., Neugebauer, V. **Porreca, F.** Kappa opioid signaling in the central nucleus of the amygdala promotes disinhibition and aversiveness of chronic neuropathic pain. *Pain*. 2019 Apr; 160(4):824-832. PMCID: PMC6424634

- b. Nation, K.M., De Felice, M., Hernandez, P.I., Dodick, D.W., Neugebauer, V., Navratilova, E., **Porreca, F.** Lateralized kappa opioid receptor signaling from the amygdala central nucleus promotes stress-induced functional pain. *Pain*. 2018 May; 159(5):919-928. PMCID: PMC5916844
 - c. Navratilova, E., Nation, K., Remeniuk, B., Neugebauer, V., Bannister, K., Dickenson, A.H., **Porreca, F.** Selective modulation of tonic aversive qualities of neuropathic pain by morphine in the central nucleus of the amygdala requires endogenous opioid signaling in the anterior cingulate cortex. *Pain*, 2020(Mar);161(3):609-618.PMCID: PMC7124010
 - d. De Felice M, Eyde N, Dodick D, Dussor GO, Ossipov MH, Fields HL, **Porreca F.** Capturing the aversive state of cephalic pain preclinically. *Ann Neurol*. 2013 Aug;74(2):257-65. PMCID: PMC3830648.
2. Reward motivation and pain: Measurement of pain in preclinical models has historically relied on the use of reflexive withdrawal responses to an external stimulus. In contrast, evaluation of pain in humans relied on self-report of negative pain affect that provides motivation to seek relief. We have used this principal to establish a learning assay in animals that demonstrates that the relief of ongoing pain produces reward learning. This approach can be viewed as the animals self report, analogous to human self report. Additionally, this approach can reveal that potential pharmacological pain relieving treatments are meaningful in modulating pain aversiveness in the animal providing a basis for validation of potential pain therapies.
- a. Navratilova, E., **Porreca, F.** Reward and motivation in pain and pain relief. *Nat Neurosci*. 2014 Oct;17(10):1304-12. PMCID: PMC4301417
 - b. Navratilova, E., Xie, J.Y., Okun, A., Qu, C., Eyde, N., Ci, S., Ossipov, M.H., King, T., Fields, H.L., **Porreca, F.** Pain relief produces negative reinforcement through activation of mesolimbic reward-valuation circuitry. *Proc Natl Acad Sci U S A*. 2012 Dec 11;109(50):20709-13. PMCID: PMC3528534
 - c. King, T., Vera-Portocarrero, L., Gutierrez, T., Vanderah, T.W., Dussor, G., Lai, J., Fields, H.L., **Porreca, F.** Unmasking the tonic-aversive state in neuropathic pain. *Nat Neurosci*. 2009 Nov;12(11):1364-6. PMCID: PMC3427725
 - b. Okun, A., McKinzie, D.L., Witkin, J.M., Remeniuk, B., Husein, O., Gleason, S.D., Oyarzo, J., Navratilova, E., McElroy, B., Cowen, S., Kennedy, J.D., **Porreca, F.** Hedonic and motivational responses to food reward are unchanged in rats with neuropathic pain. *Pain*. 2016 Dec;157(12):2731-2738. PMCID: PMC5108682
4. Cortical circuits and pain: The aversive qualities of pain are largely integrated in the anterior cingulate cortex (ACC). Our studies have explored mu (MOR) and kappa (KOR) opioid receptor expressing cells in the ACC and have demonstrated that activation of the MOR is associated with pain relief likely through output projections to other brain areas. In contrast, KOR activation is associated with increased pain mediated primarily through local circuits. Disruption of these circuits by chronic pain is also associated with cognitive consequences of chronic pain.
- a. Navratilova, E., Xie, J.Y., Meske, D., Qu, C., Morimura, K., Okun, A., Arakawa, N., Ossipov, M., Fields, H.L., **Porreca, F.** Endogenous opioid activity in the anterior cingulate cortex is required for relief of pain. *J Neurosci*. 2015 May 6; 35(18):7264-71. PMCID: PMC4420787
 - b. Gomtsian, L., Bannister, K., Eyde, N., Robles, D., Dickenson, A.H., **Porreca, F.**, Navratilova, E. Morphine effects within the rodent anterior cingulate cortex and rostral ventromedial medulla reveal separable modulation of affective and sensory qualities of acute or chronic pain. *Pain* 2018; 159: 2512-2521. PMCID: PMC6320264
 - c. Cowen, S.L., Phelps, C.E., Navratilova, E., McKinzie, D.L., Okun, A., Husain, O., Gleason, S.D., Witkin, J.M., **Porreca, F.** Chronic pain impairs cognitive flexibility and engages novel learning strategies in rats. *Pain* 2018; 159(7): 1403-1412. PMCID: PMC6008204
 - d. Phelps, C.E., Navratilova, E., **Porreca, F.** Chronic Pain Produces Reversible Memory Deficits That Depend on Task Difficulty in Rats. *J Pain*, 20:S1526-5900, 2021(May). PMCID: PMC8578143
5. Sexually-dimorphic mechanisms of opioid induced hyperalgesia and pain: For reasons that are not well understood, women represent the great majority of the worlds' pain patients. Many of the pain conditions with high female prevalence are not associated with obvious tissue pathology and are therefore classified as "functional". In these conditions, the threshold for nociceptor activation may be altered so that pain can be

elicited by normally innocuous stimuli including stress. Our work has focused on sexually dimorphic mechanisms of nociceptor sensitization by prolactin. We showed that sensitization of nociceptors by prolactin is profoundly female selective and can occur in injury free conditions including opioid-induced hyperalgesia.

- a. Chen Y, Moutal A, Navratilova E, Kopruszinski C, Yue X, Ikegami M, Chow M, Kanazawa I, Bellampalli SS, Xie J, Patwardhan A, Rice K, Fields H, Akopian A, Neugebauer V, Dodick D, Khanna R, **Porreca F**. The prolactin receptor long isoform regulates nociceptor sensitization and opioid-induced hyperalgesia selectively in females. *Sci Transl Med*. 2020 Feb 5;12(529). PubMed PMID: 32024801; PubMed Central PMCID: PMC7523341.
- b. Chen, Y., Navratilova, E., Dodick, D. W., **Porreca, F.** An Emerging Role for Prolactin in Female-Selective Pain. *Trends Neurosci.*, 2020 (Aug);43(8):635-648. PMID: 32620290
- c. Ikegami, D., Navratilova, E., Yus, X., Moutal, A., Kopruszinski, C.M., Khanna, R., Patwardhan, A., Dodick, D.W. and **Porreca, F.** , A prolactin dependent sexually dimorphic mechanism of migraine chronification. *Cephalgia* 2021(Sep) Epub ahead of print. PMID: 34510920
- d. Navratilova, E., Fillingim, R. and Porreca, F., Sexual dimorphism in functional pain syndromes, *Sci Transl Med*. 2021Nov;13(619) PMID:34757805

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/1pY6CePo1TBQ6/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: Salvemini, Daniela

ERA COMMONS USER NAME (credential, e.g., agency login): SALVEMD

POSITION TITLE: Professor of Pharmacological and Physiological Science

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Kings College, London, UK	BS	05/1987	Pharmacology
University of London, UK	PHD	05/1990	Pharmacology
William Harvey Research Institute, London, UK	Postdoctoral Fellow	05/1992	Pharmacology
Monsanto Discovery Research, St Louis, USA	Postdoctoral Fellow	05/1994	Pharmacology

A. Personal Statement I am an *in vivo* pharmacologist and a recognized leader in translational pain research. I am interested in understanding the molecular mechanisms involved in neuropathic pain and the discovery of novel non-opioid based targets for therapeutic interventions. Work in my lab combines approaches from behavioral pharmacology, drug discovery and development, biochemistry, molecular biology, genetics and toxicology. Before joining SLU, I spent 15 years in the private sector where I led drug discovery efforts on novel anti-inflammatory agents and analgesics that contributed to the advancement of several therapeutics into clinical trials. I have strong leadership skills, the ability to multitask, a track record of successful collaborations and continuous productivity and a clear appreciation and expertise to execute our goals within the proposed timeline and budget constraints.

Ongoing projects:

RO1NS128004

7/1/2022-6/30/2027

Salvemini (contact) and Arnatt (MPI)

Uncovering the roles of oxysterols in neuropathic pain

DOD W81XWH-21-1-0486

6/1/2021-5/30/2025

Salvemini and Ackerman (contact) (MPI)

Sphingosine-1-Phosphate Receptor Subtype 1: A Novel Target in the Treatment of Chronic Migraine

RO1NS113257

Salvemini (contact) and Yosten (MPI)

7/01/2019-6/30/2024

Discovery and validation of a novel orphan GPCR as a target for therapeutic intervention in neuropathic pain

RO1CA230512

Salvemini (contact) and Heijnen (MPI)

8/09/2018-7/31/2023

A3AR agonists as a novel approach to mitigate chemotherapy-induced neurotoxicity

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022 - present	Professor, Psychiatry and Behavioral Neuroscience (secondary appointment)
2021 - present	William Beaumont Professor and Chair, Department of Pharmacology and Physiology, Saint Louis University, St. Louis, MO
2019 - 2021	Interim Chair, Department of Pharmacology and Physiology, Saint Louis University, St. Louis, MO
2018 - present	Director, Henry and Amelia Nasrallah Center for Neuroscience, Saint Louis University, St. Louis, MO
2015 - present	Vice Chair of Research, Department of Pharmacology and Physiology, Saint Louis University, St. Louis, MO
2014 - present	Founder and Chief Scientific Advisor, BioIntervene, A SLU-based venture backed start-up company committed to the clinical development of small molecule A3AR agonists.
2012 - present	Professor of Internal Medicine, Department of Pulmonary, Critical Care and Sleep Medicine (secondary), Saint Louis University, St. Louis, MO
2012 - present	Adjunct Professor, Pharmaceutical Sciences, SIUE, Edwardsville, IL
2012 - present	Professor of Pharmacology and Physiology, Saint Louis University, St. Louis, MO
2009 - 2012	Associate Professor, Department of Pharmacology and Physiology, Saint Louis University, St. Louis, MO
2005 - 2009	Research Professor of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, Saint Louis University, St. Louis, MO
2005 - present	Visiting Professor, University of Florence, Florence
2003 - 2005	Vice President of Research, Metaphore Pharmaceuticals, St. Louis, MO
2001 - 2003	Vice President of Biological and Pharmacological Research, Metaphore Pharmaceuticals, St. Louis, MO
2000 - present	Adjunct Professor of Molecular Pharmacology, Faculty of Pharmacy, Catanzaro
1999 - 2001	Director of Biology, Metaphore Pharmaceuticals, St. Louis, MO
1997 - 1999	Research Scientist II and Project Leader, G.D Searle (Pfizer), St. Louis, MO
1996 - 2009	Adjunct Professor, Department of Pharmacological and Physiological Science, Saint Louis University, St. Louis, MO
1995 - 1997	Research Scientist I, G.D Searle (Pfizer), St. Louis, MO
1994 - 1995	Senior Research Investigator, G.D. Searle (Pfizer), St. Louis, MO

Honors

2022	h index=80, 24,000 citations (Google Scholar)
2022	Saint Louis University Faculty Scholarly Works Winner
2020	Fellow, National Academy of Inventors
2020	Pharmacia-ASPET Award in Experimental Therapeutics
2020	Saint Louis University Faculty Innovation Award
2019	Saint Louis University Senior Faculty Grant winner
2018	Saint Louis University School of Medicine Distinguished Faculty Award in Research
2018	Member, External Consultant Board, Preclinical Screening Platform for PAIN (PSPP; NINDS)
2016	Elected Faculty Member, Hope Center for Neurological Disorders, Washington University School of Medicine, Saint Louis
2014	Fellows Award- Outstanding Scientist Award, Saint Louis Academy of Science
2010	Premio Internazionale Maria Luisa de'Medici Award for excellence in research, Italy
2002	Society of Experimental Biology and Medicine Award for research in free radical biology, FRBM
2000	Magna Graecia Prize for contribution of the scientific advancement of research in Southern Italy, University of Catanzaro, Italy
1997	Novartis Prize in Pharmacology for research on free radicals, nitric oxide and cyclooxygenases in inflammation. British Pharmacological Society

- 1997 Searle Discovery Research Achievement Award for achievements and leadership in the nitric oxide and superoxide dismutase project, G.D. Searle (Pfizer)
- 1996 Searle Discovery Research Achievement Award for achievements and leadership in the superoxide dismutase project, G.D. Searle (Pfizer)
- 1991 William Julius Mickle Fellowship for "An investigator under 35 years of age, who has in the opinion of the Committee, done most to advance medical art or science within the preceding five years", University of London
- 1989 Young Investigator Award for the excellence and originality of the work presented on the interactions between nitric oxide, platelets and vascular smooth muscle cells, The British Pharmacological Society of Thrombosis and Haemostasis

Other Experience and Professional Memberships

Editorial Board Experience

2021-present ACS Pharmacology and Translational Science

2018-present American Journal of Physiology

Reviewer (*Ad hoc*): Nature, Journal of Neuroscience, PAIN, Neuroscience, Molecular Pain, J. Biol. Chemistry, Journal of Pharmacology and Experimental Therapeutics, Proc. Natl. Acad. Sci. USA, Cannabis and Cannabinoid Research, Free Radical Biology and Medicine, Critical Care Medicine, Molecular and Cell Biology of Lipids, Journal of Cellular and Molecular Medicine, American Journal of Physiology, Pharmacological Research, British Journal of Pharmacology, European Journal of Pharmacology, Inflammation Research, American Journal of Physiology, Brain Behavior and Immunity, Experimental Neurology, Acta Neuropathology, J Neurochemistry.

Study sections

Ad Hoc Reviewer for NIH Study Section (NIDA; NCI; NINDS; NHLBI) and DOD.

Ad Hoc Reviewer for various foundations (i.e., Mayday Fund).

Professional Societies

ASPET; USAPS; AAAS; Society for Neuroscience; ANC; Hope Center Washington University St Louis; Institute of Clinical and Translational Sciences at Washington University; Peripheral Neurotoxic Society

C. Contributions to Science of >200 peer reviewed papers, review articles and book chapters

1. Results from my early studies led to the discovery that nitric oxide (NO) signaling in pathophysiological settings is mediated to a large extent to activation of cyclooxygenase (COX) enzymes. This work was the first to establish the concept that COX enzymes are important endogenous "receptor" targets for NO and provided the mechanistic understanding for the cardiovascular and anti-inflammatory/analgesic actions of NO-based therapeutics. These findings moved the field beyond the original concept that soluble guanylate cyclase is the sole receptor target for NO's effects, thus opening a new field of investigation.
 - a. **Salvemini D**, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P. Nitric oxide activates cyclooxygenase enzymes. *Proc Natl Acad Sci U S A*. 1993 Aug 1;90(15):7240-4. PubMed PMID: [7688473](#); PubMed Central PMCID: [PMC47112](#).
 - b. **Salvemini D**, Seibert K, Masferrer JL, Misko TP, Currie MG, Needleman P. Endogenous nitric oxide enhances prostaglandin production in a model of renal inflammation. *J Clin Invest*. 1994 May; 93(5):1940-7. PubMed PMID: [7514189](#); PubMed Central PMCID: [PMC294301](#).
 - c. **Salvemini D**, Manning PT, Zweifel BS, Seibert K, Connor J, Currie MG, Needleman P, Masferrer JL. Dual inhibition of nitric oxide and prostaglandin production contributes to the antiinflammatory properties of nitric oxide synthase inhibitors. *J Clin Invest*. 1995 Jul; 96(1):301-8. PubMed PMID: [7542281](#); PubMed Central PMCID: [PMC185201](#).
 - d. **Salvemini D**, Currie MG, Mollace V. Nitric oxide-mediated cyclooxygenase activation. A key event in the antiplatelet effects of nitrovasodilators. *J Clin Invest*. 1996 Jun 1; 97(11):2562-8. PubMed PMID: [8647949](#); PubMed Central PMCID: [PMC507342](#).
2. My studies significantly contributed to the identification of small molecule synthetic enzymes that catalytically decompose superoxide (SO) and its bioactive mediator, peroxynitrite (PN). We used these agents as pharmacological probes to precisely dissect the role(s) of SO/PN in disease states. Results provided critical insight on how these nitroxidative species impact the course of inflammation by modulating for example enzymes (i.e. MnSOD, COX2), transcription factors (i.e., NFkB) and MAPKs (i.e., p38 kinase). These results

provided the pharmacological basis that supported the advancement of such synthetic enzymes in clinical trials.

- a. **Salvemini D**, Wang ZQ, Stern MK, Currie MG, Misko TP. Peroxynitrite decomposition catalysts: therapeutics for peroxynitrite-mediated pathology. *Proc Natl Acad Sci U S A*. 1998 Mar 3; 95(5):2659-63. PubMed PMID: [9482943](#); PubMed Central PMCID: [PMC19452](#).
 - b. **Salvemini D**, Wang ZQ, Zweier JL, Samoilov A, Macarthur H, Misko TP, Currie MG, Cuzzocrea S, Sikorski JA, Riley DP. A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. *Science*. 1999 Oct 8; 286(5438):304-6. PubMed PMID: [10514375](#).
 - c. Macarthur H, Westfall TC, Riley DP, Misko TP, **Salvemini D**. Inactivation of catecholamines by superoxide gives new insights on the pathogenesis of septic shock. *Proc Natl Acad Sci U S A*. 2000 Aug 15; 97(17):9753-8. PubMed PMID: [10944234](#); PubMed Central PMCID: [PMC16937](#).
 - d. Samlowski WE, Petersen R, Cuzzocrea S, Macarthur H, Burton D, McGregor JR, **Salvemini D**. A nonpeptidyl mimic of superoxide dismutase, M40403, inhibits dose-limiting hypotension associated with interleukin-2 and increases its antitumor effects. *Nat Med*. 2003 Jun; 9(6):750-5. PubMed PMID: [12730689](#).
3. I have contributed to the understanding of the roles of superoxide and peroxynitrite in 1) neuropathic pain states, 2) side effects exerted by opioids for the treatment of neuropathic pain states, and 3) the development of chemotherapy-induced neurotoxicity's. We identified sites of action, unraveled enzymatic pathways leading to the overt production of these species and defined molecular signaling pathways. These studies established the role of nitroxidative stress in these areas, opened a new field of investigation and provided the impetus for therapeutic development of drugs that propelled clinical development of inhibitors of nitroxidative stress for pain and opioid adjuncts.
- a. Ndengele MM, Cuzzocrea S, Masini E, Vinci MC, Esposito E, Muscoli C, Petrusca DN, Mollace V, Mazzon E, Li D, Petrache I, Matuschak GM, **Salvemini D**. Spinal ceramide modulates the development of morphine antinociceptive tolerance via peroxynitrite-mediated nitroxidative stress and neuroimmune activation. *J PET* 2009 Apr; 329(1):64-75. PubMed PMID: [19033555](#); PubMed Central PMCID: [PMC2670603](#).
 - b. Doyle T, Chen Z, Muscoli C, Bryant L, Esposito E, Cuzzocrea S, Dagostino C, Ryerse J, Rausaria S, Kamadulski A, Neumann WL, **Salvemini D**. Targeting the overproduction of peroxynitrite for the prevention and reversal of paclitaxel-induced neuropathic pain. *J Neurosci*. 2012 May 2; 32(18):6149-60. PubMed PMID: [22553021](#); PubMed Central PMCID: [PMC3752044](#).
 - c. Little JW, Chen Z, Doyle T, Porreca F, Ghaffari M, Bryant L, Neumann WL, **Salvemini D**. Supraspinal peroxynitrite modulates pain signaling by suppressing the endogenous opioid pathway. *J Neurosci*. 2012 Aug 8; 32(32):10797-808. PubMed PMID: [22875915](#); PubMed Central PMCID: [PMC3511865](#).
 - d. Little JW, Cuzzocrea S, Bryant L, Esposito E, Doyle T, Rausaria S, Neumann WL, **Salvemini D**. Spinal mitochondrial-derived peroxynitrite enhances neuroimmune activation during morphine hyperalgesia and antinociceptive tolerance. *Pain*. 2013 Jul; 154(7):978-86. PubMed PMID: [23590939](#); PubMed Central PMCID: [PMC4874243](#).
4. I have contributed to the understanding of the roles of the sphingolipid S1P and its receptor subtype (S1PR1) in 1) neuropathic pain states, 2) side effects exerted by opioids for the treatment of neuropathic pain states, and 3) the development of chemotherapy-induced neurotoxicity's. Our studies identified S1PR1 as a novel target for therapeutic intervention in these areas with S1PR1 antagonists, established molecular signaling pathways engaged downstream of S1PR1, identified the cellular locus of S1PR1 activity and provided the rationale to initiate clinical trials for proof of concept.
- a. Janes K, Little JW, Li C, Bryant L, Chen C, Chen Z, Kamocki K, Doyle T, Snider A, Esposito E, Cuzzocrea S, Bieberich E, Obeid L, Petrache I, Nicol G, Neumann WL, **Salvemini D**. The development and maintenance of paclitaxel-induced neuropathic pain require activation of the sphingosine 1-phosphate receptor subtype 1. *J Biol Chem*. 2014 Jul 25; 289(30):21082-97. PubMed PMID: [24876379](#); PubMed Central PMCID: [PMC4110312](#).
 - b. Stockstill K, Doyle TM, Yan X, Chen Z, Janes K, Little JW, Braden K, Lauro F, Giancotti LA, Harada CM, Yadav R, Xiao WH, Lionberger JM, Neumann WL, Bennett GJ, Weng HR, Spiegel S and **Salvemini, D**. Dysregulation of sphingolipid metabolism contributes to bortezomib-induced neuropathic pain. *J Exp Med*. 2018 May 7; 215(5):1301-13. PubMed PMID: [29703731](#); PubMed Central PMCID: [PMC5940258](#)

- c. Grenald SA, Doyle TM, Zhang H, Slosky LM, Chen Z, Largent-Milnes TM, Spiegel S, Vanderah TW, **Salvemini D**. Targeting the S1P/S1PR1 axis mitigates cancer-induced bone pain and neuroinflammation, *Pain*. 2017; 158(9):1733-1742. PMID:28570482
 - d. Cuzzocrea S, Doyle T, Campolo M, Paterniti I, Esposito E, Farr SA, **Salvemini D**. Sphingosine 1-Phosphate Receptor Subtype 1 as a Therapeutic Target for Brain Trauma. *J Neurotrauma*. 2018 Jul 1;35:1452-1466.
 - e. S. Squillace, M.L. Niehoff, T.M. Doyle, M. Green, E. Esposito, S. Cuzzocrea, C.K. Arnatt, S. Spiegel, S.A. Farr, and **D. Salvemini**. Sphingosine-1-phosphate receptor 1 activation in the central nervous system drives cisplatin-induced cognitive impairment. (2022). *J. Clinical. Investigation* (In Press).
5. My work on purinergic signaling moved the field beyond the original dogma that only A₁AR or A_{2A}AR contribute to the nociceptive signaling pathway when we established the critical role of the A₃AR in the development of chronic neuropathic pain of diverse etiologies. This work led to the discovery of 1) the first highly selective A3AR agonist and 2) A3AR signaling pathways involved in chronic pain and development of chemotherapy-induced neurotoxicity's. This work enabled preclinical development of selective A3AR agonists identified in my lab for Phase 1 clinical trials for pain and neuroinflammatory diseases. These efforts are led by BiolIntervene Inc, a company that I founded in 2014.
- a. Little JW, Ford A, Symons-Liguori AM, Chen Z, Janes K, Doyle T, Xie J, Luongo L, Tosh DK, Maione S, Bannister K, Dickenson AH, Vanderah TW, Porreca F, Jacobson KA, **Salvemini D**. Endogenous adenosine A₃ receptor activation selectively alleviates persistent pain states. *Brain*. 2015 Jan; 138(Pt 1):28-35. PubMed PMID: [25414036](#); PubMed Central PMCID: [PMC4285194](#).
 - b. Ford A, Castonguay A, Cottet M, Little JW, Chen Z, Symons-Liguori AM, Doyle T, Egan TM, Vanderah TW, De Konnick Y, Tosh DK, Jacobson KA, **Salvemini D**. Engagement of the GABA to KCC2 Signaling Pathway Contributes to the Analgesic Effects of A3AR Agonists in Neuropathic Pain. *J Neurosci*. 2015 Apr 15; 35(15):6057-67. PubMed PMID: [25878279](#); PubMed Central PMCID: [PMC4397603](#).
 - c. Durante M, Squillace S, Lauro F, Giancotti LA, Coppi E, Cherchi F, Di Cesare Mannelli L, Ghelardini C, Kolar G, Wahlman C, Opejin A, Xiao C, Reitman ML, Tosh DK, Hawiger D, Jacobson KA, Salvemini D, Adenosine A3 agonists reverse neuropathic pain via T cell-mediated production of IL-10. *J Clin Invest* 2021, Apr 1; 131(7): e139299 PubMed PMID: [33621215](#); PubMed Central PMCID: [PMC8011899](#).

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/daniela.salvemini.1/bibliography/40689556/public/?sort=date&direction=ascending>

BIOGRAPHICAL SKETCH

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

NAME: Soltesz, Ivan

ERA COMMONS USER NAME (credential, e.g., agency login): ISoltesz

POSITION TITLE: Professor and Vice Chair, Neurosurgery (with courtesy appointment in Neurology and Neurological Sciences)

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
L. Eotvos University, Hungary	Diploma	1988	Biology
L. Eotvos University, Hungary	PHD	1989	Physiology
Oxford University, Oxford, UK	Postdoctoral	1990	Neuroscience
University of London, London, UK	Postdoctoral	1991	Neuroscience
Université Laval, Quebec, Canada	Postdoctoral	1992	Neuroscience
Stanford University, Stanford, CA	Postdoctoral	1993	Neuroscience
UT Southwestern School of Medicine, Dallas, TX	Postdoctoral	1994	Neuroscience

A. Personal Statement

I am interested in inhibition in the CNS, focusing on the synaptic and cellular organization of interneuronal microcircuits in the hippocampus under normal conditions and in temporal lobe epilepsy. My laboratory employs a combination of closely integrated experimental and theoretical techniques, including closed-loop *in vivo* optogenetics, *in vivo* juxtacellular recordings from identified interneurons in awake mice, 2P functional calcium imaging, paired patch clamp recordings, AI-aided segmentation of behavior, 24/7 long-term video-EEG recordings to track seizures over time, a variety of behavioral approaches, and large-scale computational modeling methods using supercomputers. In terms of synergistic activities, I wrote a monograph book on GABAergic microcircuits (Oxford University Press), co-edited a book on Computational Neuroscience in Epilepsy (Academic Press/Elsevier), co-founded the first Gordon Research Conference on the Mechanisms of neuronal synchronization and epilepsy, and taught for several years in the Ion Channels Course at Cold Springs Harbor. I have over 34 years of research experience, with 27 years as a faculty involved in the training of graduate students (total of 14, 6 of them MD/PhDs) and postdoctoral fellows (30), several of whom received fellowship awards, K99 grants, joined prestigious residency programs and became independent faculty.

Ongoing and recently completed projects that I would like to highlight include:

U19 NS104590

PIs: Soltesz, Buzsaki, Losonczy, Schnitzer

09/25/17-06/30/23

Towards a Complete Description of the Circuitry Underlying Sharp Wave-Mediated Memory Replay

R01 NS099457

Soltesz (PI); Katona (Co-PI)

07/01/17-03/31/23

Cannabinoid Control of Epilepsy.

R01 NS114020

Soltesz (PI); Datta (Co-PI)

09/30/19-06/30/24

Automated Phenotyping in Epilepsy

R01 NS121106

Soltesz (PI); Losonczy (Co-PI)

04/15/21-03/31/26

Control of the Axon Initial Segment in Epilepsy

R01 NS124590

Schnitzer (PI); Role: Co-Investigator

09/25/21-08/31/24

Dissecting neocortical field potential dynamics using optical voltage imaging in genetically targeted cell-types

NNX15AI22G (NASA)

Limoli (PI); Role: Co-Investigator

07/01/15-02/28/23

Mechanisms Underlying Charged Particle-induced Disruption of CNS Function

N00014-19-1-2373O (ONR)

Chowdhary (PI); Role: Co-Investigator

06/01/19-09/30/22

A CyberOctopus that Learns, Evolves, Adapts

2123781 (NSF)

Gazzola (PI); Role: Co-Investigator

04/15/22-03/31/29

Expeditions: Mind in Vitro – Computing with Living Neurons

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

7/2015-present Professor and Vice Chair, Neurosurgery (with courtesy appointment in Neurology and Neurological Sciences), Stanford University

2006-2015 Chair, Anatomy & Neurobiology, University of California, Irvine, CA

2003-2015 Professor, Anatomy & Neurobiology (with joint appointments in Physiology & Biophysics, Neurobiology & Behavior), University of California, Irvine, CA

2001-2015 Fellow, Center for Neurobiology of Learning & Memory, UC Irvine, CA

1999-2003 Associate Professor, Anatomy and Neurobiology, University of California, Irvine, CA

1995-1999 Assistant Professor, Anatomy and Neurobiology, University of California, Irvine, CA

Honors

2022 Keynote, ICTALS conference, Bern, Switzerland

2022 Founder's Lecture, UCSD

2022 Ruth K. Broad Foundation Seminar, Duke University

2019 Distinguished Seminar, Allen Brain Institute

2019 Keynote, Epilepsy Conference, Park City, Utah

2019 Keynote, Inhibition in the CNS Gordon Research Symposium

2018 Keynote, Gordon Research Conference on Epilepsy

2016 Keynote, Brain Informatics & Health conference

2015 James R. Doty Professor of Neurosurgery and Neurosciences, Stanford University

2011-2015 Chancellor's Professor, UCI

2011 Research Recognition Award, American Epilepsy Society (highest honor from AES)

2011-2013 Chair, Clinical Neuroplasticity and Neurotransmitters (CNNT) NIH study section

2011 Keynote, Organization of Computational Neurosciences meeting, Stockholm

2010-2012	Chair, Grants and Fellowship Review Panel, Epilepsy Foundation
2010-2012	Scientific Advisory Board, Citizens United for Research in Epilepsy (CURE)
2009	Michael Prize (top international award for basic epilepsy research)
2006-2009	Chair, Basic Science Committee, American Epilepsy Society
2005	Javits Neuroscience Investigator Award, NINDS
2005	Athalie Clark Research Award, UC Irvine

C. Contribution to Science

1. GABAergic interneurons play critical roles in virtually all aspects of cortical circuit function and dysfunction. Research in my laboratory over the years has exerted significant and lasting impact on the field and resulted in the discovery of novel principles underlying the organization of GABAergic inhibition in cortical circuits. Recently, we demonstrated that inhibition in the hippocampus is not homogenous, or uniform as previously thought, but that it is organized in a highly specific manner, where individual interneurons selectively form local circuits with only specific subsets of pyramidal cells that are defined by their long-distance projection patterns. My past and recent work has significantly contributed to our understanding of the spatio-temporal organization of network oscillations in the hippocampus and resulted in the identification of new interneuronal subtypes.
 - a) **Soltesz, I.** & Losonczy, A. CA1 pyramidal cell diversity enabling parallel information processing in the hippocampus. *Nature Neuroscience* (2018) 21:484-493. PMCID: PMC5909691.
 - b) Dudok, B., Klein, P.M., Hwaun, E., Lee, B.R., Yao, Z., Fong, O., Bowler, J.C., Terada, S., Sparks, F.T., Szabo, G.G., Farrell, J.S., Berg, J., Daigle, T.L., Tasic, B., Dimidschstein, J., Fishell, G., Losonczy, A., Zeng, H. & **Soltesz, I.** Alternating sources of perisomatic inhibition during behavior. *Neuron* (2021) 109(6):997-1012. PMCID: PMC7979482.
 - c) Dudok, B., Szoboszlay, M., Paul, A., Klein, P.M., Liao, Z., Hwaun, E., Szabo, G.G., Geiller, T., Vancura, B., Wang, B., McKenzie, S., Homidan, J., Klaver, L.M.F., English, D.F., Huang, Z.J., Buzsáki, G., Losonczy, A. & **Soltesz, I.** Recruitment and inhibitory action of hippocampal axo-axonic cells during behavior. *Neuron* (2021) 109(23):3838-3850.e8. PMCID: PMC8639676.
 - d) Farrell J.S., Lovett-Barron M., Klein P.M., Sparks F.T., Gschwind T., Ortiz A.L., Ahanonu B., Bradbury S., Terada S., Oijala M., Hwaun E., Dudok B., Szabo G., Schnitzer M.J., Deisseroth K., Losonczy A., **Soltesz I.** Supramammillary regulation of locomotion and hippocampal activity. *Science* (2021) 374(6574):1492-1496. PMCID: PMC9154354.
2. Cannabinoid receptor mediated signaling provides powerful and versatile regulation of synaptic transmission in the brain. My work on the endocannabinoid control of synaptic transmission in normal and epileptic circuits resulted in several major advances, including the identification of novel mechanisms regulating tonic control of GABA release by cannabinoid receptors, discovery of cell type-specificity of cannabinoid control of GABA release, and the recognition that cannabinoid signaling undergoes robust long-term plasticity in epilepsy.
 - a) Dudok, B., Barna, L., Ledri, M., Szabó, S.I., Szabadits, E., Pintér, B., Woodhams, S.G., Henstridge, C.M., Balla, G.Y., Nyilas, R., Varga, C., Lee, S.H., Matolcsi, M., Cervenak, J., Kacskovics, I., Watanabe, M., Sagheddu, C., Melis, M., Pistis, M., **Soltesz, I.** & Katona, I. Cell-specific STORM superresolution imaging reveals nanoscale organization of cannabinoid signaling. *Nature Neuroscience* (2015) 18(1):75-86. PMCID: PMC4281300.
 - b) **Soltesz, I.**, Alger, B.E., Kano, M., Lee, S.H., Lovinger, D.M., Ohno-Shosaku, T. & Watanabe, M. Weeding out bad waves: Towards selective cannabinoid circuit control in epilepsy. *Nature Reviews Neuroscience* (2015) 6(5):264-77. PMID: 25891509. NIHMSID: NIHMS1749285.
 - c) Maroso, M., Szabo, G.G., Kim, H.K., Alexander, A., Bui, A.D., Lee, S-H., Lutz, B. & **Soltesz, I.** Cannabinoid control of learning and memory through HCN channels. *Neuron* (2016) 89,1059-1073. PMCID: PMC4777634.
 - d) Farrell, J.S., Colangeli, R., Dong, A., George, A.G., Addo-Osafo, K., Kingsley, P.J., Morena, M., Wolff, M.D., Dudok, B., He, K., Patrick, T.A., Sharkey, K.A., Patel, S., Marnett, L.J., Hill, M.N., Li, Y., Teskey, G.C. & **Soltesz, I.** In vivo endocannabinoid dynamics at the timescale of physiological and pathological neural activity. *Neuron* (2021) 109(15):2398-2403. PMCID: PMC8351909.
3. There are over 65 million people world-wide with epilepsy, and current treatment options for epilepsy are inadequate. Research in my laboratory over the last two decades has significantly contributed to our

understanding of the basic mechanisms of epilepsy. More recently, my laboratory has developed new technologies that enabled the closed-loop, on-demand control of temporal lobe epilepsy in mice with unprecedented temporal, spatial and cell type-specificity.

- a) Krook-Magnuson, E., Armstrong, C., Oijala, M. & **Soltesz, I.** On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nature Communications* (2013) 4:1376. PMCID: PMC3562457.
 - b) Krook-Magnuson, E. & **Soltesz, I.** Beyond the hammer and the scalpel: selective circuit control for the epilepsies. *Nature Neuroscience* (2015) 18(3): 331-8. PMCID: PMC4340083.
 - c) Bui, A.D., Nguyen, T.M., Limouse, C., Kim, K.H., Szabo, G.G., Felong, S., Maroso, M. & **Soltesz, I.** Dentate gyrus mossy cells control spontaneous convulsive seizures and spatial memory. *Science* (2018) 359(6377):787-790. PMCID: PMC6040648.
 - d) Farrell, J.S., Nguyen, Q.-A. & **Soltesz, I.** Resolving the micro-macro disconnect to address core features of seizure networks. *Neuron* (2019) 101:1016-1028. PMCID: PMC6430140.
4. Normal and abnormal brain dynamics involve multiple levels of biological organization. Though there exists a wealth of data at each of these levels, the challenge of drawing connections across levels stands in the way of developing greater understanding and new treatments for the disorder. To meet this challenge, my laboratory has been at the forefront of biological data-driven modeling of control and epileptic hippocampal networks using supercomputers.
- a) Morgan, R.J. & **Soltesz, I.** Non-random connectivity of the epileptic dentate gyrus predicts a major role for neuronal hubs in seizures. *Proceedings of the National Academy of Sciences, USA* (2008) 105(16): 6179-6184. PMCID: PMC2299224.
 - b) Schneider, C.J., Cuntz, H. & **Soltesz, I.** Linking macroscopic with microscopic neuroanatomy using synthetic neuronal populations. *PLoS Comput. Biol.* (2014) 10(10):e1003921. PMCID: PMC4207466.
 - c) Bezaire, M.J., Raikov, I., Burk, K., Dhrumil, V. & Soltesz, I. Interneuronal mechanisms of hippocampal theta oscillations in full-scale models of the CA1 circuit. *eLife* (2016) 5:e18566. PMCID: PMC5313080.
 - d) Hadjibabadi, D., Lovett-Barron, M., Raikov, I.G., Sparks, F.T., Liao, Z., Baraban, S.C., Leskovec, J., Losonczy, A., Deisseroth, K. & **Soltesz, I.** Maximally selective single-cell target for circuit control in epilepsy models. *Neuron* (2021) 109(16):2556-2572. PMCID: PMC8448204.

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/ivan.soltesz.1/bibliography/public/>

BIOGRAPHICAL SKETCH

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

NAME: Xiaofei Sun**ERA COMMONS USER NAME (credential, e.g., agency login): SUNR1T****POSITION TITLE: Associate Professor**

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Zhejiang University, China	B.Sc.	1998-2002	Biology
Vanderbilt University, TN, USA	Ph.D.	2005-2010	Pharmacology

A. Personal Statement

The goal of the proposed research is to investigate the impact of cannabis/endocannabinoids on HIV infected microglia. I have the expertise to carry out the projected work due to my training and experience. I pursued my PhD degree in the Department of Pharmacology at Vanderbilt, which is known for its excellence in studying G protein coupled receptors mediated signal transduction. I completed my postdoctoral fellowship in Dr. S.K. Dey's lab, one of the leaders in endocannabinoid signaling research in female reproduction. This training has endowed me with valuable skills and knowledge in uterine biology and endocannabinoid system. As a postdoctoral fellow and research associate at Cincinnati Children's Research Foundation, I have built a strong intellectual base and developed the necessary techniques to study aberrant endocannabinoid signaling using *in vivo* and *in vitro* models. During my training, I found that either higher or lower endocannabinoid signaling compromises decidualization, trophoblast invasion and normal parturition. My role as PI or co-Investigator on foundation and NIH grants, demonstrate my ability to administer research projects. With my knowledge of endocannabinoid system and proficiency in handling genetically modified cells and mouse models, I am confident that I am capable of executing the proposed research project.

Ongoing and recently completed projects that I would like to highlight include:

NIH R01 HD103475

Dey, Sudhansu K. (PI), Role: co-investigator
08/1/2020 - 05/31/2025
Endocannabinoid signaling during early pregnancy

NIH R01 HD068524

Dey, Sudhansu K. (PI), Role: co-investigator
09/26/2011-06/30/2027
Molecular signaling in uterine receptivity to implantation

Citations:

1. Kim, Y.S., Li, Y., Yuan J., Borg, J.P., **Sun, X.***, and Dey, S. K.* (2021) Cannabinoid and planar cell polarity signaling converges to direct placentation. *Proceedings of the National Academy of Sciences of the United States of America* 118(38):e2108201118, PMID: 34521753 *co-corresponding author

2. Li, Y., Dewar A., Kim, Y. S., Dey, S. K. *, **Sun, X.*** (2020) Pregnancy success in mice requires appropriate cannabinoid receptor signaling for primary decidua formation. *Elife* 2020 (9) Epub 2020/09/30. PMID: 32990600 *co-corresponding author
3. Li, Y., Bian, F., **Sun, X.***, and Dey, S. K.* (2019) Mice Missing Cnr1 and Cnr2 Show Implantation Defects. *Endocrinology* 160, 938-946 PMID: 30776303 *co-corresponding author
4. **Sun, X.**, Xie, H., Yang, J., Wang, H., Bradshaw, H. B., and Dey, S. K. (2010) Endocannabinoid signaling directs differentiation of trophoblast cell lineages and placenta. *Proceedings of the National Academy of Sciences of the United States of America* 107, 16887-16892. PMID: 20837524

B. Positions, Scientific Appointments, and Honors

Professional Experience

Oct, 2021 – present	Research Associate Professor, Division of Developmental Biology, Center of Reproductive Sciences, Cincinnati Children's Hospital, OH, USA
Jul, 2016-Oct, 2021	Research Assistant Professor, Reproductive Sciences, Cincinnati Children's Hospital, OH, USA
Jul, 2013-Jun, 2016	Instructor, Reproductive Sciences, Cincinnati Children's Hospital, OH, USA
Apr, 2012-Jun, 2013	Research Associate, Reproductive Sciences, Cincinnati Children's Hospital, OH, USA
Jul, 2010-Apr, 2012	Postdoctoral research fellow, Reproductive Sciences, Cincinnati Children's Hospital, OH, USA
2003-2004	Research assistant, Civil and Environmental Engineering, Nanyang Technological University, Singapore

Honors

- 2020, Hidden Gem Award, Cincinnati Children's Hospital
2012-13, The Lalor Foundation postdoctoral fellowship award, Cincinnati, OH
2011-12, The Lalor Foundation postdoctoral fellowship award, Cincinnati, OH
2002, Outstanding Dissertation for Bachelor of Science, Zhejiang University, China

Professional Memberships

- 2011- Member, International Cannabinoid Research Society

C. Contributions to Science

1. **Cannabinoid signaling in pregnancy.** Majority of my research works focused on the roles of endocannabinoid signaling in reproduction. Marijuana is one of the world's most popular recreational drugs, and the most commonly abused illicit drug in pregnant women in western societies. In recent years, the recreational use of synthetic cannabinoids is increasing rapidly. The effects of maternal use of synthetic cannabinoids during pregnancy are ambiguous due to limited studies in humans and a relative short history of the drugs. However, our research suggests that abnormal endocannabinoid signaling has adverse effects on multiple pregnancy events. We identified the uterus as a major target for endocannabinoid signaling and layout the presence of endocannabinoid system in mouse uterus. We showed that besides oviductal embryo transport and implantation, placenta is compromised under abnormal endocannabinoid signaling, due to deficient trophoblast stem cell differentiation. Either silenced or amplified endocannabinoid signaling compromises the invasion of trophoblast cells. In male reproduction, we showed that sperm exposed to higher cannabinoids/endocannabinoids have lower fertility.

- a. Kim, Y.S., Li, Y., Yuan J., Borg, J.P., **Sun, X.***, and Dey, S. K.* (2021) Cannabinoid and planar cell polarity signaling converges to direct placenta. *Proceedings of the National Academy of Sciences of the United States of America* 118(38):e2108201118, PMID: 34521753 *co-corresponding author
- b. Li, Y., Dewar A., Kim, Y. S., Dey, S. K. *, **Sun, X.*** (2020) Pregnancy success in mice requires appropriate cannabinoid receptor signaling for primary decidua formation. *Elife* 2020 (9) Epub 2020/09/30. PMID: 32990600 *co-corresponding author
- c. **Sun, X.**, Xie, H., Yang, J., Wang, H., Bradshaw, H. B., and Dey, S. K. (2010) Endocannabinoid signaling directs differentiation of trophoblast cell lineages and placenta. *Proceedings of the National Academy of Sciences, USA* 107, 16887-16892 PMID: 20837524
- d. **Sun, X.***, Deng, W., Li, Y., Tang, S., Leishman, E., Bradshaw, H. B., and Dey, S. K.* (2016) Sustained Endocannabinoid Signaling Compromises Decidual Function and Promotes Inflammation-induced

Preterm Birth. **The Journal of biological chemistry** 291, 8231-8240 PMID: 26900150 *co-corresponding author

2. **Signaling pathways in implantation.** I pursued research on molecular signaling pathways critical to implantation, which advanced the understanding of signaling networks in implantation. I identified the transcriptional factor KLF5 as a critical factor in mouse implantation and revealed appropriate uterine epithelial responses to implanting embryos is a prerequisite for successful implantation. Our study using mice with uterine specific deletion of Gp130 and Stat3 showed that LIF signaling is mediated by Gp130 and Stat3. I also participated in studies on the roles of MSX homeobox genes in the uterus that show that Msx1/2 is critical to establish appropriate crypt formation in the uterus before implantation and modulate uterine receptivity by regulating the cell polarity in luminal epithelial cells.
- Matsuo, M., Yuan, J., Kim, Y.S., Dewar, A., Fujita, H., Dey, S.K.*, **Sun, X.*** (2022) Targeted depletion of uterine glandular Foxa2 induces embryonic diapause in mice. **Elife** 2022(11) e78277; PMID: 35861728 *co-corresponding author
 - Sun, X.**, Zhang, L., Xie, H., Wan, H., Magella, B., Whitsett, J. A., and Dey, S. K. (2012) Kruppel-like factor 5 (KLF5) is critical for conferring uterine receptivity to implantation. **Proceedings of the National Academy of Sciences, USA** 109, 1145-1150; PMID: 22233806
 - Sun X**, Park CB, Deng W, Potter SS, Dey SK. (2016) Uterine inactivation of muscle segment homeobox (Msx) genes alters epithelial cell junction proteins during embryo implantation. **FASEB J.** PMID: 26667042
 - Cha, J., **Sun, X.**, and Dey, S. K. (2012) Mechanisms of implantation: strategies for successful pregnancy. **Nature medicine** 18, 1754-1767 PMID: 23223073
3. **A novel event discovered in embryo attachment.** During implantation, luminal epithelial (LE) cells surrounding the blastocyst in the implantation chamber (crypt) disappear, allowing trophoblast cells to make direct physical contact with the underneath stroma for successful implantation. The mechanism for the extraction of LE cells was thought to be mediated by apoptosis. However, our work shows that LE cells in direct contact with the blastocyst are endocytosed by trophoblast cells by adopting the nonapoptotic cell-in-cell invasion process (entosis) in the absence of caspase 3 activation. Our in vivo observations were reinforced by the results of co-culture experiments with primary uterine epithelial cells with trophoblast stem cells or blastocysts showing internalization of epithelial cells by trophoblasts. We have identified entosis as a mechanism to remove LE cells by trophoblast cells in implantation, conferring a role for entosis in an important physiological process.
- Li Y, **Sun X***, Dey SK*. Entosis allows timely elimination of the luminal epithelial barrier for embryo implantation. **Cell Rep.** 2015;11(3):358-65. *co-corresponding author. PMID: 25865893
4. **In search of stem cells in the uterus.** Leucine-rich repeat-containing G-protein-coupled receptor 5 (*Lgr5*) is a stem cell marker in the stomach, intestinal epithelium, hair follicles, and ovarian surface epithelium. To search for stem cells in the mouse uterus, we studied the *Lgr5* expression in the uterus. The dynamic expression of *Lgr5* under various physiological and pathological states of the uterus prompted us to delete *Lgr5* using a Pgr-Cre driver. To our surprise, the deletion of *Lgr5* does not compromise uterine integrity, but the result reveals that *Lgr5* is critical to normal corpus luteum function. The deletion of *Lgr5* in corpora lutea causes insufficient ovarian progesterone secretion that compromises decidualization and terminates pregnancy.
- Sun X.**, Terakawa, J., Clevers, H., Barker, N., Daikoku, T., and Dey, S.K. (2014) Ovarian LGR5 is critical for successful pregnancy. **FASEB J.** 28(5):2380-9; PMID: 24469993
 - Sun, X.**, Jackson, L., Dey, S. K., and Daikoku, T. (2009) In pursuit of leucine-rich repeat-containing G protein-coupled receptor-5 regulation and function in the uterus. **Endocrinology** 150, 5065-5073 PMID: 19797400

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/18oqkWfwSMU/bibliography/40637588/public/?sort=date&direction=descending>

BIOGRAPHICAL SKETCH

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

NAME: Vanderah, Todd

ERA COMMONS USER NAME (credential, e.g., agency login): VANDERAH

POSITION TITLE: Department Head and Professor, Pharmacology

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Arizona, Tucson, AZ	B.S.	05/1991	Molecular and Cellular Biology
University of Arizona, Tucson, AZ	Ph.D.	01/1995	Pharmacology and Toxicology
University of Colorado, Denver, CO	NIH Training Grant	03/1997	The Study of Alcohol and Opioid Interactions

A. Personal Statement

I have worked in the area of Neuropharmacology for over 20 years with approximately 200 peer-reviewed publications in the area of opioids and non-opioids, including cannabinoids, in models of acute and chronic pain. In the last ten years we have worked on testing novel compounds at new and well-known molecular targets in order to attenuate pain without resulting in the activation of the reward pathway or having behavioral signs of reward/addiction. I have years of experience in behavioral measurements in rodents, pharmacokinetics and pharmacodynamics with previous experience in drug development that has resulted in the advancement of three compounds from the preclinical side that were pushed into positive clinical phase testing, approved by the FDA and onto the market.

My recent studies have focused on metastatic cancer pain utilizing a syngeneic murine model of breast cancer pain. We have identified several new targets within bone and on primary afferents that when activated significantly reduce bone cancer pain without the unwanted side effects caused by opioids. In addition, we have investigated the effects of novel treatments in combination with opioids as a mechanism of opioid sparing. These types of studies have been pursued in small but significant clinical trials. Recently we have investigated the role of CB2 receptors on microglial cells of the CNS and their ability to significantly alter morphine-induced changes in the reward pathways and respiratory centers of a mouse. We have taken on studies to investigate the endogenous cannabinoid system in the respiratory systems of the brainstem. The funding of this P30 and its ability to image at the cellular level would be exceptionally useful to our studies on how endogenous cannabinoids regulate respiration in the absence and presence of opioids including fentanyl. Relevant publications are included below.

Citations:

- 1) Zhang H, Lund DM, Ciccone HA, Staatz WD, Ibrahim MM, Largent-Milnes TM, Seltzman HH, Spigelman I, and **Vanderah TW**. A peripherally selective cannabinoid 1 receptor agonist as a novel analgesic agent in cancer-induced bone pain., *Pain* 159(9):1814-1823, 2018 PMID 29781960, PMCID: PMC6095738
- 2) Beth M. Wiese, Erika Liktor-Busa, Sarah A. Couture, Spyros P. Nikas, Lipin Ji, Yingpeng Liu, Alexandros Makriyannis, Igor Spigelman, Todd W. Vanderah, Tally M. Largent-Milnes, Brain Penetrant, but not Peripherally Restricted, Synthetic Cannabinoid 1 Receptor Agonists Promote Morphine-Mediated Respiratory Depression. *Cannabis and Cannabinoid Research*, accepted with minor revision.
- 3) Grenald SA, Young MA, Wang Y, Ossipov MH, Ibrahim MM, Largent-Milnes TM and Vanderah TW. Synergistic attenuation of chronic pain using mu opioid and cannabinoid receptor 2 agonists. *Neuropharmacology*, 2017, (116) 59-70, PMID: 28007501
- 4) Lozano-Ondoua AN, Hanlon KE, Symons-Liguori AM, Largent-Milnes TM, Havelin JJ, Ferland III HL, Chandramouli A, Owusu-Ankomah M, Nikolich-Zugich T, Bloom AP, Jimenez-Andrade JM, King T, Porreca F,

Nelson MA, Mantyh PW, and Vanderah TW. Disease Modification of Breast Cancer-induced Bone Remodeling by Cannabinoid CB2 receptor Agonists. *Journal of Bone and Mineral Research*, 28(1); 92-107, 2013.

5) Thompson AL, Grenald SA, Ciccone H, Neemah BassiriRad, Staatz WD, Niphakis MJ, Cravatt BF, Largent-Milnes TM, and Vanderah TW, The Endocannabinoid System Alleviates Pain in a Murine Model of Cancer-Induced Bone Pain, *J Pharmacol Exp Ther*. 2020 373 (2), 230-238 PMID: 32054717.

Ongoing projects that I would like to highlight include:

1R01 DA056608 (NIH/NIDA)

Largent-Milnes & Vanderah (MPIs) 7/2022–6/2027

Endocannabinoid Targeting for Opioid Induced Respiratory Depression

NIH/NCI R01CA142115

Vanderah, Todd W (PI) 4/1/2017-3/31/2022 NCE

Cannabinoid CB2 Agonists for Treatment of Breast Cancer-Induced Bone Pain

NIH/NIDA 1P30DA051355-01A1

Porreca and Vanderah (MPIs) 8/2021-7/2026

"Core Center of Excellence in Addiction Studies"

NIH R21CA245411 Ibrahim(PI) Vanderah Co-I. 07/01/2020-06/30/2023

(granted extension by NIH for patient enrollment)

Repurposing Sulfasalazine in a Two-Arm Phase Two Double-Blind Randomized Clinical Trial for the Adjunct Management of Breast Cancer-Induced Bone Pain

NIH/NIGMS T32 MSTP 1T32GM141830-01

Vanderah (MPI) 7/2021-6/2026

"Interdisciplinary Training of Future Physician Scientists"

B. Positions and Honors

Positions and Employment

2020 - Present	Director of the Comprehensive Pain and Addiction Center
2019 - Present	co-Director of the MD-PhD Program
2011 - Present	Professor and Chair, Pharmacology, University of Arizona, Tucson, AZ
2010 - Present	Professor, Pharmacology & Anesthesiology, University of Arizona, Tucson, AZ
2010 - Present	Professor, Neurology, University of Arizona, Tucson, AZ
2005 - 2010	Associate Professor, Pharmacology, University of Arizona, Tucson, AZ
2004 - Present	Member, International Association for the Study of Pain (IASP)
2000 - 2019	Member, American Pain Society (APS)
2000 - 2005	Assistant Professor, Pharmacology, University of Arizona, Tucson, AZ
1998 - Present	Director, Spring Pain
1997 - 2000	Pharmacology Group Leader, Ferring Inc., San Diego, CA
1995 - Present	Member, Society for Neuroscience
2000 - Present	member, Arizona State Governor Counsel for Healthcare

Other Experience and Professional Memberships

1995 -	Member, Society for Neuroscience
1998 -	Director, Spring Pain
2000 -	Member, American Pain Society (APS)
2004 -	Member, International Association for the Study of Pain (IASP)

Honors

2021 - Present	Human Health & Services, Member of the Pain Research Coordinating Committee of the National Institutes of Health (June 2022-June 2024)
2022	NIH ZDA1 SXC-G (01) Special emphasis, High-throughput Discovery & Validation of Novel Signal Transducers or Small Molecules that Modulate Opioid or other Substance Use Disorder Relevant Pathways

2021	NIH/NPI Neurobiology of Pain and Itch Study Section,
2020	NIH/ZRG1 IFCN-N Discovery and Validation of Novel Safe & Effective Pain Treatment
2019	NIH/ZRG1 IFCN-E Discovery and Validation of Novel Safe & Effective Pain Treatment
2018	NIH/NCI ZCA1 SRB-A (M1) Chair - NCI Provocative Questions SEP-2
2017	NIH/MNPS <i>Molecular Neuropharmacology and Signaling</i> Study Section
2017	NIH ZRG1 IFCN-B (03) M meeting Special Emphasis Panel/Scientific Review Group
2016	NIH/PMDA <i>Pathophysiological Basis of Mental Disorders and Addictions</i> Study Section
2015	NIH/IFCNI SCS <i>Somatosensory and Chemosensory Systems</i> Study Section
2014	P01 ZCA1 RPRB-J Study Section, NIH/NCI SEP
2014	NIH ZRG1 HDM-V (04) M, Special Emphasis Panel, <i>DABP/HDM/DIRH</i>
2013 - 2016	STTR/SBIR Study Section, NIH/ ZRG1 ETTN-M
2010	Inducted into the Academy of Medical Education Scholars
2008 - 2012	Study Section, Member, NIH/IFCNI SCS <i>Somatosensory and Chemosensory Systems</i>
2007	Outstanding Speaker Award, AACC, Continuing Educations Program
2006	Top Ten most cited manuscripts, <u>Pain</u>

C. Contributions to Science

1. My early work as a graduate student significantly contributed to the discovery of the delta opioid receptor subtypes based on pharmacological activity. These studies were pivotal in identifying the analgesic potential of the delta opioid receptor that lacked the unwanted side effects seen with mu opioid receptor activity.
 - a. Wild KD, Vanderah T, Mosberg HI, Porreca F. (1991). Opioid delta receptor subtypes are associated with different potassium channels. *Eur J Pharmacol.* 193(1):135-6. (100 citations)
 - b. Mattia A, Vanderah T, Mosberg HI, Porreca F. (1991). Lack of antinociceptive cross-tolerance between [D-Pen2, D-Pen5]enkephalin and [D-Ala2]deltorphin II in mice: evidence for delta receptor subtypes. *J Pharmacol Exp Ther.* 258(2):583-7. (312 citations)
 - c. Vanderah TW, Wild KD, Takemori AE, Sultana M, Portoghesi PS, Bowen WD, Mosberg HI, Porreca F. (1992). Mediation of swim-stress antinociception by the opioid delta 2 receptor in the mouse. *J Pharmacol Exp Ther.* 262(1):190-7. (87 citations)
 - d. Vanderah T, Takemori AE, Sultana M, Portoghesi PS, Mosberg HI, Hruby VJ, Haaseth RC, Matsunaga TO, Porreca F. (1994). Interaction of [D-Pen2,D-Pen5]enkephalin and [D-Ala2,Glu4]deltorphin with delta-opioid receptor subtypes in vivo. *Eur J Pharmacol.* 252(2):133-7. (68 citations)
2. My studies have significantly contributed to the pharmacological activation of a descending pain facilitatory pathway from the rostral medulla to the spinal cord. My work found that not only does dynorphin play a role in promoting pain in the CNS but also put forth the idea that sustained opioids results in the activation of this pathway, explaining a physiological tolerance of opioid analgesia.
 - a. Vanderah TW, Laughlin T, Lashbrook JM, Nichols ML, Wilcox GL, Ossipov MH, Malan TP Jr, Porreca F. (1996). Single intrathecal injections of dynorphin A or des-Tyr-dynorphins produce long-lasting allodynia in rats: blockade by MK-801 but not naloxone. *Pain.* 68(2-3):275-81. (225 citations)
 - b. Vanderah TW, Gardell LR, Burgess SE, Ibrahim M, Dogru A, Zhong CM, Zhang ET, Malan TP Jr, Ossipov MH, Lai J, Porreca F. (2000). Dynorphin promotes abnormal pain and spinal opioid antinociceptive tolerance. *J Neurosci.* 20(18):7074-9. (380 citations)
 - c. Vanderah TW, Suenaga NM, Ossipov MH, Malan TP Jr, Lai J, Porreca F. (2001). Tonic descending facilitation from the rostral ventromedial medulla mediates opioid-induced abnormal pain and antinociceptive tolerance. *J Neurosci.* 21(1):279-86. (356 citations)
 - d. Vanderah TW, Ossipov MH, Lai J, Malan TP Jr, Porreca F. (2001). Mechanisms of opioid-induced pain and antinociceptive tolerance: descending facilitation and spinal dynorphin. *Pain.* 92(1-2):5-9. (347 citations)
3. I have made significant contributions in the role of Cannabinoid 2 receptors in acute and chronic pain. My work has investigated the CB2 receptors as a site of action to significantly inhibit pain while lacking unwanted side effects seen with other clinically approved therapeutics. In addition, my work with CB2 receptor activation has resulted in a disease modification in advanced stages of breast-induced bone cancer pain. My contributions included demonstrations that CB2 agonists significantly inhibit bone loss and slow the proliferation of breast cancer cells in a murine model of bone cancer.

- a. Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, Vanderah TW, Lai J, Porreca F, Makriyannis A, Malan TP Jr. (2003). Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci U S A*. 100(18):10529-33. PMCID: PMC193595 (citations 555)
 - b. Ibrahim MM, Porreca F, Lai J, Albrecht PJ, Rice FL, Khodorova A, Davar G, Makriyannis A, Vanderah TW, Mata HP, Malan TP Jr. (2005). CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc Natl Acad Sci U S A*. 102(8):3093-8. PMCID: PMC549497 (citations 589)
 - c. Lozano-Ondoua AN, Wright C, Vardanyan A, King T, Largent-Milnes TM, Nelson M, Jimenez-Andrade JM, Mantyh PW, Vanderah TW. (2010). A cannabinoid 2 receptor agonist attenuates bone cancer-induced pain and bone loss. *Life Sci*. 86(17-18):646-53. PMCID: PMC2871326 (citations 82)
 - d. Lozano-Ondoua AN, Hanlon KE, Symons-Liguori AM, Largent-Milnes TM, Havelin JJ, Ferland HL 3rd, Chandramouli A, Owusu-Ankomah M, Nikolich-Zugich T, Bloom AP, Jimenez-Andrade JM, King T, Porreca F, Nelson MA, Mantyh PW, Vanderah TW. (2013). Disease modification of breast cancer-induced bone remodeling by cannabinoid 2 receptor agonists. *J Bone Miner Res*. 28(1):92-107. PMCID: PMC4745976 (citations 61)
4. I have contributed to the pharmacological characterization of novel bifunctional compounds for acute and chronic pain while lacking the unwanted side effects of mu opioid agonists. This is work done with my collaborators Drs. Salvemini, Hruby, Yamamura and Porreca. We have successfully designed, synthesized and tested over a 1000 novel compounds with a drive to find new analgesics that lack unwanted side effects.
- a. Ford A, Castonguay A, Cottet M, Little JW, Chen Z, Symons-Liguori AM, Doyle T, Egan TM, Vanderah TW, De Konnick Y, Tosh DK, Jacobson KA, Salvemini D. (2015). Engagement of the GABA to KCC2 Signaling Pathway Contributes to the Analgesic Effects of A3AR Agonists in Neuropathic Pain. *J Neurosci*. 35(15):6057-67. PMCID: PMC4397603 (citations 59)
 - b. Largent-Milnes TM, Yamamoto T, Nair P, Moulton JW, Hruby VJ, Lai J, Porreca F, Vanderah TW. (2010). Spinal or systemic TY005, a peptidic opioid agonist/neurokinin 1 antagonist, attenuates pain with reduced tolerance. *Br J Pharmacol*. 161(5):986-1001. PMCID: PMC2998681 (citations 62)
 - c. Little JW, Ford A, Symons-Liguori AM, Chen Z, Janes K, Doyle T, Xie J, Luongo L, Tosh DK, Maione S, Bannister K, Dickenson AH, Vanderah TW, Porreca F, Jacobson KA, Salvemini D. (2015). Endogenous adenosine A3 receptor activation selectively alleviates persistent pain states. *Brain*. 138(Pt 1):28-35. PMCID: PMC4285194 (citations 113)
 - d. Gavva, N.R., Klionsky, L., Qu, Y., Shi, L., Tamir, R., Edenson, S., Zhang, T.J., Viswanadhan, V., Toth, A. (2004) Vanderah, T.W., Porreca, F., Blumberg, P.M., Lile, J., Sun, K., Louis, J.-C. and Treanor, J.J.S., Molecular determinants of vanilloid sensitivity in TRPV1 *J Biol Chem*, 279(19): 20283-20295. (citations 390)
5. My lab has worked on identifying the role of cholecystokinin in the RVM and its ability to activate the descending pain pathway. This pathway may be activated in states of chronic, neuropathic pain. CCK is thought to act at CCK_B receptor on pain facilitatory pathways, resulting in an increase release of prostaglandins, serotonin and dynorphin at the level of the spinal cord. This work has led to novel hypotheses on the pain facilitatory pathway.
- a. Xie JY, Herman DS, Stiller CO, Gardell LR, Ossipov MH, Lai J, Porreca F, Vanderah TW. (2005). Cholecystokinin in the rostral ventromedial medulla mediates opioid-induced hyperalgesia and antinociceptive tolerance. *J Neurosci*. 25(2):409-16. (citations 217)
 - b. Wang, Z, Gardell, LR, Ossipov, MH, Vanderah, TW, Brennan, MB, Hochgeschwender, U, Hruby VJ, Malan Jr, TP, Lai, J and Porreca, F. (2001). Pronociceptive actions of dynorphin maintain chronic neuropathic pain. *J Neuroscience*. 21(5): 1779-1786. PMCID: PMC2724921 (citations 330)
 - c. King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. (2009). Unmasking the tonic-aversive state in neuropathic pain. *Nature Neurosci*. 12(11):1364-6. PMCID: PMC3427725 (citations 471)
 - d. Gardell, L.R., Ehrenfels, C., Ossipov, M.H., Rossomando, A.J., Miller, S., et.al., Vanderah, TW, Lai, J., Sah, D.W.Y. and Porreca, F. (2003). Normalization of Experimental Neuropathic Pain by Systemic Artemin. *Nature Medicine*. 9(11): 1383-9. (180 citations)

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/todd.vanderah.1/bibliography/42454396/public/?sort=date&direction=ascending>

BIOGRAPHICAL SKETCH

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

NAME: Marina E. Wolf

eRA COMMONS USER NAME (credential, e.g., agency login): wolfmarina

POSITION TITLE: Professor of Behavioral Neuroscience, Oregon Health & Science University

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

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Northwestern University, Evanston, IL	BA	1981	Biochemistry
Yale University Sch. Med., New Haven, CT	MPhil	1983	Pharmacology
Yale University Sch. Med., New Haven, CT	PhD	1986	Pharmacology
Center for Cell Biology, Sinai Hospital, Detroit, MI	Postdoc	1989	Cell Biology

A. Personal Statement

I am pleased to serve on the External Advisory Board of the **IUB Center for Cannabis, Cannabinoids and Addiction**. I am well qualified to contribute to this Center. My lab was among the first to study the role of neuronal plasticity in drug addiction (see Contribution #1 below). We have focused on the regulation of glutamate receptor expression, subunit composition, and trafficking in reward-related brain regions and how alterations in these processes contribute to addiction-related behavioral change. We use a combination of cell biological and electrophysiological approaches to study synaptic plasticity and its functional significance in rat models of psychostimulant and opioid addiction, and primary neuronal cultures for mechanistic studies that are difficult to undertake *in vivo*. Our overarching goal is to understand synaptic mechanisms in the nucleus accumbens and related circuits that maintain drug craving, and thus vulnerability to relapse, even after long periods of abstinence. We are also interested in the role of protein translation in supporting this plasticity. We use the ‘incubation of drug craving’ model, in which cue-induced drug craving in rats progressively intensifies (‘incubates’) over weeks to months of forced abstinence from drug self-administration. This model has translational relevance because incubation of craving also occurs in human drug users. Over the years, I have published a number of comprehensive reviews that have been useful to the field. Four are listed below. In addition to scientific accomplishments, I have made substantial contributions in the realms of academic leadership, service to the NIH and professional societies, and graduate education.

Ongoing projects that I would like to highlight include:

R01 DA049930-03 Wolf (PI) 03/15/20-12/31/24

Retinoic acid, homeostatic plasticity and cocaine craving

The goal is to test the hypothesis that decreased Ca^{2+} signaling in the NAc core during early withdrawal from cocaine self-administration leads to disinhibition of retinoic acid synthesis and increased GluA1 translation, accounting for the increase in synaptic levels of homomeric GluA1 CP-AMPARs that ultimately maintains incubation of cocaine craving.

R01 DA009621-25 Wolf (PI) 03/01/17-11/30/22 (NCE)

Glutamate receptor plasticity underlying incubation of methamphetamine craving

The goal is to determine how plasticity of AMPA, NMDA and group I metabotropic glutamate receptor transmission in the rat nucleus accumbens contribute to incubation of methamphetamine craving. DA009621 has been funded continuously since 1996. A competitive renewal application has been submitted (July 2022).

U18 DA052488-01 Wolf (PI), with Ingram S & Janowsky A 09/30/20-09/29/21 (NCE)
Reduction of psychostimulant craving through positive allosteric modulation of mGlu1

This U18 application was submitted in response to RFA: Step Up for Substance Use Disorders (SUD): A Drug Target Initiative for Scientists Engaged in Fundamental Research. In keeping with the “progressive value buildup model” of this RFA, we proposed two Aims designed to increase the value of mGlu1 as a target, bringing it one step closer to commercialization. A phase I STTR application has been submitted to continue this work (pending).

R13 DA051058 Wolf (PI) 2022

2020 Neurobiology of Drug Addiction Gordon Research Conference and Seminar

This R13 provides support for the 2022 Neurobiology of Addiction GRC (Chairs: Marina Wolf and Yavin Shaham).

Citations:

- a. Wolf ME (1998) The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Progress in Neurobiology* 54:679-720. PMID: 9560846
 - b. Wolf ME (2010) The Bermuda triangle of cocaine-induced neuroadaptations. *TINS* 33:391-398. PMC2935206
 - c. Wolf ME, Tseng KY (2012) Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: when, how and why? *Front Molecular Neurosci* 5:72. PMC3384237
 - d. Wolf ME (2016) Synaptic mechanisms underlying persistent cocaine craving. *Nat Rev Neurosci* 17:351-365. PMC5466704

B. Positions, Scientific Appointments, and Honors (most significant contributions are bolded)

Positions and Scientific Appointments

2018-	Professor of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR
2003-2018	Chair of Neuroscience, The Chicago Medical School at Rosalind Franklin University of Medicine and Science (university name change in 2004)
2002-2003	Acting Chair of Neuroscience, The Chicago Medical School
2000-present	Professor of Neuroscience, The Chicago Medical School
1993-1999	Associate Professor of Neuroscience, The Chicago Medical School, N. Chicago, IL
1990-1992	Assistant Professor of Psychiatry, Wayne State Univ. School of Medicine, Detroit, MI

NIH Service

2022: Ad hoc Member, CEBRA Special Emphasis Panel (7/2022); Ad hoc Member, NIH Initial Review Group NMB (6/2022); Ad hoc Member, Special Emphasis Panel ZDA1 SXC-G (03) R (2/2022)

2020: Ad hoc Member, Special Emphasis Panel ZRG1 IFCN-C (02) (7/2020)

2019: Ad hoc Member, Special Emphasis Panel ZRG1 BDCN-W (91) (12/2019); Chair, Special Emphasis Panel (Serious Adverse Drug Reaction Research PAR-16-274 & 16-275) (7/2019)

2018: Ad hoc Member, NIH Initial Review Group MNPS (10/2018); Stage 1 Reviewer, NIH Director's Early Independence Award (DP5) (12/2018); Ad hoc Member, NIDA Centers Review Group (3/2018)

2015-2017: Chair, ZRG1 IDM-C, PAR16-274: Adverse Drug Reaction Review Group (7/2017); Ad hoc Member, NIDA Centers Review Group (3/2017); Chair, NIH Initial Review Group, MNPS, 2015-2017

2010-2016: Regular Member, NIH Initial Review Group, MNPS, 2013-2014; NIDA Board of Scientific Counselors 2012-2016; NIH Council of Councils 2007-2011

Councilors, 2012-2013; NIH Council of Councils, 2007-2011
Before 2010: NIDA Advisory Council, 2006-2009 (Steering Committee, 2007-2009); NIH Neurosciences Blueprint Advisory Committee, 2004; Special Emphasis Panel ZRG1 MDCN-A, 2004; NIH Special Emphasis Panels, 2001, 2002, 2003; Regular Member, NIH Initial Review Group, MDCN-5, 1998-2001; Ad hoc Member, NIH Initial Review Group, MDCN-4, 1998; Regular Member, NIDA Initial Review Group, NIDA-A, 1997-1998; NIMH Special Review Committee & Site Visit Team, 1995

Other Professional Experience and Accomplishments

2020-present	American Brain Coalition, Board Member
2017-present	Scientific Council of the Brain & Behavior Research Foundation
2017-present	Co-Founder of GRC on Neurobiology of Drug Addiction (Co-Vice-Chair 2017, Co-Chair 2022)
2017-2021	Member F1000 Prime
2000-present	Journal Service: Journal of Neurochemistry (Editorial Board 2000-2013); Neuropsychopharmacology (Field Editor 2002-2007) ; Journal of Neuroscience (Associate Editor 2009-2014); Neuropharmacology [Editorial Board 2020-2022 and Editor (with S.L. Ingram and A. Keller) of 2023 special issue titled “Opioid-induced changes in addiction and pain circuits”]; Neuropsychopharmacology Reviews [Editor (with A. Abi-Dargham) of 2023 volume titled “Advances in plasticity of synapses and circuits related to psychiatric disorders and therapeutics”]

1997-present	American College of Neuropsychopharmacology (ACNP) (Member 1997, Fellow 2009, ACNP Council 2013-2018 & 2020-2022, ACNP President 2019)
1997-2000	Secretary, Chicago Chapter of the Society for Neuroscience
1993-2003	Founding Director, Neuroscience Graduate Program , The Chicago Medical School
1983-present	Society for Neuroscience (including service on Program Committee and others, 2007-2013)

Honors and Awards

2021	ACNP Paul Hoch Distinguished Service Award
2017	Fellow, American Association for the Advancement of Science
2017	Chicago Society for Neuroscience Career Achievement Award
2010-2015	NIDA Senior Scientist and Mentorship Award (K05)
2003-2013	NIDA MERIT Award for DA015835 (R37)
2000-2010	NIDA Career Development Award (K02)
1992-1997	NIDA First Award (R29)
1990-2006	NARSAD Young Investigator Award (1990), Independent Investigator Award (1999), and Distinguished Investigator Award (2006)
1988-1990	Postdoctoral Individual National Research Service Award, NINCDS
1981-1986	Predoctoral Fellowships, National Science Foundation and PMAF (Yale University)
1977-1981	National Merit Scholarship, Phi Beta Kappa (Northwestern University)

C. Contributions to Science

1. Pioneered the idea that synaptic plasticity contributes to drug addiction.

My interest in the role of synaptic plasticity in drug addiction developed in the 1980's through a combination of dissatisfaction with current theories of addiction, which focused exclusively on adaptations within the DA neurons themselves, and the exciting work on NMDARs and LTP that was coming out at the time. Based on studies from other labs showing that behavioral sensitization to psychostimulants was initiated within the ventral tegmental area (VTA) and was associated with increased DA cell firing, I hypothesized that sensitization involved LTP onto DA neurons. Our first relevant paper (1991) showed that the NMDAR antagonist MK-801 prevented the development of sensitization. Ralph Karler's lab actually published this first (1989), but did not pursue it on a mechanistic level as we did (see 2-5 below). Two important advances we made during these early years were the demonstration that NMDAR-dependent plasticity in the VTA is required for subsequent sensitization-related adaptations in the nucleus accumbens (NAc) (1994) and the demonstration that prefrontal cortical (PFC) projections are important for sensitization (1995). In that same year, we collaborated with Frank White's lab to test for the potentiation of AMPAR transmission that is diagnostic of LTP. Using extracellular recordings, we showed that, following sensitization, VTA DA neurons were more responsive to iontophoretic glutamate. Shortly afterwards, we published 2 papers showing that VTA DA neurons were also more responsive to locally applied AMPA. This work presaged patch-clamp studies (Ungless, Whistler, Malenka & Bonci, 2001) that definitively demonstrated cocaine-induced LTP onto VTA DA neurons.

- a. Wolf ME, White FJ, Hu X-T (1994) MK-801 prevents alterations in the mesoaccumbens dopamine system associated with behavioral sensitization to amphetamine. *J Neurosci* 14:1735-1745. PMID: 8126567
- b. White FJ, Hu X-T, Zhang X-F, Wolf ME (1995) Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *J Pharmacol Exp Ther* 273:445-454. PMID: 7714800
- c. Wolf ME, Dahlin SL, Hu X-T, Xue C-J, White K (1995) Effects of lesions of prefrontal cortex, amygdala, or fornix on behavioral sensitization to amphetamine: Comparison with N-methyl-D-aspartate antagonists. *Neurosci* 69:417-439. PMID: 8552239
- d. Zhang X-F, Hu X-T, White FJ, Wolf ME (1997) Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves non-NMDA receptors. *J Pharmacol Exp Ther* 281(2):699-706. PMID: 9152375

2. Conducted the first studies of AMPA receptor trafficking in striatal neurons.

In the late 1990's, tremendously excited by the Malenka/Nicoll proposal that insertion of new AMPARs into synapses mediated LTP, I set up methods for measuring AMPAR trafficking in primary cultures. This line of work, originally supported by a Merit Award, provided the first information about the regulation of AMPAR trafficking in reward-related brain regions including the nucleus accumbens (NAc), the mPFC, and the VTA. Our first major finding (2002) was that D1R stimulation increased surface expression of GluA1-containing AMPARs in the NAc. We went on to show, in different cell types (mPFC and hippocampal pyramidal neurons, and NAc medium spiny neurons), that PKA phosphorylation of GluA1 accelerates the rate of its insertion into extrasynaptic storage pools,

increasing the size of these pools and thereby priming AMPARs for subsequent NMDAR-dependent synaptic insertion (2005-2008). Beyond significance for understanding DA/glutamate interactions in drug addiction, these results have broad significance for synaptic plasticity, as the initial paper in this series (Sun et al., 2005), along with Oh et al. (2006) from the Soderling lab, provided the first direct evidence for this priming role of PKA. More recently, we demonstrated that synaptic scaling, a form of homeostatic plasticity, occurs in NAc neurons. We've published 15 papers using culture models, including PFC-NAc and PFC-VTA co-cultures, and continue to use them to test mechanistic hypotheses that are difficult to investigate in tissue from adult animals.

- a. Chao SZ, Ariano MA, Peterson DA, Wolf ME (2002) D1 dopamine receptor stimulation increases GluR1 surface expression in nucleus accumbens neurons. *J Neurochem* 83:704-712. PMID: 12390532
- b. Sun X, Zhao Y, Wolf ME (2005) Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. *J Neurosci* 25:7342-7351. PMID: 16093384
- c. Sun X, Milovanovic M, Zhao Y, Wolf ME (2008) Acute and chronic dopamine receptor stimulation modulates AMPA receptor trafficking in nucleus accumbens neurons co-cultured with prefrontal cortex neurons. *J Neurosci* 28:4216-4230. PMC2667279
- d. Sun X, Wolf ME (2009) Nucleus accumbens neurons exhibit synaptic scaling that is occluded by repeated dopamine pre-exposure. *Eur J Neurosci* 30:539-550. PMID: 19674091

3. Demonstrated that changes in AMPA receptor surface expression occur in animal models of addiction.

We were the first to study the role of changes in AMPAR surface expression in behavioral sensitization. These studies primed the field to examine changes in surface and synaptic AMPARs in more sophisticated behavioral models. As a starting point, we used biochemical techniques to define AMPAR subunit composition in the NAc of drug-naïve adult rats (mainly GluA1A2, some GluA2A3, and a small contribution of homomeric GluA1 Ca²⁺-permeable AMPARs or CP-AMPARs). This provided important basic information to all investigators interested in glutamate transmission in the NAc. Then, using a protein crosslinking assay which we developed, we showed that GluA1A2-containing AMPARs increase on the surface of NAc neurons after withdrawal from a sensitizing regimen of cocaine (2005) and that this is reversed by cocaine challenge (2007). Surprisingly, we found that similar plasticity does not occur in amphetamine-sensitized rats. Subsequently (2013), we showed that AMPAR upregulation tracks with incentive sensitization rather than locomotor sensitization to cocaine. Of 9 papers relevant to the findings described here, 4 are listed below.

- a. Boudreau AC, Wolf ME (2005) Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J Neurosci* 25: 9144-9151. PMID: 16207873
- b. Boudreau AC, Reimers JM, Milovanovic M, Wolf ME (2007) Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize upon cocaine challenge in association with altered activation of mitogen-activated protein kinases. *J Neurosci* 27:10621-10635. PMC2856315
- c. Reimers JM, Milovanovic M, Wolf ME (2011) Quantitative analysis of AMPA receptor subunit composition in addiction-related brain regions. *Brain Res* 1367:223-233. PMC3005033
- d. Wang X, Cahill ME, Werner CT, Christoffel DJ, Golden SA, Xie Z, Loweth JA, Marinelli M, Russo SJ, Penzes P, Wolf ME (2013) Kalirin-7 mediates cocaine-induced AMPA receptor and spine plasticity, enabling incentive sensitization. *J Neurosci* 33:11012-11022. PMC3718375

4. Characterized novel forms of cocaine-induced plasticity in the NAc that mediate incubation of cocaine craving.

In partnership with Dr. Kuei-Yuan Tseng, we characterized changes in synaptic transmission in the NAc after withdrawal from extended-access cocaine self-administration and their relationship to the incubation of cocaine craving. First, we used biochemical and electrophysiological approaches to demonstrate that CP-AMPARs accumulate in NAc synapses after ~1 month of withdrawal from extended-access cocaine self-administration; thereafter, CP-AMPAR activation is required for the expression of incubated cocaine craving. These findings raised awareness of the significance of CP-AMPARs for behavioral plasticity in the addiction field and beyond. Next we showed that incubation is accompanied by a dramatic rearrangement of group I mGluR plasticity in the NAc (2011). The normally occurring mGlu5- and CB1R-dependent LTD is abolished, whereas robust mGlu1-dependent LTD (expressed postsynaptically via CP-AMPAR internalization) emerges. By targeting the latter mechanism, mGlu1 positive allosteric modulators reduce incubated cocaine craving, suggesting these drugs might help recovering cocaine users maintain abstinence (2014). In 2016, we found similar CP-AMPAR plasticity after incubation of methamphetamine craving, and ongoing work suggests this may also hold for oxycodone. Our incubation work has resulted in 23 papers on which I am senior or co-senior (*) author. Four are listed below:

- a. Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature* 454:118-121. PMC2574981

- b. Loweth JA, Scheyer AF, Milovanovic M, LaCrosse AL, Flores-Barrera E, Werner CT, Li X, Ford KA, Le T, Olive MF, Szumlinski KK, Tseng KY, Wolf ME (2014) Synaptic depression via positive allosteric modulation of mGluR1 suppresses cue-induced cocaine craving. *Nat Neurosci* 17(1):73-80. PMC3971923
- c. Scheyer AF, Loweth JA, Christian DT, Uejima J, Rabe R, Le T, Dolubizno H, Stefanik MT, Murray CH, Sakas C, Wolf ME (2016) AMPA receptor plasticity in accumbens core contributes to incubation of methamphetamine craving. *Biological Psychiatry* 80: 661-670. PMC5050076
- d. Christian DT, Stefanik MT, Bean LA, Loweth JA, Wunsch AM, Funke JR, Briggs CA, Lyons J, Neal D, Milovanovic M, D'Souza GX, Stutzmann GE, Nicholson DA, Tseng K-Y, Wolf ME (2021) GluN3-containing NMDA receptors in nucleus accumbens core are required for incubation of cocaine craving. *J Neurosci* 41: 8262-8277. PMC8482856

5. Characterized regulation of protein translation in the NAc under control conditions and after incubation.

In 2014, we made the unexpected observation that inhibiting protein translation in brain slices prepared from rats that had undergone incubation of cocaine craving led to a normalization of CP-AMPAR levels in NAc MSNs. This led us to hypothesize that dysregulation of protein translation contributed to CP-AMPAR accumulation and incubation of cocaine craving. Glutamate receptor-mediated regulation of protein translation is important in hippocampal synaptic plasticity but nothing was known about such regulation in the NAc until a series of studies we conducted using both cultured NAc MSNs and adult NAc tissue. We found that protein translation in the NAc is regulated by group I mGluRs and NMDARs, with both similarities and differences compared to mechanisms established in hippocampus, and that translation is dysregulated at several levels after incubation of cocaine craving. A particularly interesting finding is that GluA1 translation is increased in the NAc after incubation of craving. This likely contributes to increased abundance and synaptic insertion of homomeric GluA1 CP-AMPARs. We are extending this work by studying the role of retinoic acid-mediated translational regulation and through the use of translating ribosome affinity purification (TRAP).

- a. Werner CT, Stefanik MT, Milovanovic M, Caccamise A, Wolf ME (2018) Protein translation in the nucleus accumbens is dysregulated during cocaine withdrawal and required for expression of incubation of cocaine craving. *J Neurosci* 38(11):2683-2697. PMC5852654
- b. Stefanik MT, Milovanovic M, Werner CT, Spainhour JCG, Wolf ME (2018) Withdrawal from cocaine self-administration alters the regulation of protein translation in the nucleus accumbens. *Biol Psychiatry* 84(3):223-232. PMC6054574
- c. Loweth JA, Reimers JM, Caccamise A, Stefanik MT, Woo KKY, Chauhan NM, Werner CT, Wolf ME (2018) mGlu1 tonically regulates levels of calcium-permeable AMPA receptors in cultured nucleus accumbens neurons through retinoic acid signaling and protein translation. *Eur J Neurosci* 50(3):2590-2601. PMC7556732
- d. Kawa AB, Hwang EK, Funke JR, Zhou H, Costa-Mattioli M, Wolf ME (2022) Positive allosteric modulation of mGlu1 reverses cocaine-induced behavioral and synaptic plasticity through the integrated stress response and oligophrenin-1. *Biological Psychiatry* 2022 May 14:S0006-3223(22)01245-8. doi: 10.1016/j.biopsych.2022.05.008. PMID 35871097.

Complete List of Published Work in MyBibliography (113 primary papers and >30 invited reviews/book chapters):

<http://www.ncbi.nlm.nih.gov/sites/myncbi/marina.wolf.1/bibliography/40537600/public/?sort=date&direction=asc>
ending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Yasuda, Ryohei

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POSITION TITLE: Research Group Leader, Scientific Director

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Keio University, Japan	BS	1990-1994	Physics
Keio University, Japan	MS	1994-1996	Physics
Keio University, Japan	PhD	1996-1998	Physics
Keio University, Japan		1999-2000	Biophysics
Cold Spring Harbor Laboratory, NY		2000-2005	Neuroscience

A. Personal Statement

I have the expertise, in particular optical measurements of biological systems, as well as the leadership and motivation necessary to successfully carry out the proposed work. As a graduate student in physics with Dr. Kazuhiko Kinoshita, I showed that the F1-ATPase is a rotary motor using a novel single-molecule optical assay. As a postdoctoral fellow at Cold Spring Harbor with Dr. Karel Svoboda, I studied calcium signaling using 2-photon microscopy in combination with electrophysiology. I also have developed 2-photon fluorescence lifetime imaging microscopy. As the PI of several projects, I have developed techniques to image signaling proteins in single dendritic spines using 2-photon fluorescence lifetime imaging microscopy. More recently, I have developed new genome-editing methods to insert tag sequences at specific loci of the genome using homology-directed repair. Furthermore, I have developed new optogenetic methods to manipulate proteins during learning and memory. These molecular tools are distributed to the community through Addgene. In addition, I collaborate broadly to conduct multidisciplinary research combining optics, biophysics, biochemistry, and molecular biology. Thus, I have a demonstrated record of successful and productive research projects in an area of high relevance in optical imaging of synaptic signaling in the healthy and diseased brain, and my expertise and experience have prepared me to conduct research of deciphering molecular mechanisms underlying synaptic, circuit and behavioral plasticity.

- a) H. Nojita†, **R. Yasuda**†, M. Yoshida, and K. Kinoshita, Jr. (1997) Direct observation of the rotation of F₁-ATPase. **Nature** 386 : 299-302.
- b) S. C. Harward†, N. G. Hedrick†, C. E. Hall, P. Parra-Bueno, T. A. Milner, E. Pan, T. Laviv, B. L. Hempstead, **R. Yasuda*** & J. O. McNamara (2016) Autocrine BDNF-TrkB signalling within a single dendritic spine. **Nature**, 538: 99-103. PMID: 27680698
- c) Mikuni T†, Nishiyama J†, Sun Y, Kamasawa N, **Yasuda R*** (2016) High-throughput, high-resolution mapping of protein localization in mammalian brain by in vivo genome editing. **Cell**. 165: 1803-17. doi: 10.1016/j.cell.2016.04.044. PMCID: PMC4912470
- d) Murakoshi H†, Shin ME†, Parra-Bueno P, Szatmari EM, Shibata ACE, **Yasuda R*** (2017) Kinetics of endogenous CaMKII required for synaptic plasticity revealed by optogenetic kinase inhibitor. **Neuron**. 94: 37-47. doi: 10.1016/j.neuron.2017.04.027. PMCID: PMC5425291

*Corresponding author, †Co-first author

B. Positions and Honors

Positions

7/2005 – 5/2012	Assistant Professor, Duke University Medical Center
9/2009 – 5/2012	Early Career Scientist, Howard Hughes Medical Institute
6/2012 – now	Scientific Director, Max Planck Florida Institute for Neuroscience

Awards and fellowships

2/2000	Young Fluorescence Investigators Award, Fluorescence Subgroup of the Biophysical Society of USA. (New Orleans, LA)
6/2004	Distinguished Alumni Award, Faculty of Science and Technology, Keio University
1/2003 – 6/2008	Career Award at the Scientific Interface, Burroughs Wellcome Fund
1/2006 – 12/2007	Program in Brain and Immuno-imaging, Dana Foundation
7/2006 – 6/2009	Research Award, Whitehall Foundation
9/2006 – 9/2008	Alfred P. Sloan Fellow, Alfred P. Sloan Foundation
8/2008 – 7/2010	New Investigator Award, Alzheimer's Association
9/2009 – 5/2012	Early Career Scientist, Howard Hughes Medical Institute
10/2009	Research Award for Innovation in Neuroscience, Society for Neuroscience
5/2010, 5/2011	Albert and Ellen Grass Faculty award, Marine Biological Laboratory
5/2011	Ruth and A. Morris Williams Faculty Research Prize, Duke University
9/2015	NIH Director's pioneer award
2017	Mikuni et al (2016, Cell) is selected as "Best of Cell 2016".
7/2017	Nakaakira Tsukahara Award, Japanese Neuroscience Society
1/2018	Scientific Innovation Award, Brain Research Foundation
9/2018	Gill Transformative Investigator Award, Indiana University
6/2020	NIH/NINDS, Outstanding Investigator Research Program Award (R35)

Activities

2007 – now	Co-organizer, Fluorescent proteins and biological sensors (Janelia farm, HHMI)
2008	Guest editor, Special issue "Optogenetic probes" Brain Cell Biology.
2008 – 2017	Faculty, Neurobiology course, Marine Biological Laboratory (Woods Hole, MA)
2015 – now	Organizer, Neuroimaging Techniques course, Max Planck Florida Institute for Neuroscience
2015, 2017	Chair (2017), Vice-Chair (2015) Gordon Research Conference, Dendrites: Molecules, Structure & Function
2019 – now	Review editor, eLife

C. Contributions to Science

1. Imaging signal transduction during synaptic plasticity

Long-term potentiation (LTP) of synaptic connections is an important mechanism for learning and memory. LTP is induced by intracellular signaling triggered by Ca^{2+} elevation in dendritic spines, tiny ($\sim 0.1 \text{ fL}$) postsynaptic compartments. The mechanisms that transduce this transient Ca^{2+} elevation ($\sim 100 \text{ ms}$) into a long-lasting synaptic change have been elusive. To address this question, we developed a new technique based on fluorescence resonance energy transfer (FRET) to visualize the spatiotemporal dynamics of signaling activity in single dendritic spines undergoing LTP. Using this new technique, we succeeded in imaging the activity of several signaling proteins in neurons during LTP. Our results indicated that Ca^{2+} elevation in spines is relayed in multiple stages. First, Ca^{2+} elevation in the spine activates a signaling protein CaMKII, the activity of which decays over ~ 10 seconds. Then, CaMKII activates downstream signaling proteins Cdc42, Rho and Rac, which relay this transient signal into signals lasting tens of minutes. These long-lasting signals are necessary to induce sustained increase in postsynaptic sensitivity. In addition, our approach has revealed the spatial regulation of signaling processes. Notably, it was found

that the activation of Cdc42 and Rac is regulated by autocrine BDNF-TrkB signaling within the stimulated spine, suggesting the complexity of signal integration in dendritic spines.

- a) S.-J. Lee, Y. Escobedo-Lozoya, E. M. Szatmari and **R. Yasuda*** (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* (Article) 458: 299-304. PMCID: PMC2719773
- b) Murakoshi H, Wang H, **Yasuda R*** (2011) Localized, persistent activation of Rho GTPases during long-term structural plasticity induced in single dendritic spines. *Nature*. 472:100-4. PMCID: PMC3105377
- c) Harward SC†, Hedrick NG†, Hall CE, Parra-Bueno P, Milner TA, Pan E, Laviv T, Hempstead BL, **Yasuda R***, McNamara JO. (2016) Autocrine BDNF–TrkB signalling within a single dendritic spine. *Nature*. 538: 99-103 doi: 10.1038/nature19766. PMCID: PMC5398094
- d) Hedrick NG†, Harward SC†, Hall CE, Murakoshi H, McNamara JO, **Yasuda R*** (2016) Rho GTPase complementation underlies BDNF-dependent homo- and heterosynaptic plasticity. *Nature*. 538: 104-108 doi: 10.1038/nature19784. PMCID: PMC5361895

2. The mechanisms and roles of signal compartmentalization and spreading

During synaptic plasticity, some signals are compartmentalized in the stimulated spines while others spreads into the shaft and surrounding spines. The compartmentalization of signaling is presumably required for synapse specificity while the spread of signals is important for hetero-synaptic plasticity as well as signals to subcellular compartments in dendritic shafts such as endosomes. The degree of spreading can be explained by the competition between inactivation and diffusion of the signaling protein. We found that the PKC, CaMKII and Cdc42 pathways are compartmentalized in spines undergoing LTP, producing synapse-specific signaling. In contrast, Rho, Rac and Ras activity spreads along a short stretch (micrometers) of dendrite, influencing plasticity of neighboring synapse. Ras signaling further activates ERK signaling, which is transmitted a long distance from potentiated synapses to the nucleus to regulate gene transcription. The nuclear signaling integrates biochemical signaling distributed over multiple dendritic branches. Thus, we demonstrated how the spatiotemporal dynamics of signaling are orchestrated during LTP from milliseconds to tens of minutes and from single synapses to the nucleus.

- a) Zhai S, Ark ED, Parra-Bueno P, **Yasuda R*** (2013) Long-distance integration of nuclear ERK signaling triggered by activation of a few dendritic spines. *Science*. 342: 1107-11. PMCID: PMC4318497
- b) Tang A, **Yasuda R*** (2017) Imaging ERK and PKA activation in single dendritic spines during structural plasticity. *Neuron*. 93: 1315-1324. doi: 10.1016/j.neuron.2017.02.032. PMCID: PMC6042854.
- c) Colgan LA*, Mo H, Misler JA, Parra-Bueno P, Moran MC, Leitges M, **Yasuda R*** (2018) PKC α integrates spatiotemporally distinct calcium and BDNF signaling to facilitate plasticity. *Nat Neurosci*. 21: 1027-1037. doi: 10.1038/s41593-018-0184-3.
- d) Chang J-Y, Nakahata Y, Hayano Y, **Yasuda R** (2019) Mechanisms of Ca^{2+} /Calmodulin-dependent kinase II activation in single dendritic spines. *Nat Commun*. 10:2784. doi: 10.1038/s41467-019-10694-z.

3. Development of new tools for analyzing function and localization of synaptic molecules

Biochemical signaling in dendritic spines plays an important role in synaptic plasticity. To elucidate the operation principle of the complex signaling network, we have been developing several tools to image and manipulate signal transduction in single dendritic spines. Collaborating with Dr. Lin, we develop new fluorophores that is suited to multi-color 2pFLIM imaging, which was used for imaging CREB activity in vivo. Furthermore, we developed a method to image endogenous proteins using CRISPR-based in vivo genome editing (single-cell labeling of endogenous proteins via CRISPR-assisted homology directed repair or SLENDR). In addition, we developed an optogenetic inhibitor for CaMKII. These tools we developed are expected to help make sense of biochemical computation in dendritic spines.

- a) Mikuni T†, Nishiyama J*†, Sun Y, Kamasawa N, **Yasuda R*** (2016) High-throughput, high-resolution mapping of protein localization in mammalian brain by in vivo genome editing. *Cell*. 165: 1803-17. doi: 10.1016/j.cell.2016.04.044. PMCID: PMC4912470
- b) Nishiyama J†, Mikuni T*†, **Yasuda R*** (2017) Virus-mediated genome editing via homology-directed repair in mitotic and postmitotic cells in mammalian brain. *Neuron*. 96: 755-768, doi: 10.1016/j.neuron.2017.10.004 PMCID: PMC5691606
- c) Murakoshi H*†, Shin ME†, Parra-Bueno P, Szatmari EM, Shibata ACE, **Yasuda R*** (2017) Kinetics of

- endogenous CaMKII required for synaptic plasticity revealed by optogenetic kinase inhibitor. *Neuron*. 94: 37-47. doi: 10.1016/j.neuron.2017.04.027. PMCID: PMC5425291
- d) Laviv T*, Scholl B, Parra-Bueno P, Foote B, Zhang C, Yan L, Hayano Y, Chu J, **Yasuda R*** (2020) In Vivo Imaging of the Coupling between Neuronal and CREB Activity in the Mouse Brain. *Neuron*. 105(5):799-812.e5. doi: 10.1016/j.neuron.2019.11.028.

4. Contributions to other projects

Using our tools to image and manipulate the activity of signaling proteins and protein-protein interaction in single synapses, we broadly collaborate with other groups. These projects provided insights into the molecular assembly and signaling ex vivo and in vivo in development, synaptic plasticity, and learning.

- a) Zou W, Dong X, Broederdorf TR, Shen A, Kramer DA, Shi R, Liang X, Miller DM Third, Xiang YK, **Yasuda R**, Chen B, Shen K (2018) Dendritic guidance receptor complex brings together distinct actin regulators to drive efficient F-actin assembly and branching. *Dev Cell*. 45: 362-375. doi: 10.1016/j.devcel.2018.04.008.
- b) Adler A, Zhao R, Shin ME, **Yasuda R**, Gan WB (2019) Somatostatin-expressing interneurons enable and maintain learning-dependent sequential activation of pyramidal neurons. *Neuron*. 102: 202-216. doi: 10.1016/j.neuron.2019.01.036
- c) Saneyoshi T, Matsuno H, Suzuki A, Murakoshi H, Hedrick NG, Agnello E, O'Connell R, Stratton MM, **Yasuda R**, Hayashi Y (2019) Reciprocal Activation within a Kinase-Effector Complex Underlying Persistence of Structural LTP. *Neuron*. 102: 1199-1210. doi: 10.1016/j.neuron.2019.04.012.
- d) Chen LF, Lyons MR, Liu F, Green MV, Hedrick NG, Williams AB, Narayanan A, **Yasuda R**, West AE (2020) The NMDA receptor subunit GluN3A regulates synaptic activity-induced and myocyte enhancer factor 2C (MEF2C)-dependent transcription. *J Biol Chem*. jbc.RA119.010266. doi: 10.1074/jbc.RA119.010266.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/ryohei.yasuda.1/bibliography/40596890/public/?sort=date&direction=asc>

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01MH080047	(Yasuda, PI)	4/5/07 – 12/30/23
NIH / NIMH		
'Mechanisms of Ras Signaling in Single Synapses'		
The goal of this project is to identify the factors that control Ras spatiotemporal regulation in dendrites and spines.		
Role: PI		
R35NS116804	(Yasuda, PI)	5/1/20 – 4/30/2028
Title: Neuronal Intracellular Signaling Underlying Synaptic, Circuit and Behavioral Plasticity.		
The goal of this project is to develop tools that allows us to define spatiotemporal dynamics of endogenous postsynaptic intracellular signaling during synaptic plasticity and learning and memory in behaving animals.		
Role: PI		

Completed Research Support

1DP1NS096787	(Yasuda, PI)	9/30/15 – 7/31/20
NIH / NINDS		

'Deciphering Biochemical Networks in Single Dendritic Spines'

The goal of this project is to establish a high-throughput system for the development and optimization of signaling sensors, and a fully automated system for imaging signal transduction during plasticity in single dendritic spines

Role: PI

Scientific Innovation Award (Yasuda, PI)

1/1/18 – 12/31/20

Brain Research Foundation

The goal of this project is to image the dynamics of interactions between endogenous proteins with the resolution of single dendritic spines.

Role: PI

R01GM094483 (Izard, PI)

7/15/16 – 4/30/20

(NIH / GM / The Scripps Research Institute Subaward 5-20927)

'Mechanisms directing adherens junctions and actin network interactions'

The goal of this project is to determine to what degree alpha-catenin homodimer and alpha-catenin/beta-catenin heterodimer exist near the adherens junctions at or near the plasma membrane.

Role: Co-Investigator

R01MH111486 (Yasuda, Gan, MPIs)

9/15/16 – 6/30/19

NIH / NIMH (BRAIN)

'Optogenetic signaling inhibitors for studying brain plasticity'

The goal of this proposal is to develop a new technique based on genetically encoded light- inducible kinase inhibitors to resolve the spatiotemporal dynamics of signaling required for synaptic and behavioral plasticity *in vivo*.

Role: PI

Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: TRUSTEES OF INDIANA UNIVERSITY

UEI: YH86RTW2YVJ4

Street1*: 702 N Walnut Grove Ave

Street2:

City*: BLOOMINGTON

County: MONROE

State*: IN: Indiana

Province:

Country*: USA: UNITED STATES

Zip / Postal Code*: 474052201

Project/Performance Site Congressional District*: IN-009

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* Yes No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes NoIf YES, check appropriate exemption number: 1 2 3 4 5 6 7 8If NO, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number D16-00587

3. Is proprietary/privileged information included in the application?* Yes No**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an Yes No environmental assessment (EA) or environmental impact statement (EIS) been performed?

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* Yes No

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* 2022_Overview_Abstract_20221001.pdf**8. Project Narrative*** Project_Narrative_final.pdf**9. Bibliography & References Cited** 2022_References_Overviw_20221001.pdf**10. Facilities & Other Resources** Facilities_and_resources_2022.pdf**11. Equipment** Major_Equipment_Available_to_the_C3A-final.pdf**12. Other Attachments** Center_Organizational_Structure.pdf
Table_of_Research_Core_Utilization.pdf

Abstract

We are proposing to establish the IU Bloomington Center for Cannabis, Cannabinoid, and Addiction (IUB-C3A) as a NIDA Core Center for Excellence to serve addiction researchers both in the central Midwest and across the nation. This Center will offer core services to further our understanding of fundamental brain processes leading to or following the use of addictive drugs, particularly cannabis. The IUB-C3A will consist of two service cores, a pilot project core, and an administrative core. The Administrative Core will provide a well-defined structure for efficient center management, for public outreach, for organizing the Center's courses, as well as for preserving and making easily accessible the data generated by the Center's scientists. The Bioactive Lipid Mediators Core (BLMC) will provide analytical service for detecting cannabinoids and other bioactive lipid mediators in biological samples and run a summer course directed towards underserved minority college students interested in STEM careers. The MultiScale Imaging Core (MSIC) will offer services and training across a range of light microscopic imaging modalities. The multiphoton resource of the MSIC will include training and access to longitudinal *in vivo* imaging of calcium and other sensors (e.g., neuromodulators such as endocannabinoids, dopamine, and serotonin) from very young ages as well as long-range pathway tracing in "cleared" brain specimens. The STORM/confocal resource of the MSIC will offer users the opportunity to perform correlative structure/function studies from the macro- to nanoscale level and will also offer courses on these techniques. Both cores will emphasize innovation and integration of their respective techniques, as outlined in the proposal. The Pilot Project Core will solicit pilot projects from C3A Affiliates and investigators outside of the drug abuse field and mentor them through the process of obtaining data and NIDA support for their research ideas. The PIs for the IUB-C3A have a long history of productive collaborations, including publishing more than sixty papers together and holding several MPI NIH grants. The IUB-C3A is conceptualized as a resource that will offer opportunities for other addiction investigators across the Central Midwest (Southern Illinois, Indiana, Kentucky, Ohio, and West Virginia), a region strongly affected by drug addiction, and across the nation. The IUB-C3A aims to increase diversity in addiction research through a combination of summer experiences and pilot project programs targeted to under-represented populations in neuroscience and addiction. A core goal of these programs is to bring talented individuals into the field of addiction research. We anticipate that they will apply creative directions, rigorous experimental approaches, and novel ways of thinking to a major public health problem. The strong support of Indiana University to this endeavor is evident by generous matching funds for both equipment purchases and our diversity programs. All of these factors predict that the IUB-C3A will become a regional and national resource for better understanding and developing treatments for addictive disorders and their consequences.

Project Narrative

We are proposing to establish the *Indiana University Bloomington Center for Cannabis, Cannabinoids, and Addiction* to support drug abuse researchers across the central Midwest and around the nation. The Center consists of research cores to support the work of these researchers as they measure lipid biomarkers associated with addiction and apply cutting-edge optical imaging to learn how drug addiction affects brain anatomy and function. The Center will also have a strong educational component to bring more researchers into drug addiction research, particularly drawing from populations not well-represented in the field and to train scientists in the cutting-edge technologies used in the Center.

Facilities and Resources

Indiana University at Bloomington: Founded in 1820, Indiana University (IU) is the largest public university in the State of Indiana, consisting of two core campuses— Bloomington (IUB) and Indianapolis—and six statewide regional campuses. IUB is a public research university in Bloomington, Indiana. It is the flagship institution of the IU system with over 40,000 students. It has numerous schools and programs, including the Jacobs School of Music, the Luddy School of Informatics, Computing, and Engineering, the O'Neill School of Public and Environmental Affairs, the Kelley School of Business, the School of Public Health, the School of Nursing, the School of Optometry, the Maurer School of Law, the School of Education, the Media School, and the Hamilton Lugar School of Global and International Studies. IUB is home to multiple research centers including the Gill Center for Biomolecular Science, which serves as the home for the IU Bloomington Center for Cannabis, Cannabinoids, and Addiction. Drs. Hájos, Lu and Mackie are core members of the Gill Center and Drs. Bradshaw and Katona are Gill Center Affiliates, with full access to Gill Center resources. All four PIs of the C3A are members of the Department of Psychological and Brain Sciences.

Gill Center: The Gill Center is a research center embedded within the College of Arts and Sciences at IUB and consists of six endowed chairs and their labs. Its primary goals are to conduct innovative neuroscience research, strengthen the IUB neuroscience community, train and educate neuroscientists with the tools and skills to prosper in a wide range of employment settings, and to engage with academia, industry, and the public. Research in the Gill Center has a strong emphasis in addressing drug addiction, particularly aspects of drug addiction that involves cannabis, synthetic cannabinoids, and the endocannabinoid system. The Gill Center was established in 1999 through a generous gift from Linda and Jack Gill. The central goals of the Center are to establish a community of scientists that: (1) conduct innovative molecular and cellular neuroscience research, disseminate those findings in premier journals, and lead the national research agenda through their participating in societies and funding agencies; (2) foster and participate in the broad neuroscience community at Indiana University, building relations that enable new solutions to major research questions. (3) recruit, educate, and train outstanding students and post-doctoral fellows in molecular and cellular neuroscience, and provide them with the community in which to pursue translational and other forms of collaborative and multi-level research; (4) encourage collaborations between IUB and other research universities and bioscience companies as a means of increasing IUB's national reputation as an outstanding campus for neuroscience research. Drs. Hájos, Hohmann (IUB Affiliate), Lu, and Mackie all hold endowed chairs in the Gill Center, while Drs. Bradshaw and Katona are affiliate members of the Gill Center.

Department of Psychological & Brain Sciences: The Department is home to 63 faculty and over 50 research laboratories. PBS has expertise in molecular, cellular, behavioral, cognitive, and computational neuroscience; developmental, cognitive, social psychology; clinical and translational science. The Department embodies a culture of interdisciplinary collaboration among discrete sub-discipline areas. PBS serves over 110 graduate students and 36 post-doctoral fellows and researchers, indicating a strong commitment to scientific training. In addition, the Department instructs 1,436 undergraduate Psychology major students and 368 Neuroscience major students, which represents the largest major in the College of Arts and Sciences. The Department currently ranks 6th in the nation in external funding among Psychology Departments. PBS has world-class equipment and laboratories. Lastly, some PBS faculty have labs within other IU schools and even other campuses, which has cultivated unique opportunities for research and practicum experiences for our students. All of the PIs of the C3A are full professors in PBS.

A. The Bradshaw Laboratory

Heather Bradshaw is a Professor of Psychological and Brain Sciences at Indiana University, Bloomington. Her lab is located on the first floor of MSBII adjacent to Katona's, Lu's and Mackie's lab and next to the shared Neuroscience Core Lab. The Bradshaw laboratory occupies ~1000 square feet of laboratory space. In this space are 3 fume hoods, 4 wet bench areas with chemical-safe sinks, and the following instruments that will be used to conduct the research outlined in this proposal. API 3000 triple quadrupole mass spectrometer with attached computer analytical station; Shimadzu HPLC system comprised of 2 10AdVP pumps, SIL 20AC Prominence Autosampler, CBM-20A controller; Compressed air, nitrogen, and zero air generators; Revco 24.4 cu ft -80 Freezer; SterilGARD III Advance laminar flow hood; Thermo Corp Forma series II water-jacketed CO₂ incubator; Sorvall RT-7 plus table-top centrifuge; 2-20 cu ft refrigerator (4C)/freezer (-20C) units. In an adjacent shared Neuroscience Core lab, the following equipment is used for lipid extraction techniques as needed for a minimal fee: Beckman Coulter Avanti J-25 I Centrifuge and Tuttnauer Brinkman 3870E Autoclave.

Dr. Bradshaw occupies a 125sq ft office on the same floor as her laboratory and has an additional 200sq ft office for research assistants and students on the same floor. In addition, there are multiple desktop computers equipped with Microsoft Office, Prism, SPSS 16, Adobe Suite, ChemDraw, and Analyst software for mass spectrometric analysis.

B. The Katona Laboratory

Istvan Katona is an endowed Naus Family Chair in Addiction Sciences and Professor at the Department of Psychological and Brain Sciences at Indiana University, Bloomington. His lab is located adjacent to the Bradshaw lab and next to the Lu and Mackie labs. The Katona lab (MSBII-154) occupies 1539 sq ft. In this space there are 2 fume hoods, 9 wet bench areas with chemical-safe sinks. Two adjacent research rooms host the N-STORM super-resolution imaging setup (MSBII-165) and the A1-HD25 confocal microscope (MSBII-169). These equipment and rooms are organized in accordance with the relevant laser safety regulations. The opposite room hosts a high-power analysis computer (MSBII-178), where the slide scanner and its computer will also be located. These rooms have fiber cable data transfer capacity at GB/s speed and give access to IU-based computing resources. Additional instruments that will be used to conduct the research outlined in this proposal are located in MSBII-153: a patch-clamp set-up that will be used to fill individual neurons, the Vibratome for slicing and the incubators for Pharmacostorm pharmacoprobe exposure; in MSBII-163: a fume hood, a Gilson perfusion setup and a Vibratome for slicing of 10 µm-thick free-floating brain sections; in MSBII-154: a wet lab with all necessary small equipment to carry out immunostaining including shakers, incubators, fridges, freezers. Dr. Katona has a 125 sq ft office on the same floor as his laboratory and has an additional 200 sq feet office for research scientists and students on the same floor. In addition, there are multiple desktop computers equipped with Microsoft Office, Prism, SPSS 16, Adobe Illustrator for data analysis and data visualization.

C. The Lu Laboratory

Hui-Chen Lu is an endowed Linda and Jack Gill Chair and Professor of Neuroscience at Indiana University. Her lab is located in the Multiple Science Building II (MSBII). Dr. Lu's lab occupies 2000 sq ft of the 20,000 sq ft devoted to neuroscience research. In addition to lab space, the building offers dedicated office space for faculty, research scientists, post-docs, and graduate students, undergraduate students as well as ample space for shared equipment (see below). The Lu laboratory is composed of several rooms: MSBII-149 (700 sq ft) for general molecular/biochemistry/histology, MSBII-153 for (600 square feet) for our electrophysiology set ups, MSBII-159 (200 sq ft) for multiphoton microscope, MSBII-174 (100 sq ft) for cell cultures, MSBII-155 (100 sq ft) for storage, MSBII-143 (100 sq ft) for surgical procedures, MSBII-149 (100 sq ft) for mini-confocal microscope. In addition, we have four offices for PI, scientists, post-docs, and students (MSBII 108, 112,114,132). The lab is outfitted with the following equipment that will be used for this proposal: multiple -20°C and -80°C freezers and fridge/freezer combinations are available for tissue and reagent storage. This space has the necessary equipment (PCR machines, bench top centrifuge, benchtop ultraspeed centrifuge, shakers, gel apparatus, plate readers, water baths, mixers, temperature blocks, etc) for molecular biological work. This is also where biochemical experiments (e.g., Western blotting) are done. Tissue culture rooms with two incubators, two laminar flow hoods, and one inverted microscope. Electrophysiology room has with two whole-cell patch clamp setups, each include a TMC vibration table, Zeiss Axioskop FS 2+ microscopes (with epifluorescence and Gibraltar stages), Axon 700B amplifiers, digitata 1440A, Scientifica micromanipulators and LED lights for optogenetics. There is also a Sutter pipette puller and Leica vibratome. In addition, a Leica mini-confocal microscope for routine immunofluorescence imaging at gross levels and a Nikon multiphoton microscope connected to Insight laser for imaging live animals have been set up. We use a computerized Stereotaxic Injection device (Leica) that has been set up in the animal procedure room (within the vivarium) for AAV injections.

The Lu lab has eighteen computers with internet connections and printers and the necessary programs are available for data analysis, image analysis, correspondence and manuscript preparation. Two Dell Pentium systems are linked to the electrophysiology rigs for data acquisition and analysis. One work station for an Axioskop microscope and one station for a Leica mini-confocal data acquisition have been set up. Two advanced work stations are for multiple photon microscopy data acquisition and analysis. Imaris software on Mac Pro is used for cell counting and neuronal morphological analysis.

D. Other Resources

Animal: The Laboratory Animal Resources (LAR) unit is 5500 sq-ft temperature and humidity controlled animal facility with secured access located one floor below Lu laboratory. LAR is directed by Dr. Karen Rogers, DVM and is AAALAC accredited. Animal housing occupies 3000 sq ft, and 2500 sq ft is for support resources (cage cleaning, surgery, procedure rooms, food/bedding storage, office space, etc.). The facility is maintained by a staff of full-time animal caretakers and two full time veterinarians. They are available seven days per week. Facility staff will order, house, care for, and dispose of the rodents used in this project. They are licensed by the USDA, and adhere strictly to the animal welfare guidelines established by the NIH. Two rooms (052 and 063) in the facility for animal housing are dedicated for the Lu laboratory's research use and four room (048, 056, 064, and 066) are reserved for Dr Mackie's lab's use. There are also shared procedure rooms and a 1000 sq ft suite of animal behavior testing rooms (see below). Our Department of Comparative Medicine, in coordination with Bloomington Institutional Animal Care and Use Committee (BIACUC), is responsible for the health and care of all animals used in University teaching and research.

Core neuroscience lab: This shared facility located adjacent to Lu and Mackie labs includes the following equipment: low and ultrahigh speed preparative centrifuges, a LiCor Odyssey scanner, a Flexstation 3, a Spectramax, a Leica cryostat, a BioRad spinning disk confocal microscope, one Nikon upright microscope outfitted with Neurolucida, one upright Nikon microscope outfitted with a Stereo Investigator package, a recently installed Nikon A1HD25 confocal system, a recently installed Nikon STORM/C2 super resolution microscope, a custom-modified Intracellular Imaging single cell calcium imaging station, and a scintillation counter. In addition, Perkin-Elmer Enspire multi-modal plate reader was acquired in 2013 for ultrasensitive luminometer measurement, dynamic mass redistribution, time-resolved fluorescence measurements, and Alphascreen (proximity based) second messenger assays, and an ABI QuantStudio 7 Flex Real-time PCR system was purchased in 2017. A senior research associate oversees the METACyt Neuroscience lab to ensure proper user training and to coordinate use, maintenance and repair of the equipment.

Behavioural testing suite: An eight room behavioral suite (042A-K), designed by Dr. Mackie and his colleagues (Drs. Hohmann and Crystal) in 2010, is located on the ground floor of MSBII, contiguous with the animal facility. The behavioral suite is fully equipped to permit simultaneous testing in six adjacent procedure rooms. The behavioral suite houses MSBII investigator lab equipment consisting of: computer control and interface equipment (042A, 042E), 6 mouse operant chambers equipped for drug self administration (042C), mouse memory assessment (Morris water maze), elevated plus maze, rotarod (042J), mechanical (electronic vonFrey) and cold allodynia testing (042H), activity boxes, automated mouse pain assays including hot/cold plate, and Hargreaves test of plantar thermal sensitivity (042J), and procedure room with sink (042K). We have 6 custom configured three chamber conditioned place preference chambers for mice (042C) designed by Dr. Hohmann to permit assessment of evoked pain, spontaneous pain and locomotor activity. Most behavioral tests are recorded by Ethovision software for off line, blinded analysis.

In addition, we have 12 rat operant chambers (042B), 16 rat self-administration chambers (042D), and 6 custom-designed rat conditioned place preference chambers (042F), (rat/mouse). The conditioned place chambers were custom-designed by Hohmann and fabricated by Med Associates (to permit assessment of spontaneous and evoked pain as well as locomotor activity within the chambers) and are analogous to our mouse chambers.

IUB shared core facilities: IUB has outstanding and well-supported core facilities. The three most relevant for this work include the Light Microscopy Imaging Center (LMIC) at IUB as well as the Center for Medical Genomics and the Center for Computational Biology and Bioinformatics at IUSM. The LMIC is an IU subsidized imaging center that includes an Applied Precision (AP) OMX, super high resolution microscope, a Leica SP5 scanning confocal, a Leica SP8 scanning confocal equipped with a resonance scanner mode for fast imaging and modules for FRAP, PRET, FLIM and spectral imaging, etc. This facility is available for use to qualified investigators 24/7 and is supervised by a PhD-level scientist who provides oversight, training for new users, as well as performing equipment upkeep. The Center for Medical Genomics is a state-of-the-art technology center that provides high-quality and high-throughput genomic services in timely manner. The center is equipped with Agilent bioanalyzer for RNA and DNA quality checking, next generation sequencing instruments, including Illumine HiSeq 4000, Illumine NextSeq 500, and life tech ion proton sequencing system. This core has extensive experience for both regular RNA-seq and single-cell RNA-seq. The Center for Computational Biology and Bioinformatics (CCBB) host many faculties and bioinformatics analysts to provide advanced bioinformatic analysis, such as data analysis for single cell RNA-seq to identify biological pathways and signaling cascades.

Machine shop: As members of the Department of Psychological and Brain Sciences, Drs. Lu, Mackie, and Bradshaw and their lab personnel have access to a completely equipped machine shop staffed by two full-time technicians who are available to custom fabricate testing equipment, perfusion chambers, dialysis blocks, etc. For example, they made a desk-top air-table to allow a mouse to be head-fixed clamped for multiphoton imaging, set up to move freely on a light-weight container as well as different sizes head frames to restrain mice of different ages, including young pups. This service is provided at the cost of materials. Similarly, the department also provides two full time computer technicians and an electronics technician for equipment repair. Again, these services are provided for the cost of materials.

Licensure: Dr. Mackie holds a DEA Schedule I license (RM0365684, expires 1-31-2023) and Schedule II-V license (RM0298504, expires 1-31-2023) as well as an Indiana State Pharmacy license (61100562B, expires 12/31/2023). The DEA Schedule I license covers the most relevant DEA drug codes for cannabinoid-related work: 7350, 7360, and 7370. Additional drug codes will be added if needed by specific experiments performed in the C3A.

Hazardous waste: IU maintains facilities to properly process and dispose of any hazardous waste generated in the course of these experiments. In addition, the IU regulations that mandate IU employees engaged in laboratory work undergo task-specific training (provided by IU) will be followed.

IU Bloomington Computing Resources

Central Information Technology organizations. The Indiana University Office of the Vice President for Information Technology (OVPIT) and University Information Technology Services (UITS) are responsible for delivery of core information technology and cyberinfrastructure services and support. OVPIT and UITS collectively have an annual budget of more than \$110,000,000 and employ more than 1,100 full-time staff members.

Federal systems security policy and federal funding agency policy compliance. The IU high performance computing and storage systems described here are managed and administered in ways that meet National Institute of Standards and Technology (NIST) 800 security standards. OVPIT and UITS also comply with the NIH Grants Policy Statement.

1. Physical facilities

IU's cyberinfrastructure leverages the university's unusual arrangement of two major research campuses separated by 50 miles and connected by university-owned optical networks. This creates resilience in case of natural or man-made disaster and provides an outstanding testbed for development of grid and distributed computing innovations.

1.1. IU Bloomington Data Center

The IU Bloomington Data Center provides a highly secure and green environment for IU's largest computational and storage systems. The facility is secured with card-key access, biometric authentication, and 7 x 24 x 365 video surveillance. Only staff with systems or network administration privileges have access to the machine room. Fire suppression is provided by a double-interlock system accompanied by a Very Early Smoke Detection Apparatus (VESDA). Three circuits feed the Data Center, travelling redundant physical paths. Any two circuits can fully power the building.

1.2. Informatics & Communications Technology Complex

The Informatics and Communications Technology Complex (ICTC) houses IU's Data Center in Indianapolis. The ICTC is secured with card-key access and 7 x 24 x 365 video surveillance. Fire suppression is provided by a dry-pipe, pre-action sprinkler system in accordance with university risk management policy. The electrical design for the ICTC includes UPS service and generator backup for the entire facility.

1.3. Cyberinfrastructure Building

The Cyberinfrastructure Building (CIB) on the Bloomington campus opened in August 2011. Located in Technology Park East, along with the IU Bloomington Data Center and the IU Innovation Center, the CIB houses University Information Technology Services (UITS) staff. There is ample desk space and administrative support for project activities.

1.4. Sustainability of physical facilities

IU Bloomington's Data Center is significantly more efficient than former facilities. The walls are made of 9,000 cubic yards of poured concrete that offers several sustainability features including: longevity; thermal mass that

decreases heating and cooling needs; recycled content; minimal waste; and regional production. The single-story facility is surrounded by an earthen berm, offering added insulation and protection from weather events, including tornadoes up to and including category 5

2. Equipment

Federal systems security policy and federal funding agency policy compliance. The IU high-performance computing and storage systems described here are managed and administered in ways that meet National Institute of Standards and Technology (NIST) 800 security standards. OVPIT and UITS comply with the NIH Grants Policy Statement.

1. Facilities for handling sensitive data. IU has put in place appropriate administrative, technical, and physical controls to protect data in accordance with the HIPAA security rule. Electronic Personal Health Information may be stored on all of the HPC and storage facilities described in this document.
2. Services lists and disaster recovery planning. IU has a written disaster recovery plan for every service and system it provides, which is by definition an experimental facility. IU has a contract in place for use of an off-site disaster recovery facility in case of a disaster affecting one or more of IU's campuses. If a disaster strikes one core campus (IUPUI or IUB), the disaster recovery plans call for restoring service at the core campus that remains operational. Plans are also in place for service recovery if a disaster strikes both core campuses simultaneously.

2.1. High performance computing (HPC) systems

IU has the following production high-performance computing systems.

- *Big Red 3* is a 5-cabinet XC40 supercomputer from Cray, Inc. Big Red 3 has a theoretical peak performance (Rpeak) of 928 trillion floating-point operations per second (928 teraFLOPS). Big Red 3 consists of 930 XC40 compute nodes, each with two Intel Haswell 12-Core 2.6 GHz processors and 64GB of DDR4-2133 RAM. All nodes are connected via Cray's Aries Dragonfly-topology interconnect, a very high-speed, low-latency network. This system is connected to our Slate storage system via 6 FDR InfiniBand links, providing an aggregate of 42GB/s of I/O to that filesystem.
- *Big Red 200* is an [HPE Cray EX](#) supercomputer designed to support scientific and medical research, and advanced research in artificial intelligence, machine learning, and data analytics. Installed at Indiana University in January 2020, Big Red 200 entered early access in 2021 and production in 2022. Big Red 200 features 640 compute nodes, each equipped with 256 GB of memory and two 64-core, 2.25 GHz, 225-watt [AMD EPYC 7742](#) processors. In 2021 an expansion added 64 GPU-accelerated nodes, each with 256 GB of memory and four NVIDIA A100 GPUs. Big Red 200 has a theoretical peak performance (Rpeak) of nearly 7 petaFLOPS, making it one of the fastest university-owned supercomputers in the nation.
- *Quartz* is a high-throughput computing cluster. Designed to deliver large amounts of processing capacity over long periods of time, Quartz provides the advanced supercomputing performance needed to run high-end, data-intensive applications that are critical to scientific discovery and innovation. Quartz features 92 compute nodes, each equipped with two 64-core AMD EPYC 7742 2.25 GHz CPUs and 512 GB of RAM, with a peak per-node performance of greater than 4,608 gigaFLOPS. All Quartz nodes are housed in the IU Bloomington Data Center, run Red Hat Enterprise 8.x, and are connected to the IU Science DMZ via 10-gigabit Ethernet. The Slate and Slate-Project file systems are mounted for temporary storage of research data. The Lmod environment management package allows users to dynamically customize their shell environments. Quartz uses Slurm to coordinate resource management and job scheduling.
- *Carbonate* is a high-throughput cluster with a peak theoretical capability of 83.8 TFLOPS, and an aggregate RAM of 28 TB. Carbonate consists of 96 nodes total each with 256 GB of RAM or larger. An additional 8 nodes are dedicated to specific research areas. Each node is a Lenovo NeXtScale nx360 M5 server equipped with two 12-core Intel Xeon E5-2680 v3 CPUs and four 480 GB solid-state drives.
 - *Deep Learning Nodes.* Carbonate has been expanded to include 12 Lenovo ThinkSystem SD530 servers with NVIDIA GPUs. Eight of these nodes have dual NVIDIA 16GB Tesla P100s per server and four of these nodes have dual NVIDIA 32GB Tesla V100s per server. All twelve servers have dual Intel Xeon Gold 6126 12-core2.6GHz processors, 192GB RAM and 7.68TB of local disk.
 - *GPU-centric Expansion.* During the first quarter of 2020, Carbonate was expanded to include an additional 24 Apollo 6500 nodes with 8 NVLink-capable V100s per node.

Summary of computational resources at Indiana University				
Name	Architecture	TFLOPS	Total RAM (TB)	Local disk (TB)
Big Red 200	Cray Shasta	6,955.0	180.0	N/A
Big Red 3	Cray XC40	934.0	59.5	N/A
Carbonate	IBM NeXtScale nx360 cluster	83.9	28.0	180.0
- Deep Learning	Lenovo ThinkSystem SD530 (CPU, GPU)	89.6	2.4	92.0
- GPU-centric	Apollo 6500	755.0	18.4	92.2
Quartz	Gigabyte H262-Z63	423.0	14.0	368.0
Totals		9,240.5	302.3	732.2

- *Jetstream*. Jetstream is the National Science Foundation's first production cloud for science and consists of 640 nodes geographically dispersed into two 320 node systems. One is housed at Indiana University's Data Center. The second system is at the Texas Advanced Computing Center (TACC). Each 320 node system has 640 Intel Haswell CPUs with 7,680 cores, 40GB of memory, 640TB of local disk, and 960TB of additional available storage. The two systems are connected to Internet2 via 100Gbps links and via 10Gbps links to XSEDE resources. Jetstream provides a user selectable library of virtual machines for research use or allow for customized environments. System images can be archived for long-term storage and restored on demand. Jetstream is currently open for allocations and is in full production mode as of September 1, 2016 and has been in operation through August 2022.
- *Jetstream2*. Jetstream2, the latest NSF-supported hybrid-cloud platform, will expand the availability of flexible, on-demand, programmable cyberinfrastructure tools. Jetstream2 features a core cloud computing platform at Indiana University with a mix of more than 500 CPU (AMD EPYC 3rd generation), GPU (NVIDIA A100), and 1TiB large-memory nodes paired to a 14PB hybrid storage environment. The project also includes four regional partners with single-rack systems at Arizona State University, Cornell University, the University of Hawai'i, and the Texas Advanced Computing Center. Jetstream2, available to the US research community in 2021Q4, is configured to allow all users AI accessibility through virtual GPU (vGPU) capabilities as well as pre-built containers that include machine learning (ML) and deep learning (DL) tools. Jetstream2's prioritization of flexible user experience and programmatic interfaces will ease cross-disciplinary collaborations, cross-platform workflows, and workforce development.

2.2. Data storage systems

IU has five major disk-based file systems and one archival storage system that serve local and remote users. These include:

- *Research File System (Geode)*. A Lenovo storage solution using Spectrum Scale/GPFS provides 7.2PB of raw (2.7PB usable, replicated) storage that is asynchronously replicated for high availability. This environment provides home directory space for IU's HPC systems. Using the Samba, mapped drive, interface users are also using this storage for desktop access across campus. The system is integrated with campus Active Directory to allow for use of existing permission schemes. Geode also provides Condo Storage, primarily accessed by Samba. Condo storage is departmentally purchased storage with 70+ departments and projects utilizing the storage. All data on Geode are replicated on identical hardware at the IUB and IUPUI. This replication provides high availability, and in the case of a power or other event at one of the sites, the remaining site will serve the filesystem. Snapshots allow users to restore data going back 30 days. Currently only the home directories are backed up to alternate storage, and other DR backup solutions are being explored. Users can archive large data sets on Geode to the SDA for extra data protection.
- *The Data Capacitor Wide Area Network 2 (DC-WAN2)* file system is a high-speed/high-bandwidth Lustre/ZFS storage system for research computing that serves all IU campuses and other sites throughout the country, primarily by wide area network (remote) Lustre file system mounts. DC-WAN has a total formatted capacity of over 1.1 PB with a 160 Gbps maximum I/O. DC-WAN consists of Supermicro servers running the Lustre file system. DC-WAN uses four servers for object storage equipped with 40-gigabit Ethernet cards, and two servers used for Lustre metadata that use Gigabit Ethernet. DC-WAN can map remote users to local users, allowing machines with heterogeneous namespaces to communicate seamlessly. This system has permitted users to harness the power of geographically distributed resources to provide novel solutions to their workflow issues. Data stored on the Data Capacitor II and DC-WAN are not backed up automatically. These systems were designed primarily for short-term data storage. However,

data from the Data Capacitor can easily be transferred to the SDA from any of IU's compute resources, so replica copies may easily be maintained.

- *Slate*. Slate is a high-speed/high-bandwidth Lustre storage system comprised of three separate file systems, Slate, Scratch, and Project, that serve the high-performance computing systems at Indiana University Bloomington. Slate is a 1.9 PB Lustre file system (1.3 PB usable), Project is an 17.7 PB Lustre file system (15 PB usable), and Scratch is a Lustre file system built on a mix of solid state and spinning disk storage which will replicate data from 1 PB of SSD to HDD providing an aggregate of 2.5 PB usable. Scratch has exceeded 50 GB/s reads and writes and has achieved over 500K creates/destroys per second. Slate was purchased from Data Direct Networks (DDN) and runs on two of their SFA18KX machines with an additional 4 servers running metadata for Scratch. Slate and Project each have eight virtual Lustre metadata servers backed by solid state storage while Scratch has 4 physical multicore machines. The default user quota for Slate is 800 GB and can be doubled once to 1.6 TB. Project accounts are created on demand and users can request up to 15 TB per project with additional space provided upon request at a nominal rate per TB per month. Data stored on Slate and Project are not backed up by default and were designed for persistent storage are therefore not subject to a purge policy. Scratch is subject to a 30-day purge policy. Data from all three file systems can easily be transferred to the SDA from any of IU's compute resources, so replica copies may easily be maintained
- *IU's Scholarly Data Archive (SDA)*. SDA uses High Performance Storage System (HPSS) software to make available to IU researchers a total storage capacity exceeding 79 PB. Data are written to a fast, front-end disk cache and migrated over time to IBM TS3500 tape libraries on the Indianapolis and Bloomington campuses. Data written to IU's HPSS system are copied simultaneously to both locations, providing highly reliable disaster protection. Users can access data over the network from central research systems or from personal workstations, using SFTP, HSI/HTAR, CIFS, and Globus. The default allowance is 50 TB of mirrored data, with additional space provided upon request at a nominal rate per GB per year. SDA stores and provides access to data for the IUScholarWorks Repository, a document and data archiving system created using DSpace software. By default, data stored within the IU Scholarly Data Archive are stored in duplicate copies – one in the tape library at IU Bloomington, and one in the tape library at IUPUI in Indianapolis. User data is not backed up to other external systems. The HPSS metadata specifying which tapes contain any given file is backed up continuously; multiple copies exist in Indianapolis and Bloomington. The libraries are being upgraded to SpectraLogic TFinity ExaScale libraries which will have a capacity of 177PB (replicated).

Summary of data storage resources available at Indiana University:

Name	File system	Disk PB	Disk (PB) usable	Tape (PB)
Geode	GPFS	3.60	2.7	N/A
DC-WAN	Lustre	1.47	1.1	N/A
Scratch	Lustre	3.50	2.5	N/A
Slate	Lustre	1.90	1.3	N/A
Slate Project	Lustre	17.70	15.0	N/A
Scholarly Data Archive	HPSS	2.20	1.7	79
Totals		30.37	24.3	79

2.3. Restricted/secure data services

- *Research Database Complex*. The Research Database Complex (RDC) provides research-related Oracle and MySQL databases. The RDC serves data-intensive applications based on relational databases and web applications that rely on database back ends. The RDC consists of three nodes, and it has an aggregate RAM of 0.2 TB and 10 TB of SAN-attached storage for database hosting. The database-serving component consists of three virtual machines, each with four vCPUs and 96 GB of memory. The web serving environment is a VM with a single vCPU and 8 GB of memory. The RDC has a 10-gigabit Ethernet interconnect.
- *Restricted Access Data Remote Server*. RADaRS at Indiana University is a secure research hub that allows IU researchers to access and analyze datasets from a variety of providers that require that their data be used only within highly secure environments. Data custodians upload secure datasets to project folders and manage access control permissions. Individual researchers and collaborative project teams can access RADaRS remotely from their personal workstations to work with their datasets. Statistical packages

and productivity software, including SAS, SPSS, Stata, R, and Microsoft Office, are installed on the server. The server is a VM with 4 vCPUs and 32 GB of memory with 1.5 TB of attached storage running Windows Server 2019.

2.4. Advanced visualization resources

The IU Advanced Visualization Laboratory (AVL) serves as a university-wide resource for information and scientific visualization, virtual and augmented reality, interactive experiences and exhibits, advanced graphics and media, and object and environment digitization services for researchers, educators, students, and artists in all departments on all campuses. These advanced visualization resources include, but are not limited to:

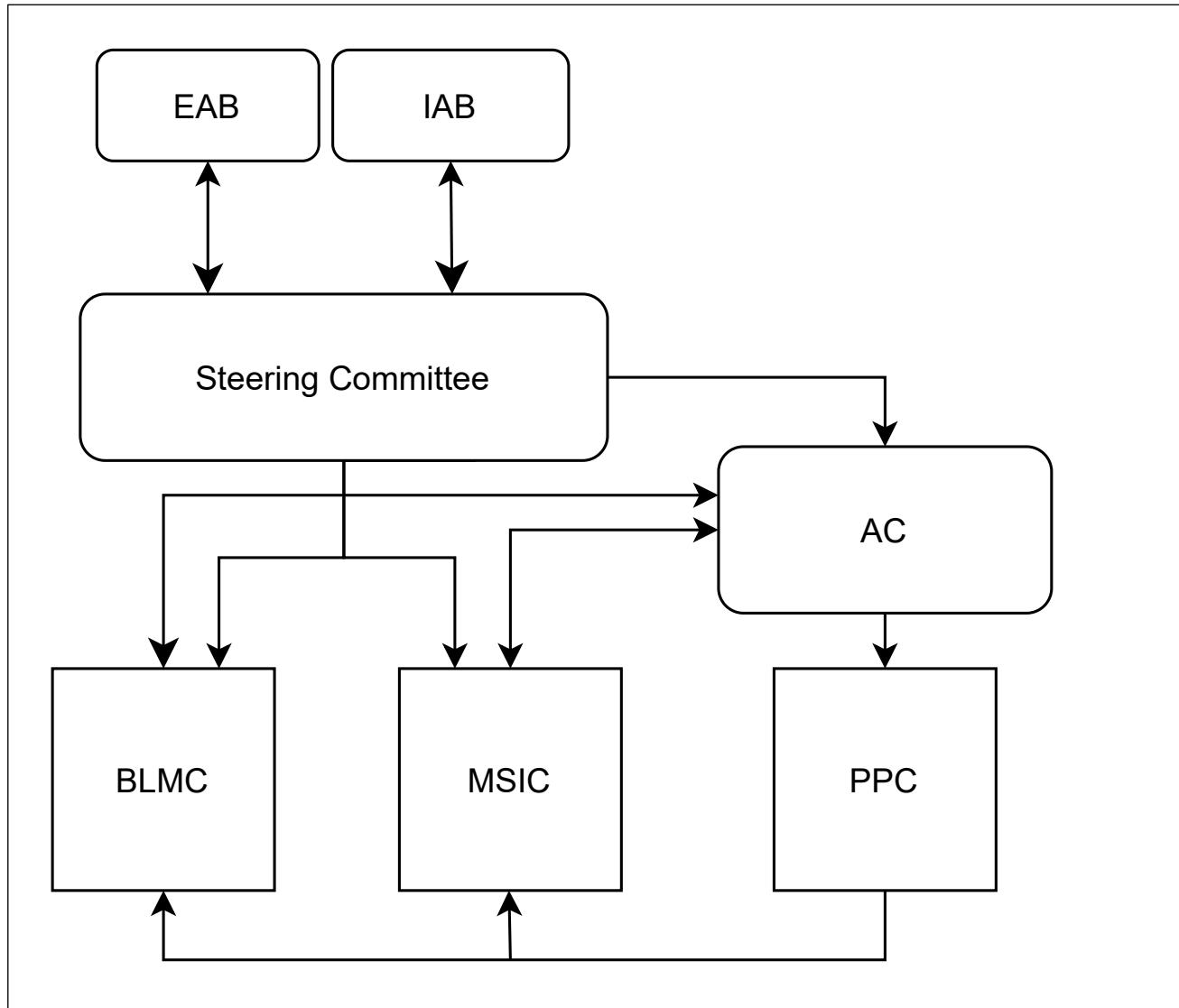
- *Crystal Wall.* The Crystal Display Wall is a large-format, ultra-high resolution display located in the Wrubel Commons of the Cyberinfrastructure Building at IU Bloomington. This display is 24' wide x 9' high at a resolution of 5760 x 2160 and supports multiple computing and video sources. The system utilizes Sony's Crystal LED technology which produces a bezel-less image capable of extremely high brightness (1,000 nits), contrast ratio (1 million : 1), and stereoscopic display. It is ideal for the most demanding tasks in scientific visualization, image analysis, group collaboration, and media presentation.
- *IQ-Wall.* The IQ-Wall is an AVL design that tiles thin, energy-efficient, flat-screen monitors into configurations that meet the requirements of the users and the space. These Walls are driven by a single Windows computer and accommodate a number of interactive and/or collaborative use cases. The premier units are located in the Informatics and Communication Complex (ICTC 403) at IUPUI and the Innovation Center (IC 105) at IUB, but IU has installed more than 20 IQ-Walls geographically distributed across 6 campuses.
- *IQ-Table.* A 65" monitor equipped with multi-touch capabilities, the IQ-Table is ideal for building lobbies, libraries, and exhibits. Using modern HTML5 web standards, AVL staff maintain a library of supporting software technologies that can be used to create custom exhibits and interactive applications.
- *3D Scanning and Digital Object Preparation.* AVL staff maintain knowledge and have established workflows broadly related to object and environment scanning. The Lab owns and operates multiple 3D scanners which can be used in AVL facilities. Lab staff are well versed in converting and manipulating all types of digital 3D models for purposes of online display, AR or VR, or 3D printing.

Major Equipment Available to the C3A

Major equipment available to the C3A

Core/Lab	Description	Manufacturer	Qty
Multi-Scale Imaging Core (MSIC)	VT-1200S Vibratome either for live and fixed tissue slicing	Leica	2
Multi-Scale Imaging Core (MSIC)	Upright Nikon Eclipse FN1 microscope equipped with infrared differential interference contrast (DIC) optics for patch-clamp recordings	Nikon	1
Multi-Scale Imaging Core (MSIC)	MultiClamp 700B amplifier	Molecular Devices	1
Multi-Scale Imaging Core (MSIC)	3DHISTECH Panoramic MIDI II slide scanner	Epredia	1
Multi-Scale Imaging Core (MSIC)	Nikon A1-HD25 confocal laser-scanning system built on a Ti-E inverted microscope operated by NIS-Elements AR software 4.50.00 for anatomical imaging	Nikon	1
Multi-Scale Imaging Core (MSIC)	CFI Apo TIRF 100x Oil 1.49 NA objective on a Ti-E inverted microscope equipped with an N-STORM system, a Nikon C2 confocal scan head, and an Andor EMCCD camera for correlated confocal and STORM imaging	Nikon/Andor	1
Multi-Scale Imaging Core (MSIC)	InSight DeepSee infrared pulsed laser with dual line (tunable IR laser 680-1300, with second fixed 1040 nm output)	Spectra Physics	1
Multi-Scale Imaging Core (MSIC)	Leica computerized stereotoxic injection device	Leica	1
Multi-Scale Imaging Core (MSIC)	Nikon A1R multiphoton microscope with Galvo and Resonant scanners	Nikon	1
Multi-Scale Imaging Core (MSIC)	Scientifica motorized stage	Scientifica	1
Multi-Scale Imaging Core (MSIC)	Axioskop microscope	Zeiss	1
Multi-Scale Imaging Core (MSIC)	Custom built mouse pup holder allowing head restrained but awake imaging with temperature control	IUB Machine Shop	3
Bioactive Lipid Mediators Core (BLMC)	24.4 Cu ft -80°C Freezer	Revco	1
Bioactive Lipid Mediators Core (BLMC)	SterilGARD III Laminar flow hood	Baker	2
Bioactive Lipid Mediators Core (BLMC)	Forma CO2 Incubator	Thermo	2
Bioactive Lipid Mediators Core (BLMC)	RT-7 plus tablet top centrifuge	Sorval	1
Bioactive Lipid Mediators Core (BLMC)	API 3000 triple quadrupole mass spectrometer	Applied Biosystems	1
Bioactive Lipid Mediators Core (BLMC)	HPLC w/2 10AdvP pumps, SIL 20AC Prominence Autosampler, CBM-20A Controller	Shimadzu	1
Bioactive Lipid Mediators Core (BLMC)	Compressed air, nitrogen, and zero air generators	Ingersol Rand, Liberty Systems, Parker	3

C3A Center Organizational Structure



Legend

AC	Administrative Core
BLMC	Bioactive Lipids Mediator Core
EAB	External Advisory Board
IAB	Internal Advisory Board
MSIC	MultiScale Imaging Core
PPC	Pilot Project Core

Table of Core Utilization

Estimated utilization of the Cores

Core	Specific Aim	Utilization (%)
BLM	#1	35
BLM	#2	35
BLM	#3	10
BLM	#4	10
BLM	#5	10
MSI	#1	45
MSI	#2	20
MSI	#3	25
MSI	#4	10

No PI or affiliate will use a core more than 50% of the time. The nature of the C3A is such that there will be many small projects (outlined in the descriptions of the two research cores) being conducted by about two dozen PIs and affiliates.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Kenneth	Middle Name P.	Last Name*: Mackie	Suffix: M.D.
Position/Title*:	Professor			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 N WALNUT GROVE AVE			
Street2:				
City*:	BLOOMINGTON			
County:	MONROE			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	812-855-2042		Fax Number:	
E-Mail*:	kmackie@indiana.edu			
Credential, e.g., agency login:	KMACKIE			
Project Role*:	PD/PI		Other Project Role Category:	
Degree Type:	MD,BS		Degree Year: 1984,1980	
Attach Biographical Sketch*:	File Name:	Mackie_biosketch_for_P30_20220911.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: HUI-CHEN	Middle Name	Last Name*: LU	Suffix: Ph.D
Position/Title*:	Linda and Jack Gill Chair of Neuroscience			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 N Walnut Grove Ave			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	812-856-4998		Fax Number:	
E-Mail*:	hclu@indiana.edu			
Credential, e.g., agency login:	HL690781			
Project Role*:	Other (Specify)	Other Project Role Category: Core Co-Lead		
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	Lu_Biosketch_P30_final.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Istvan	Middle Name	Last Name*: Katona	Suffix: Ph.D
Position/Title*:	Naus Family Chair of Addiction Sciences			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 NORTH WALNUT GROVE AVE			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	47405-2204			
Phone Number*:	(812) 855-2012		Fax Number:	
E-Mail*:	ikatona@iu.edu			
Credential, e.g., agency login:	katona			
Project Role*:	Other (Specify)	Other Project Role Category: Core Co-Lead		
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	Istvan_Katona_NIH_biosketch_09212022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Heather	Middle Name Bryte	Last Name*: Bradshaw	Suffix: Ph.D
Position/Title*:	Professor			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	1101 East 10th Street			
Street2:				
City*:	Bloomington			
County:	MONROE			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	812-856-1559		Fax Number:	
E-Mail*:	hbbradsh@indiana.edu			
Credential, e.g., agency login:	HBBRADSH			
Project Role*: Other (Specify)	Other Project Role Category: Core Lead			
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	Bradshaw_Biosketch_P30_2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Brady	Middle Name	Last Name*: Atwood	Suffix: Ph.D
Position/Title*:	Assistant Professor			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:				
Division:				
Street1*:	302 W 15th St			
Street2:	NB-400C			
City*:	Indianapolis			
County:				
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	462022266			
Phone Number*:	2062901649		Fax Number:	
E-Mail*:	bkatwood@iu.edu			
Credential, e.g., agency login:	atwoodb			
Project Role*: Other (Specify)	Other Project Role Category: External Consultant - Key			
Degree Type: PHD	Degree Year: 2010			
Attach Biographical Sketch*:	File Name:	Biosketch_Atwood_R01_Sept_2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Andrea	Middle Name Grace	Last Name*: Hohmann	Suffix: Ph.D
Position/Title*:	Linda and Jack Gill Chair			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	1101 E. 10th St.			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	812 856-0672		Fax Number: 812 856-7187	
E-Mail*:	hohmanna@indiana.edu			
Credential, e.g., agency login:	ahohmann			
Project Role*: Other (Specify)	Other Project Role Category: Significant Contributor - Key			
Degree Type: PHD,MS,BS	Degree Year: 1996,1993,1988			
Attach Biographical Sketch*:	File Name:	090522_Hohmann_BioR_P30.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Norbert	Middle Name	Last Name*: Hajos	Suffix: Ph.D
Position/Title*:	Professor			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 N Walnut Grove Ave			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	8128552012		Fax Number:	
E-Mail*:	nhajos@iu.edu			
Credential, e.g., agency login:	nhajos			
Project Role*: Other (Specify)	Other Project Role Category: Core Lead			
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	biosketch_Hajos_P30.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Sachin	Middle Name	Last Name*: Patel	Suffix:
Position/Title*:	Professor,Professor			
Organization Name*:	NORTHWESTERN UNIVERSITY AT CHICAGO			
Department:				
Division:				
Street1*:	676 N Saint Clair Street			
Street2:				
City*:	Chicago ,Chicago			
County:				
State*:	IL: Illinois			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	606110000			
Phone Number*:	3126955060		Fax Number:	
E-Mail*:	sachin.patel@northwestern.edu			
Credential, e.g., agency login:	patels2			
Project Role*:	Other (Specify)	Other Project Role Category: External advisory board		
Degree Type:	MD,PHD,BS			
Attach Biographical Sketch*:	File Name:	PATEL,_SACHIN_2022_NIH_BIOSKETCH.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: MARGARET	Middle Name M.	Last Name*: MCCARTHY	Suffix:
Position/Title*:	Professor			
Organization Name*:	University of Maryland			
Department:				
Division:				
Street1*:	Department of Physiology			
Street2:	655 West Baltimore Street			
City*:	Baltimore			
County:				
State*:	MD: Maryland			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	212010000			
Phone Number*:	410-706-2655		Fax Number: 410-706-8341	
E-Mail*:	mmccarth@umaryland.edu			
Credential, e.g., agency login:	margaretmccarthy			
Project Role*:	Other (Specify)	Other Project Role Category: External Advisory Board		
Degree Type:	Degree Year: 1989			
Attach Biographical Sketch*:	File Name:	McCarthy_NIH_Biosketch_2022-trimmed.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Michael	Middle Name R	Last Name*: Bruchas	Suffix:
Position/Title*:	Professor			
Organization Name*:	UNIVERSITY OF WASHINGTON			
Department:				
Division:				
Street1*:	1959 NE Pacific St.			
Street2:	J187a-Health Sciences			
City*:	Seattle			
County:				
State*:	WA: Washington			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	981950000			
Phone Number*:	206-543-6870		Fax Number:	
E-Mail*:	mbruchas@uw.edu			
Credential, e.g., agency login:	mbruchas			
Project Role*:	Other (Specify)		Other Project Role Category: External Advisory Board	
Degree Type:	PHD		Degree Year: 2004	
Attach Biographical Sketch*:	File Name:	Bruchas_Biosketch_2022_MR.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Cecilia	Middle Name J	Last Name*: Hillard	Suffix:
Position/Title*:	PROFESSOR			
Organization Name*:	Medical College of Wisconsin			
Department:				
Division:				
Street1*:	PHARMACOLOGY			
Street2:	DIRECTOR, NEUROSCIENCE RES CTR			
City*:	MILWAUKEE			
County:				
State*:	WI: Wisconsin			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	532260000			
Phone Number*:	4149558493		Fax Number: 4149556057	
E-Mail*:	CHILLARD@MCW.EDU			
Credential, e.g., agency login:	CHILLARD			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD		Degree Year: 1983	
Attach Biographical Sketch*:	File Name:	2022_Mackie_P30_Cece_grant.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Adam	Middle Name J	Last Name*: Kimbrough	Suffix:
Position/Title*:	Assistant Professor			
Organization Name*:	Purdue University			
Department:				
Division:				
Street1*:	625 Harrison Street			
Street2:				
City*:	West Lafayette			
County:				
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	479070000			
Phone Number*:	1-765-494-8637		Fax Number:	
E-Mail*:	kimbroua@purdue.edu			
Credential, e.g., agency login:	adam_kimbrough			
Project Role*:	Other (Specify)	Other Project Role Category: External Consultant - Key		
Degree Type:	PHD,BS	Degree Year: 2015,2007		
Attach Biographical Sketch*:	File Name:	KimbroughBiosketch.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: TODD	Middle Name W	Last Name*: VANDERAH	Suffix:
Position/Title*:	Professor			
Organization Name*:	UNIVERSITY OF ARIZONA			
Department:				
Division:				
Street1*:	UNIVERSITY OF ARIZONA			
Street2:	DEPT OF PHARMACOLOGY			
City*:	TUCSON			
County:				
State*:	AZ: Arizona			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	857245050			
Phone Number*:	(520) 626-7801		Fax Number: (520) 626-4779	
E-Mail*:	VANDERAH@EMAIL.ARIZONA.EDU			
Credential, e.g., agency login:	vanderah			
Project Role*:	Other (Specify)	Other Project Role Category: External Consultant - Key		
Degree Type:	PHD,BS	Degree Year: 1995,1991		
Attach Biographical Sketch*:	File Name:	Bio_Vanderah_2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Sudhansu	Middle Name K	Last Name*: Dey	Suffix:
Position/Title*:	Professor			
Organization Name*:	Cincinnati Children's Hospital			
Department:				
Division:				
Street1*:	3333 Burnet Avenue			
Street2:	MLC 7045			
City*:	Cincinnati			
County:				
State*:	OH: Ohio			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	452290000			
Phone Number*:	5138031158		Fax Number:	
E-Mail*:	Sk.Dey@cchmc.org			
Credential, e.g., agency login:	dey_sk			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD		Degree Year: 1972	
Attach Biographical Sketch*:	File Name:	Dey_Biosketch_2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Anna	Middle Name	Last Name*: Dunaevsky	Suffix:
Position/Title*:	Professor			
Organization Name*:	University of Nebraska Medical Center			
Department:				
Division:				
Street1*:	985960 Nebraska Medical Center			
Street2:				
City*:	Omaha			
County:				
State*:	NE: Nebraska			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	681980000			
Phone Number*:	402-559-1071		Fax Number:	
E-Mail*:	adunaeovsky@unmc.edu			
Credential, e.g., agency login:	adunaeovsky			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD,MS,BS		Degree Year: 1997,1992,1990	
Attach Biographical Sketch*:	File Name:	Dunaevsky_biosketch-9-2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Keisuke	Middle Name	Last Name*: Kawata	Suffix:
Position/Title*:	Assistant Professor			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:				
Division:				
Street1*:	1025 E. 7th Street			
Street2:				
City*:	Bloomington			
County:				
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474067508			
Phone Number*:	8702109918		Fax Number:	
E-Mail*:	kkawata@indiana.edu			
Credential, e.g., agency login:	KKawata150			
Project Role*: Other (Specify)	Other Project Role Category: Significant Contributor - Key			
Degree Type: PHD,MS	Degree Year: 2016,2013			
Attach Biographical Sketch*:	File Name:	2022_Kawata_Biosketch.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Karoly	Middle Name	Last Name*: Mirnics	Suffix:
Position/Title*:	Director of Munoe-Meyer Institute			
Organization Name*:	University of Nebraska Medical Center			
Department:				
Division:				
Street1*:	Munroe-Meyer Institute			
Street2:	Rm 2006			
City*:	Omaha			
County:				
State*:	NE: Nebraska			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	681985450			
Phone Number*:	6157274835		Fax Number:	
E-Mail*:	mirnicsk@gmail.com			
Credential, e.g., agency login:	karolymirnics			
Project Role*: Other (Specify)	Other Project Role Category: External Consultant - Key			
Degree Type: MD,PHD	Degree Year: 1986,2010			
Attach Biographical Sketch*:	File Name:	Mirnics_Biosketch_Jan_2022_R01.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: John	Middle Name A.	Last Name*: Dani	Suffix:
Position/Title*:	Chair, Dept of Neuroscience			
Organization Name*:	University of Pennsylvania			
Department:				
Division:				
Street1*:	415 Curie Boulevard			
Street2:	211 Clinical Research Building			
City*:	Philadelphia			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	191040000			
Phone Number*:	215-898-8498		Fax Number:	
E-Mail*:	johndani@pennmedicine.upenn.edu			
Credential, e.g., agency login:	jadani			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD,BS		Degree Year: 1980,1975	
Attach Biographical Sketch*:	File Name:	2022_Dani-Biosketch.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Sayeepriyadarshini	Middle Name	Last Name*: Anakk	Suffix:
Position/Title*:	Assistant Professor			
Organization Name*:	University of Illinois at Urbana-Champaign			
Department:				
Division:				
Street1*:	506 S Mathews			
Street2:	453 MSB			
City*:	Urbana			
County:				
State*:	IL: Illinois			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	618010000			
Phone Number*:	217 300 7905		Fax Number:	
E-Mail*:	anakk@illinois.edu			
Credential, e.g., agency login:	sayeepriyadarshini_anakk			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD		Degree Year: 2005	
Attach Biographical Sketch*:	File Name:	Biosketch_Anakk_2021_BLMC_HB_4_pages.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Joseph	Middle Name François	Last Name*: Cheer	Suffix:
Position/Title*:	Professor			
Organization Name*:	University of Maryland			
Department:				
Division:				
Street1*:	Department of Anatomy and Neur			
Street2:				
City*:	Baltimore			
County:				
State*:	MD: Maryland			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	212010000			
Phone Number*:	4107060112		Fax Number: 4107062512	
E-Mail*:	jchee001@umaryland.edu			
Credential, e.g., agency login:	jcheer			
Project Role*: Other (Specify)	Other Project Role Category: External Consultant - Key			
Degree Type: PHD	Degree Year: 2000			
Attach Biographical Sketch*:	File Name:	2022_CheerBio-trimmed.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Aditi	Middle Name	Last Name*: Das	Suffix:
Position/Title*:	Assistant Professor			
Organization Name*:	University of Illinois at Urbana-Champaign			
Department:				
Division:				
Street1*:	4903 Stonebridge Drive			
Street2:				
City*:	champaign			
County:				
State*:	IL: Illinois			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	618220000			
Phone Number*:	6092036924		Fax Number:	
E-Mail*:	aditidas@illinois.edu			
Credential, e.g., agency login:	aditi_das			
Project Role*: Other (Specify)	Other Project Role Category: External Consultant - Key			
Degree Type: PHD	Degree Year: 2005			
Attach Biographical Sketch*:	File Name:	Das_Biosketch_09132022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: YASMIN	Middle Name L.	Last Name*: HURD	Suffix:
Position/Title*:				
Organization Name*:	Icahn School of Medicine at Mount Sinai			
Department:				
Division:				
Street1*:	1470 Madison Avenue			
Street2:				
City*:	New York			
County:				
State*:	NY: New York			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	100290000			
Phone Number*:	212-824-9314		Fax Number:	
E-Mail*:	yasmin.hurd@mssm.edu			
Credential, e.g., agency login:	yasmin_hurd			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD		Degree Year: 1989	
Attach Biographical Sketch*:	File Name:	Hurd_Biosketch-2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Jui-Yen	Middle Name	Last Name*: Huang	Suffix: Ph.D
Position/Title*:	Assistant Research Scientist			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 N Walnut Grove Avenue			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	8128565869		Fax Number:	
E-Mail*:	juiyuan@iu.edu			
Credential, e.g., agency login:	juiyuanhuang			
Project Role*:	Other (Specify)		Other Project Role Category: Core Technical Staff	
Degree Type:	PHD		Degree Year: 2010	
Attach Biographical Sketch*:	File Name:	biosketch-Huang_20220910_P30.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Laszlo	Middle Name	Last Name*: Barna	Suffix: Ph.D
Position/Title*:	Light Microscopy Core Facility Manager			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	Sziagony u. 43.			
Street2:				
City*:	Budapest			
County:				
State*:				
Province:				
Country*:	HUN: HUNGARY			
Zip / Postal Code*:	1083			
Phone Number*:	+36203427793		Fax Number:	
E-Mail*:	larnabaci@gmail.com			
Credential, e.g., agency login:	Ibarna			
Project Role*: Other (Specify)	Other Project Role Category: Core Technical Staff			
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	Laszlo_Barna_NIH_biosketch.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Debra	Middle Name Shamala	Last Name*: Karhson	Suffix: Ph.D
Position/Title*:	Assistant Professor			
Organization Name*:	University of New Orleans			
Department:	Psychology			
Division:				
Street1*:	2000 Lakeshore Dr			
Street2:				
City*:	New Orleans			
County:				
State*:	LA: Louisiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	701480000			
Phone Number*:	6504985187		Fax Number:	
E-Mail*:	dkarhson@stanford.edu			
Credential, e.g., agency login:	karhson.debra			
Project Role*: Other (Specify)	Other Project Role Category: External Consultant - Key			
Degree Type: PHD,BS	Degree Year: 2014,2007			
Attach Biographical Sketch*:	File Name:	DKarhson_Biosketch_UPDATED_SEPT2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: DANIEL	Middle Name J	Last Name*: MORGAN	Suffix:
Position/Title*:	Assistant Professor			
Organization Name*:	Marshall University			
Department:				
Division:				
Street1*:	Mailcode H187, Room C2850			
Street2:				
City*:	Hershey			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	170330000			
Phone Number*:	732-309-8281		Fax Number:	
E-Mail*:	dmorgan1@hmc.psu.edu			
Credential, e.g., agency login:	morganda			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD,BA		Degree Year: 2004,1997	
Attach Biographical Sketch*:	File Name:	Morgan,_Daniel_P30_Biosketch_8.27.22.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Marina	Middle Name Elizabeth	Last Name*: Wolf	Suffix:
Position/Title*:	Professor of Behavioral Neuroscience			
Organization Name*:	Oregon Health and Science University			
Department:				
Division:				
Street1*:	Dept Behavioral Neuroscience			
Street2:				
City*:	Portland			
County:				
State*:	OR: Oregon			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	972390000			
Phone Number*:	8479870278		Fax Number:	
E-Mail*:	wolfmar@ohsu.edu			
Credential, e.g., agency login:	wolfmarina			
Project Role*:	Other (Specify)		Other Project Role Category: External Advisory Board	
Degree Type:	PHD,MS,BA		Degree Year: 1986,1983,1981	
Attach Biographical Sketch*:	File Name:	Wolf_IUB_Biosketch_8-19-22.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Ryohei	Middle Name	Last Name*: Yasuda	Suffix:
Position/Title*:	Scientific Director			
Organization Name*:	MAX PLANCK FLORIDA CORPORATION			
Department:				
Division:				
Street1*:	1 Max Planck Way			
Street2:				
City*:	Jupiter			
County:				
State*:	FL: Florida			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	334580000			
Phone Number*:	561-339-3036		Fax Number:	
E-Mail*:	Ryohei.Yasuda@mpfi.org			
Credential, e.g., agency login:	yasuda@neuro			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD		Degree Year: 1998	
Attach Biographical Sketch*:	File Name:	Yasuda_biosketch_2021-KM.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Xiaofei	Middle Name	Last Name*: Sun	Suffix:
Position/Title*:	Instructor			
Organization Name*:	Cincinnati Children's Hospital			
Department:				
Division:				
Street1*:	3333 Burnet Ave, S-11-234			
Street2:				
City*:	Cincinnati			
County:				
State*:	OH: Ohio			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	452290000			
Phone Number*:	513-803-2091		Fax Number:	
E-Mail*:	xiaofei.sun@cchmc.org			
Credential, e.g., agency login:	sunr1t			
Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD,BS		Degree Year: 2010,2002	
Attach Biographical Sketch*:	File Name:	2022_Sun_Biosketch_Sun.P30.2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Clare	Middle Name Therese	Last Name*: Johnson	Suffix:
Position/Title*:	Research Associate			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	515 W 6th St. Apt 3			
Street2:				
City*:	Bloomington			
County:				
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474040000			
Phone Number*:	8282428128		Fax Number:	
E-Mail*:	clthjohn@iu.edu			
Credential, e.g., agency login:	claretheresejohnson			
Project Role*: Other (Specify)	Other Project Role Category: Core Technical Staff			
Degree Type: PHD,BS	Degree Year: 2023,2018			
Attach Biographical Sketch*:	File Name:	220913_Clare_Johnson_Biosketch.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Frank	Middle Name	Last Name*: Porreca	Suffix:
Position/Title*:	Professor			
Organization Name*:	UNIVERSITY OF ARIZONA			
Department:				
Division:				
Street1*:	1501 N. Campbell Ave			
Street2:				
City*:	Tucson			
County:				
State*:	AZ: Arizona			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	857240000			
Phone Number*:	15206267421		Fax Number:	
E-Mail*:	frankp@email.arizona.edu			
Credential, e.g., agency login:	frankp			
Project Role*: Other (Specify)	Other Project Role Category: External Consultant - Key			
Degree Type: PHD,MS,BS	Degree Year: 1982,1979,1975			
Attach Biographical Sketch*:	File Name:	KenIstvanPorreca_Biosketch_rev.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Mehmet	Middle Name	Last Name*: Dalkilic	Suffix: Ph.D
Position/Title*:	Professor			
Organization Name*:	Trustees of Indiana University			
Department:				
Division:				
Street1*:	700 N Woodlawn Ave			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474050000			
Phone Number*:	8128563010		Fax Number:	
E-Mail*:	dalkilic@indiana.edu			
Credential, e.g., agency login:	dalkilic			
Project Role*: Other (Specify)	Other Project Role Category: Core Technical Staff			
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	biosketch-dalkilic_2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Shanna	Middle Name	Last Name*: Babalonis	Suffix:
Position/Title*:	Assistant Professor			
Organization Name*:	University of Kentucky			
Department:	Behavioral Science			
Division:				
Street1*:	Robert Straus Behavioral Science Bldg.			
Street2:	845 Angliana Avenue			
City*:	Lexington			
County:				
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Project Role*: Other (Specify)	Other Project Role Category: External Consultant - Key			
Degree Type: PHD,MA,BA	Degree Year: 2010,2005,2002			
Attach Biographical Sketch*:	File Name:	SBabalonis_Biosketch_Sept_14_2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
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Degree Type:	PHD,BS	Degree Year: 2002,1997		
Attach Biographical Sketch*:	File Name:	Kepecs_Biosketch_03.15.2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
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Project Role*:	Graduate Student		Other Project Role Category:	
Degree Type:	PHD,BS		Degree Year: 2024,2019	
Attach Biographical Sketch*:	File Name:	GN_Biosketch_2022.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
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State*:	MO: Missouri			
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Project Role*:	Other (Specify)		Other Project Role Category: External Consultant - Key	
Degree Type:	PHD,BS		Degree Year: 1990,1987	
Attach Biographical Sketch*:	File Name:	2022_Salvemini_BioFinal.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
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Degree Type:	PHD Degree Year: 1989			
Attach Biographical Sketch*:	File Name:	2022_Soltesz_BiosketchNew_08122022.pdf		
Attach Current & Pending Support:	File Name:			

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 09/30/2024

1. Vertebrate Animals Section

Are vertebrate animals euthanized? Yes No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

Yes No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

Yes No

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? Yes No

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? Yes No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: Yes No

If the answer is "Yes" then please answer the following:

*Previously Reported: Yes No

6. Change of Investigator/Change of Institution Section

Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001

Expiration Date: 09/30/2024

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1. Introduction to Application (for Resubmission and Revision applications)	2022_Overall_Introduction__20220925.pdf
Research Plan Section	
2. Specific Aims	2022_Overview_specific_aims_20221001.pdf
3. Research Strategy*	2022_Overview_research_strategy_20221002.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	2022_Combined_letters_of_support.pdf
10. Resource Sharing Plan(s)	2022_Resource_sharing_and_data_managment_20221001.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication_key_resources_final.pdf
Appendix	
12. Appendix	

Introduction – Overall

We thank the reviewers for their careful reading of our original proposal, thoughtful comments, and enthusiasm for the P30 proposal. For this Introduction, we will address concerns raised for the overall application and issues that cut across multiple cores. Core-specific comments will be addressed in the relevant cores.

1. More details were needed on center operations given the strong enthusiasm by the SUD community for the P30 proposal and the risk that the Center will be overwhelmed if clearly delineated processes to prioritize project and maintain rigor are not in place. We agree with this concern and have now emphasized throughout the proposal how choices will be balanced between enthusiastic affiliates and limited resources, individuals are trained, when alternative approaches will be used if unanticipated problems arise, etc. In addition, each Affiliate and their potential contributions to the C3A was reviewed and we have eliminated a few affiliates in the resubmission to better focus the proposal.

2. Stronger integration between the imaging and mass spectrometry cores. We agree that integration is a key consideration for P30. While some projects will naturally fall into one or the other core, many projects will involve tight integration. For example, changes in the levels of cortical 2-AG in specific brain regions identified in the BLMC can be mechanistically examined by using the cell-type-specific expression of the eCB-GRAB sensor (Fig 11 in MSIC research strategy) to determine the anatomical location with the greatest changes. Similarly, PharmacoSTORM can be used to determine if altered nanoscale location and abundance of eCB metabolizing enzymes underlies changes in 2-AG or anandamide levels identified by the BLMC.

3. Concern that translational opportunities were missed. Many of the proposed BLMC studies (directly address translational questions, either with human samples or translational models. For example, study 1.7 will evaluate circulating endocannabinoid levels in cannabis users enrolled in the ABCD study. Furthermore, both proposed pilot projects examine translational questions: One is the impact of THC exposure on later mild traumatic brain injury cognitive sequelae and the other explores if plasma endocannabinoids predict outcomes in individuals with opioid use disorder. Thus, at its core, the proposal is very translational in nature—both from examining translational questions in preclinical models as well as substantial human studies. We now emphasize this translational focus throughout the proposal.

4. Concern that core PI's NIH funding was weak. One co-PI of the Imaging Core, Istvan Katona, was recently awarded an R21 (DA056825), despite the chaos arising from moving his lab from Budapest to Bloomington during the pandemic. The second co-PI of the Imaging Core, Hui-Chen Lu is PI on two R01s (NS086794-08 and R01DA053746-01). By the nature of her analytical work, the PI of the MS Core, Heather Bradshaw, is supported by numerous subcontracts from NIH grants and other funding sources. These subcontracts include several from NIH grants with Hohmann, Lu, and/or Mackie as PIs. All core PIs have one or more R01-level proposal under review.

5. Multiple concerns involving the Pilot Projects Core (PPC). We have taken the resubmission as an opportunity to completely revise the PPC. Norbert Hájos, who has extensive experience in mentoring early-stage scientists (details in PPC and Administrative Core) will now lead this core. All PIs and Affiliates will assist with mentoring, as best matches the interests, experiences, needs and goals of the mentee. Our target now is to fund 2 to 4 pilot projects annually as supported by our budget. We go into more detail in the proposal how mentoring will be specifically tailored to fit the mentee, how pilot project progress will be tracked, how compliance challenges will be addressed early in a project, and how oversight and coordination will occur between the PPC and the other cores in the Administrative Core and PPC sections of the proposal.

6. Outreach by P30 mentees to their communities. This was an excellent point raised in the review. Many of our affiliates work in regions of the US particularly hard hit by SUDs and already engage in this type of outreach (some examples are now mentioned in affiliates' letters of support). We will leverage the experiences of these outreach-engaged affiliates broadly across our mentees to incorporate community outreach as a component of our mentoring program.

7. Challenges with data management. We agree that the types of data generated by the cores will be quite different, each with distinct challenges. We have increased the effort of the data scientist to 1.0 FTE to help address this. In addition, we have increased the diversity and quantity of the resources available for data management and go into more detail on how the data scientist will interface with project leads for the two service cores to most efficiently handle the large datasets generated. In addition, Core-specific data challenges are now more thoroughly discussed in the relevant section of the Core Research Strategy.

Specific Aims Overview

Addictive substances trigger plasticity at the molecular, cellular and circuit levels and their chronic use may manifest as persistent behavioral changes, leading to substance use disorders. Targeting these changes may lead to novel strategies for preventing or treating substance use disorders. However, our knowledge of the molecular changes, the cellular processes and the circuit activity patterns that underlie various aspects of substance use disorders including compulsion, loss of intake control, withdrawal, and relapse is rather limited. To obtain a better understanding of the molecular to circuit level plasticity accompanying drug abuse, the IU Bloomington Center for Cannabis, Cannabinoids, and Addiction (IUB-C3A) brings together more than two dozen exceptionally strong drug addiction researchers (*i.e.*, *Center Affiliates*) at all career stages from more than twenty institutions and a dozen states to create a research center that will foster innovative and integrated application of lipid mass spectrometry and multi-scale imaging to answer important questions in drug addiction.

When initially discussing a possible P30 Center with our colleagues across the Midwest, we were gratified and inspired by the high demand and great enthusiasm for establishing a Center like the C3A at IU Bloomington (IUB). Due to our strong cannabinoid research programs at IUB, all Center PIs share a fundamental interest in cannabis and cannabinoids. Thus, cannabinoids are a central theme of the C3A. Building on this, many of our Affiliates have, or are implementing, active research programs centered around cannabinoids. However, the Center will also apply its resources to all important questions relevant to drug addiction that can be approached using lipid mass spectrometry or multi-scale light microscopic imaging. As the supportive letters attest, the five C3A PIs (Bradshaw, Hájos, Katona, Lu, and Mackie) have already established strong collaborations with many of the Center Affiliates and the C3A naturally extends from this collaborative foundation, providing a rigorous framework within which to continue ongoing and establish new collaborations. We look forward to expanding this network by additional collaborations with new Affiliates and pilot project participants through the C3A.

The IUB-C3A is comprised of four cores: An Administrative Core, a Pilot Project Core, and two Service Cores. The *Administrative Core* ensures the efficient operations of the Center, oversees the educational components of the Center, coordinates data storage and dissemination by the Center, and serves to connect both scientists and the public with the Center and its resources. The *Pilot Project Core* (PPC) will support 2-4 pilot projects annually, selected from Center Affiliates or trainees. It will also provide a framework for mentoring of pilot project applicants (successful or not). The first Service Core is the *Bioactive Lipid Mediators Core (BLMC)*. This core will use highly sensitive mass spectrometry to determine levels of bioactive lipid mediators such as phytocannabinoids and their metabolites as well as endogenous signaling lipids (such as endocannabinoids and related molecules) in biological samples (most often, plasma, cultured cells, or brain). The second Service core is the *Multi-Scale Imaging Core (MSIC)*. This core provides imaging across multiple modalities (slide scanning, conventional confocal, super-resolution microscopy implemented as stochastic optical reconstruction microscopy (STORM, including the recently developed PharmacostORM approach), and *in vitro* and *in vivo* multiphoton imaging). Taking a multi-modality approach allows for imaging from the macro- to the nanoscale in fixed tissue as well as functional imaging in awake behaving mice with multiphoton microscopy.

The IUB-C3A has three specific aims:

1. Establish itself as a resource to provide cutting edge lipid mass spectrometry and imaging approaches to addiction researchers addressing unresolved, high impact questions in substance use disorder research. Center Affiliates will be able to access core resources at minimal expense to perform experiments that they are unable to execute at their home institutions. The focus of this use of the Cores will be to extend already funded drug abuse research, enable investigators to obtain key preliminary data for grant applications, and to encourage highly qualified non-drug addiction researchers to rapidly enter the field.

2. Provide training in mass spectrometry and imaging techniques. Our objectives are both to familiarize users with these approaches (especially their strengths and limitations) so they can be applied to high impact questions in the field of drug addiction, as well as to introduce individuals from groups historically under-represented in drug addiction research into the addiction field. This will be done both through a 8-week summer course (offered by the BLMC for under-represented groups) and a 2-week course (for STORM imaging) offered four times a year by the MSIC.

3. To innovate lipid mass spectrometry and high-resolution imaging so these techniques can be used to study important research questions that cannot be satisfactorily addressed with current mass spectrometry or imaging techniques (specific examples of innovation are given in the relevant core research strategy).

Research Strategy

A. Significance

This is a proposal to establish a P30 Core Center of Excellence (the *IU Bloomington Research Center for Cannabis, Cannabinoids and Addiction* (C3A)) at Indiana University, Bloomington. The goal of the center is to provide access to cutting edge imaging and mass spectrometry techniques to researchers (“Center Affiliates”) studying drug addiction and related topics. While most of our Center Affiliates come from across the Midwest (Indiana, Ohio, Kentucky, West Virginia, Illinois, Missouri, Nebraska, and Wisconsin), we also have scientists from as far away as Pennsylvania, New York, Maryland, and Arizona express interest in using our specialized core resources and participating in our courses (see letters of support). These researchers will be joining the C3A as Affiliates. Building on IU Bloomington’s dedicated nucleus of cannabinoid researchers, a major focus of the Center will be on cannabinoids. However, few individuals abuse only a single class of drug (and often transition between drugs during their lifetime), and many addictive drugs interact with the endocannabinoid system, so the Center will facilitate research into a broad range of addictive drugs as well as combinations of these drugs (e.g., Dr. Babalonis’ studies).

The Midwest is a region hit very hard by drug addiction in general, and opioid overdose deaths in particular. While comprehensively addressing this problem requires attention at multiple levels (treatment, prevention, social support, etc.), an important component to solving the problem of drug addiction is understanding basic mechanisms of addictive drugs with an emphasis on the long-term consequences of their use. In addition to performing this basic research, we also feel that it is equally important to identify, encourage, train, and support researchers from this region (Midwest) in researching the basic science of drug addiction. We have addressed this training and regional concern by assembling a consortium of more than 20 Midwest drug abuse researchers as *Center Affiliates* (see below and letters of support) whose existing research will be enhanced by access to the Center’s resources. These Midwest drug addiction researchers are complemented by additional Center Affiliates drawn from outstanding addiction researchers across the country (e.g., Yasmin Hurd, Joe Cheer, John Dani, etc.). In addition to facilitating research, an integral component of this P30 Center of Excellence will be in depth educational experiences designed to introduce researchers from backgrounds traditionally underrepresented in drug addiction research (many drawn from the Midwest) to the field. This will primarily be accomplished through a summer course organized by Dr. Bradshaw, PI on the Bioactive Lipid Mediators Core. This will ensure a pipeline of new substance use disorder investigators, many of whom we hope will retain their connections to the region. The second educational component of the C3A is to train more established scientists in the-cutting edge technologies used by the P30’s Service Cores, particularly STORM super-resolution imaging. One goal of this course is to introduce these researchers to the possibilities of STORM imaging, gain familiarity with the techniques, and then apply them through super resolution imaging cores in their home institutions.

Under the direction of Ken Mackie (Gill Chair, and Distinguished Professor of Psychological and Brain Sciences) and director of the Administrative Core, the Center includes the Multi-scale Imaging Core (MSIC, co-directed by Drs. István Katona and Hui-Chen Lu), the Bioactive Lipid Mediator Core (BLMC, directed by Dr. Heather Bradshaw), and the Pilot Project Core (PPC, directed by Dr. Norbert Hájos). The C3A will be housed in a series of contiguous labs on the first floor of the Multiscience Building II (MSBII) on the IU Bloomington Campus. The PI (Mackie) and Core Directors (Bradshaw, Hájos, Katona, and Lu) have their research labs on the first two floors of MSBII, along with IUB C3A Affiliate and Internal Advisory Board member Dr. Andrea Hohmann. The proximity of the C3A to the Gill Center (the major neuroscience center at IUB) and these labs facilitates sharing of resources and enhances formal and informal scientific interactions (e.g., by the C3A “piggybacking” onto the seminar series and journal club run by the Gill Center). Day-to-day running of the C3A is overseen by a steering committee consisting of the C3A PIs, the technical director of each core, and the Program Manager. The C3A is advised by an internal advisory board (IAB) (see below) that provides tactical and strategic advice for local (primarily IU Bloomington-specific) C3A issues and an external advisory board (EAB) (see below) that provide longer term strategic advice and evaluates the Center’s performance as a national resource. Both will also be involved in evaluating Pilot Core proposals. The ~two dozen Center Affiliates are supported by more than 30 federal grants, more than a dozen of which are qualifying (6 are funded by NIDA) (**Table 1**). The non-NIDA, but qualifying grants are addressing problems related to drug abuse or the consequences of drugs abuse, as explained in the footnotes to **Table 1**. Non-qualifying grants (e.g., less than two years of funding remaining, non-R mechanisms, not directly related to substance abuse, and training grants) are also shown (**Table 2**) as they give an informative picture of the breadth of funding supporting the labs and trainees of C3A PI’s and C3A Affiliates.

The strong commitment of IU Bloomington to the C3A and substance use disorder research is evident by the significant pledges of financial and administrative support that they have offered us. The IU Bloomington Campus administration has a culture of partnering with faculty to support their research, particularly research that promotes historically under-represented groups in specific disciplines. Thus, they have partnered with us to provide a financial commitment of 75% match for equipment purchased for the IUB C3A (see letters of support from Associate Dean Pohl and Vice President for Research Cate). In addition, the Gill Center has partnered with the C3A to fund five summer internship lines for our summer internship program supporting laboratory experiences for under-represented minorities (see letter of support from the Gill Center Director, Dr. Hui-Chen Lu).

B. Innovation

Continuous innovation is necessary if a technology-intense center is to continue to be relevant and useful for scientists in the field. The PIs of the C3A service cores have extensive track records of innovating their techniques and will continue to do so in the context of the C3A. Examples of innovation that will occur in the C3A include, the development and implementation of lipid extraction and more sensitive mass spectrometric analysis techniques aimed at measuring bioactive lipid signaling molecules in smaller amounts of plasma and CNS tissue, implementing machine learning algorithms for the analysis of lipidomics data, further developing PharmacoSTORM to increase the number of ligands, receptors, types of tissues, and enzymes that can be examined, extending longitudinal 2P imaging in young mouse pups, and optimizing two-photon-Fluorescent Lifetime Imaging Microscopy (2pFLIM) protocols for *in vivo* imaging of cellular metabolic changes and neuron/astrocyte proximity that occur during or after chronic exposure to drugs of abuse.

C. Approach

Organizational Structure and Administration

Figure 1 shows the organization of the IU Bloomington Center for Cannabis, Cannabinoids and Addiction (C3A). The **Steering Committee** (comprised of the PIs and Core technical directors) will be the group that is responsible for the day-to-day running of the C3A. Overall, the C3A will consist of two service/research cores, the **Pilot Project Core (PPC)** and the **Administrative Core**. One research core (**Bioactive Lipid Mediator Core, BLMC**) will focus on measurements of phytocannabinoids and bioactive lipid molecules from a variety of biological samples. The second core (**Multi-Scale Imaging Core, MSIC**) will match specific imaging techniques (whole brain scanning, conventional confocal, super-resolution (STORM) microscopy, two-photon (2P) imaging and ScaleS) to the research question being asked. The PPC will solicit pilot projects related to drug addiction from Center Affiliates, their trainees and collaborators, and the central Midwest neuroscience community. Preference in awarding the pilot projects will be to fund strong applications submitted by scientists not well represented in the drug addiction community, to investigators whose research is transitioning to questions in substance use disorders, and investigators who need pilot data for grant submissions to NIDA. The Administrative Core will ensure the smooth running of the C3A (ordering, compliance, etc.), coordinate the two training programs (mass spectrometry summer course and Four Seasons STORM imaging courses), run the seminar series, prepare the necessary reports, ensure data archiving and dissemination, and track of C3A finances.

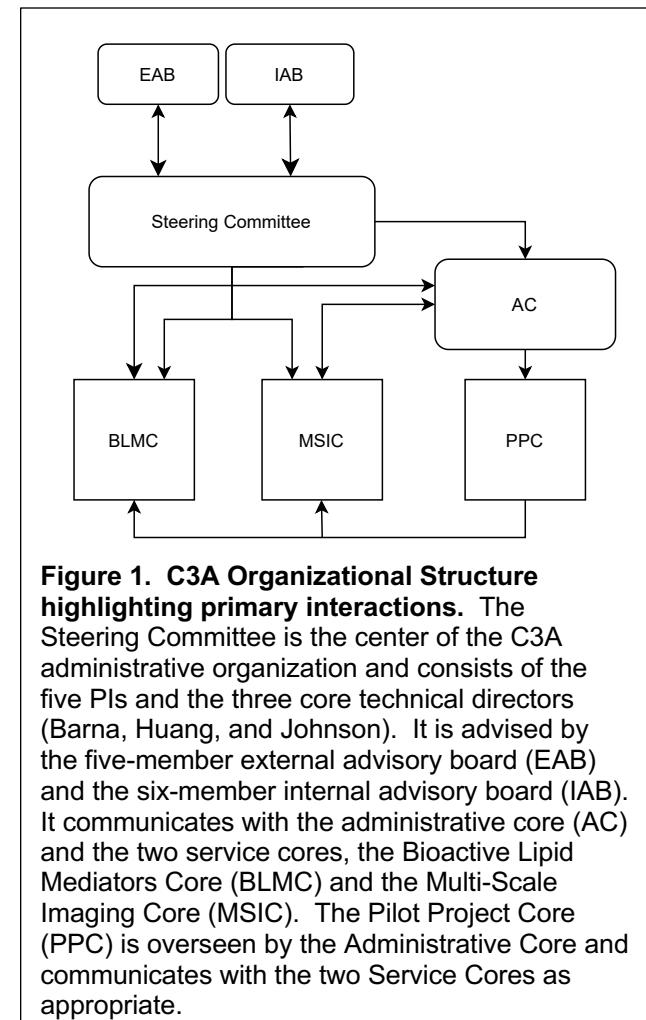


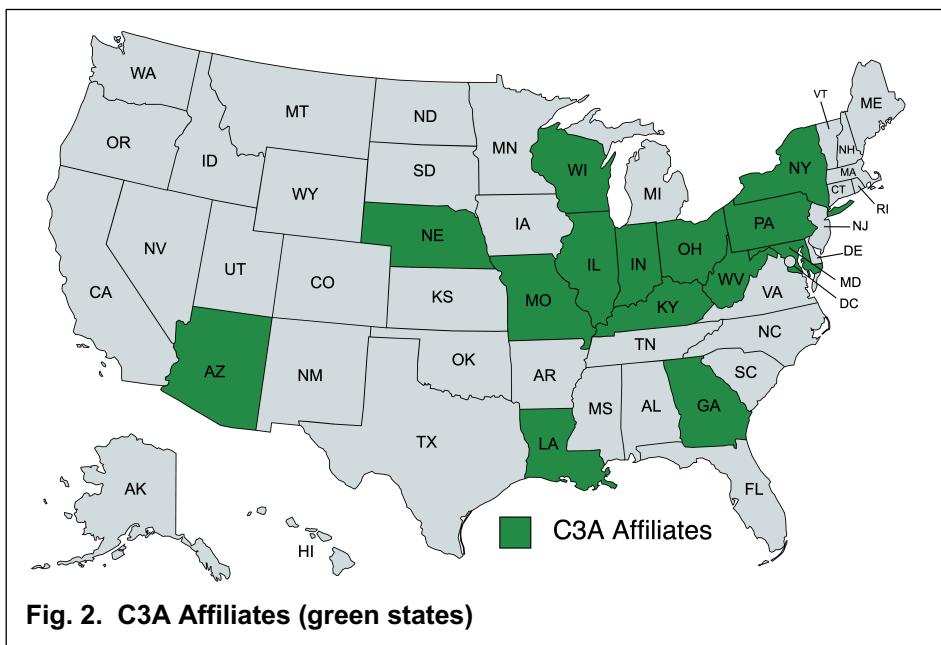
Figure 1. C3A Organizational Structure highlighting primary interactions. The Steering Committee is the center of the C3A administrative organization and consists of the five PIs and the three core technical directors (Barna, Huang, and Johnson). It is advised by the five-member external advisory board (EAB) and the six-member internal advisory board (IAB). It communicates with the administrative core (AC) and the two service cores, the Bioactive Lipid Mediators Core (BLMC) and the Multi-Scale Imaging Core (MSIC). The Pilot Project Core (PPC) is overseen by the Administrative Core and communicates with the two Service Cores as appropriate.

Overview of Scientific Focus and Integration of Ongoing Center Research

In preparing this proposal, the PIs and IUB Affiliates reached out to local, regional, and national colleagues to identify those that will both benefit from the Center's resources and enhance the intellectual climate of the Center. We are pleased to have assembled an excellent group of researchers. (See letters of support and biosketches for more details.) Many of our Center Affiliates would be considered "core" drug addiction researchers. However, others like Keisuke Kawata (IUB) and Adam Kepecs (WU) are very successful in their current fields but would like to transition part of their research programs to address key unanswered questions in the substance abuse field. Thus, investigators like Keisuke and Adam will bring their unique approaches and perspectives to studying the consequences of abused drugs, enriching the C3A. Finally, we have also included some highly talented new investigators as Affiliates, such as Drs. Karhson and Kimbrough. Our goal is to incorporate a diversity of career stages among the Affiliates with the expectation that those who will benefit from it will be mentored through their use of the C3A and by interacting with the C3A PIs, technical staff and other Affiliates. Conversely, we will also learn from them.

The five PIs on this proposal have a strong interest in how drugs of abuse, particularly cannabis, impact neurodevelopment. Thus, one unifying theme of the proposal is the impact of drugs of abuse on the developing nervous system. However, we would like to stress that the interests of the PIs are broader than this and the range of Center Affiliates reflects these broader interests that extends well beyond neurodevelopment.

The geographic reach of the Center is intentionally broad. **Fig. 2** shows the geographic distribution of C3A Affiliates. The C3A has excellent representation across the Midwest and with additional C3A Affiliates in Arizona, Georgia New York, Pennsylvania, and Maryland



Strengthening existing collaborations, initiating new collaborations

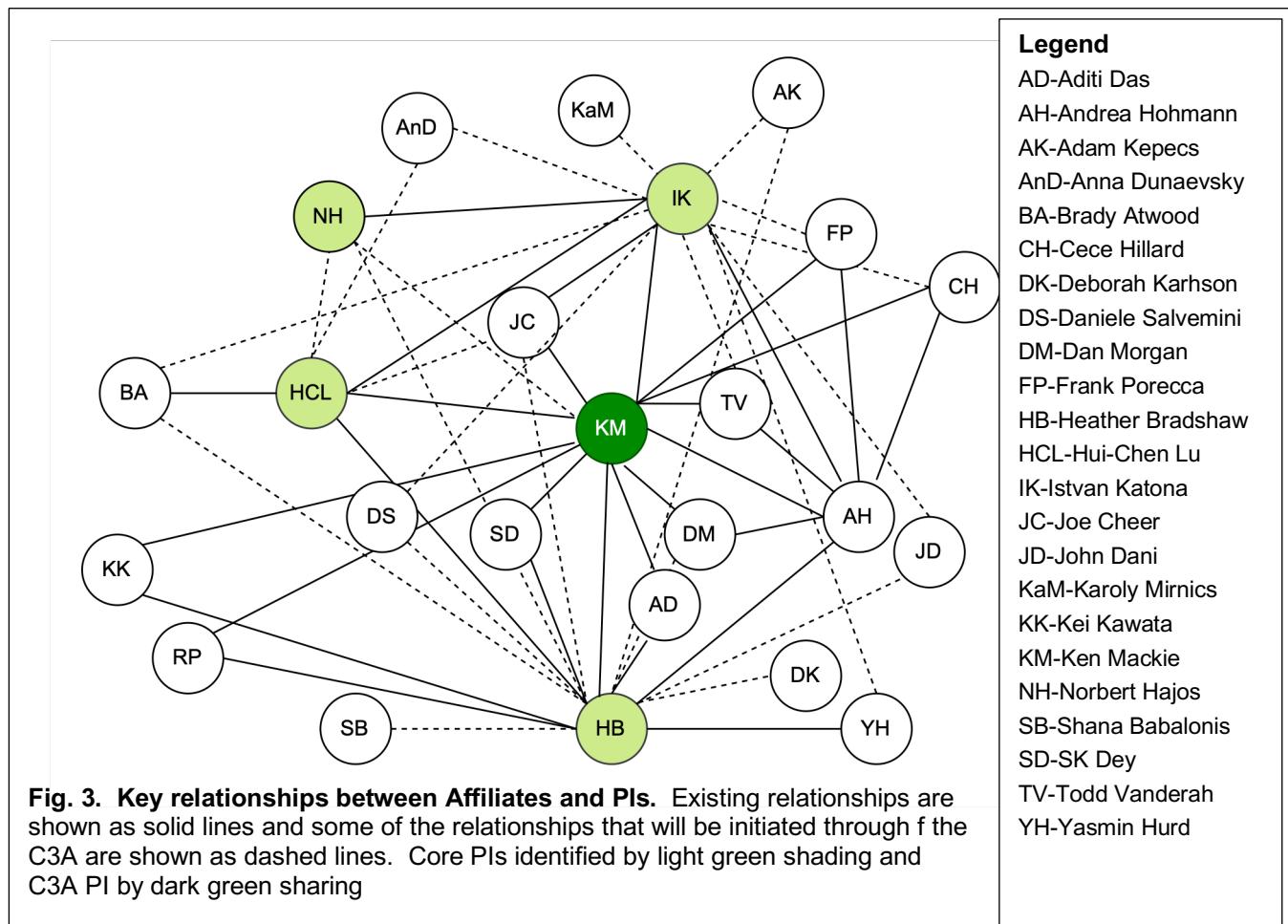
Existing, very strong collaborations form the foundation of the C3A (see Affiliate letters and **Fig 3**). However, an important added value of the C3A comes from the new collaborations that will emerge from interactions between the Core PIs and the Affiliates, and very likely between Affiliates, the latter being catalyzed by C3A activities (such has monthly C3A research meetings, held in a hybrid fashion—in person and on Zoom) and targeted outreach "AKA match making" based on the Core PIs knowledge of experiments being proposed to the C3A. **Fig. 3** provides a graphical representation of current collaborations (solid lines) and collaborations that will likely be initiated (dashed lines) if the C3A is funded.

We highlight a few representative C3A projects to better illustrate how existing collaborations will be strengthened and new collaborations initiated through the C3A. Please note that this is not an exhaustive list. However, it gives a sense of range of the cores and technologies that will be used as well as the subjects of the experiments—ranging for preclinical to translational.

Brady Atwood: Dr. Atwood (IUSM) examines the role of the striatum in alcohol abuse and binge drinking [1,2]. Recently, working with Dr. Lu and others at IUSM and IUB, they have developed a model of neonatal opioid withdrawal syndrome (NOWS, funded by the IU "Addictions Grand Challenge") and showed electrophysiological, anatomical, and behavioral abnormalities involving motor cortex in young mouse pups [3,4]. Dr. Atwood would like to extend these studies using 2P imaging in

the MSIC to determine how the maturation of cortical neuronal network activity is affected in this NOWS model.

Cecilia Hillard: Dr. Hillard (Medical College of Wisconsin) is an established cannabinoid researcher, having contributed many fundamental findings to the field [5-8] and a long-term collaborator of Dr. Mackie. While her lab has the capability to measure anandamide and 2-AG, she doesn't have the ability to measure the wide range of lipoamines and oxylipins that Dr. Bradshaw can measure in the BLMC. In a study of ABCD participants, Dr. Hillard would like to extend the initial goal of correlating endocannabinoid levels to various cognitive abilities and emotional states to examining if specific lipoamines have a correlation with any of these outcomes. This essentially extends the study from measuring 2 lipids to >100 lipids. Dr. Hillard would also like to use ScaleS and STORM imaging to extend a R01 (MH121454) examining the connectivity of the medial septum and the nucleus of the diagonal band to the medial habenula during abstinence and relapse to drugs of abuse.



Keisuke Kawata: Dr. Kawata (IUB) is an associate professor in the IUB School of Public Health interested in the long-term sequelae of repeated mild traumatic brain injury (mTBI) (R01NS113950) and how they may be predicted and prevented. In an add-on study Dr. Kawata has found that chronic cannabis users show an attenuated biomarker response (plasma protein and oculomotor function) in a standardized human model of mTBI. He will collaborate with the BLMC to determine if lipoamines and oxylipins will serve as biomarkers for predicting the severity and duration of CNS impairment following repeated mTBI.

Adam Kepcs: Dr. Kepcs' lab (Washington University, St. Louis) studies the neural circuit basis of decisions and cognitive functions and how they go awry in mental disorders (R01MH097061). Recently, he has become interested in drugs of abuse (e.g., hallucinogens, [9]), their mechanisms of action, and the consequences of chronic use (including use during development). He proposes to use PharmacoSTORM to determine which cell types and in which subcellular compartment express the

relevant receptors (for which there are not very specific antibodies) in the tail of the striatum and how their number and localizing vary following environmental challenges.

Yasmin Hurd: One research focus in Dr. Hurd's lab is the effects of cannabis on the developing brain (R01DA030359) [10-12]. She has recently initiated a collaboration with Dr. Bradshaw and the BLMC to determine the effects of phytocannabinoids on levels of endocannabinoids and related lipids in the developing brain. These studies will be enhanced if the C3A is funded by improving sensitivity of the lipid mediator measurements, thus facilitating lipid measurements in smaller brain regions. These studies would then motivate studies in the imaging core using endocannabinoid sensors to determine the spatial distribution of altered endocannabinoid signaling. Finally, if changes are found, these would then inspire anatomical studies to determine if an altered distribution of endocannabinoid synthesizing or degrading enzymes underlie the changes in measured lipids. In addition, Dr. Hurd would like to apply STORM imaging to better characterize the effects of THC on different domains of the developing placenta, a topic that is likely quite important, but has received little attention in the field. In addition, she would like to extend her previous studies on the impact of developmental THC as well as opioid use on the endogenous opioid system (R01DA051191), a system which is highly amenable to study by PharmacoSTORM. A particularly attractive and translational direction for method development by the MSIC will be the application of PharmacoSTORM to frozen human brain tissue, which Dr. Hurd has access to. These studies would nicely follow up her earlier studies that used *in situ* hybridization in human fetal tissue showing maternal cannabis-induced alteration in dopamine and opioid gene expression [13,14], by extending them to the nanoscale protein level.

Environment at Indiana University Bloomington (IUB)

Bloomington is the flagship residential, doctoral-extensive campus of Indiana University. Its mission is to create, disseminate, preserve, and apply knowledge. It does so through its commitments to cutting edge research, scholarship, arts, and creative activity; to challenging and inspired undergraduate, graduate, professional, and lifelong education; to culturally diverse and international educational programs and communities; to first-rate library and museum collections; to economic development in the state and region; and to meaningful experiences outside the classroom. The Bloomington campus is committed to full diversity, academic freedom, and meeting the changing educational and research needs of the state, the nation, and the world. There are six schools on the IUB Campus, with the College of Arts and Sciences (the College) being the largest. The College has ~10,000 undergraduates and 2,500 graduate students, more than 800 tenure track faculty, and ~50 research centers and institutes.

The PIs for the C3A are all tenured faculty of the College, members of the Department of Psychological and Brain Sciences, and three (Drs. Hájos, Lu and Mackie) hold endowed chairs in the Gill Center for Biomolecular Science and two (Drs. Bradshaw and Katona) are affiliate members of the Gill Center, the premier research center on the Bloomington Campus. We are gratified to have received strong support for this proposal from the IUB Campus (i.e., the Office of the Vice President for Research) and the College. Together, they have pledged 75% matching funds for equipment purchased for the C3A (~\$870,000 contribution from the College and Campus, details are in the budget portion of the application and spelled out in the letters of support from Associate Dean Nikki Pohl and Vice President for Research, Fred Cate). This generous partnership is typical of IUB where major institutional support at both the Campus and College level is provided for faculty initiatives after careful assessment of the proposal and how it would enhance research on the IUB campus. In addition, the STEM Summer Scholar Institute (SSI) program will provide financial support for the BLMC summer course (see letter of support). Finally, the C3A is also strongly supported by the Gill Center, who has pledged \$20,000 annually in support of under-represented minority summer undergraduate research stipends. This is consistent with the Gill Center's strong emphasis on undergraduate experiential learning. In all, IUB provides an outstanding and supportive environment to ensure the success of the C3A.

Advice and Oversight

External Advisory Board Members

We have recruited a strong and engaged external advisory board (EAB) to provide a rigorous evaluation of C3A performance and to provide thoughtful advice for long term strategic Center decisions. The five external

advisory board members are: Michael Bruchas (Professor of Anesthesiology, Pharmacology, and Bioengineering, University of Washington), Margaret McCarthy (Chair of Pharmacology, University of Maryland School of Medicine), Sachin Patel (Chair of Psychiatry, Northwestern Medical School), Ivan Soltesz (James R Doty Professor of Neurosurgery and Neurosciences, Stanford University), Marina Wolf (Professor of Behavioral Neuroscience, OHSU). Dr. Bruchas brings experience in multiphoton imaging, optogenetics and as a core director of UW's NIDA P30 on opioid addiction. Dr. McCarthy is an expert on sex differences in the developing nervous system and has considerable administrative experience as chair of the Department of Pharmacology at the University of Maryland School of Medicine. Dr. Patel is practicing psychiatrist and an expert in the role of endocannabinoids in stress, behavior, and in performing lipid measurements using mass spectrometry. Dr. Soltesz has worked in the endocannabinoid field for many years, has extensive administrative experience, and is an expert in multiphoton imaging, including imaging using the recently developed endocannabinoid sensor. Dr. Wolf has a longstanding interest in the role of synaptic plasticity in all phases of drug addiction, has served in numerous administrative roles and the university and national levels, including on the external advisory board of Yale's NIDA-supported P30 on neuroproteomics. Thus, we are confident that our EAB will be engaged and helpful in executing our P30 goals.

Internal Advisory Board Members

Local advice, oversight, and strategic planning will be provided by an internal advisory board (IAB). The IAB will consist of a key IUB Center Affiliate (Andrea Hohmann, also a holder of a Gill Chair) and several IUB faculty chosen for their technical and/or administrative experience: Katy Börner (Victor H. Yngve Distinguished Professor of Information Science in the IUB School of Informatics, Computing, and Engineering and an expert in large scale networks, data analysis/visualization, and managing large groups), David Clemmer (Distinguished Professor and Robert & Marjorie Mann Chair of Chemistry, former associate dean for Natural and Mathematical Sciences (IUB) and expert in mass spectrometric technique development), Tennisha Riley (adolescent development and decision making that may lead to problem drug use), Sid Shaw (director of the IUB Light Microscopy Imaging Center and the Integrated Freshman Learning Experience), and Jeff Zaleski (Provost Professor of Chemistry and former IUB Vice Provost for Research). Dr. Zaleski has agreed to chair the IAB. Letters of support are enclosed for all IAB members.

Operating Policies

One of Dr. Mackie's main roles as PI of this P30 will be to facilitate communications and collaborations between Center Affiliates and the cores, to engage with current and future investigators to identify optimal approaches and technologies to address important unresolved questions in drug addiction research, ensure spending of each core is in line with budgeted funds, and to work with the core directors as they institute innovative technologies in their respective

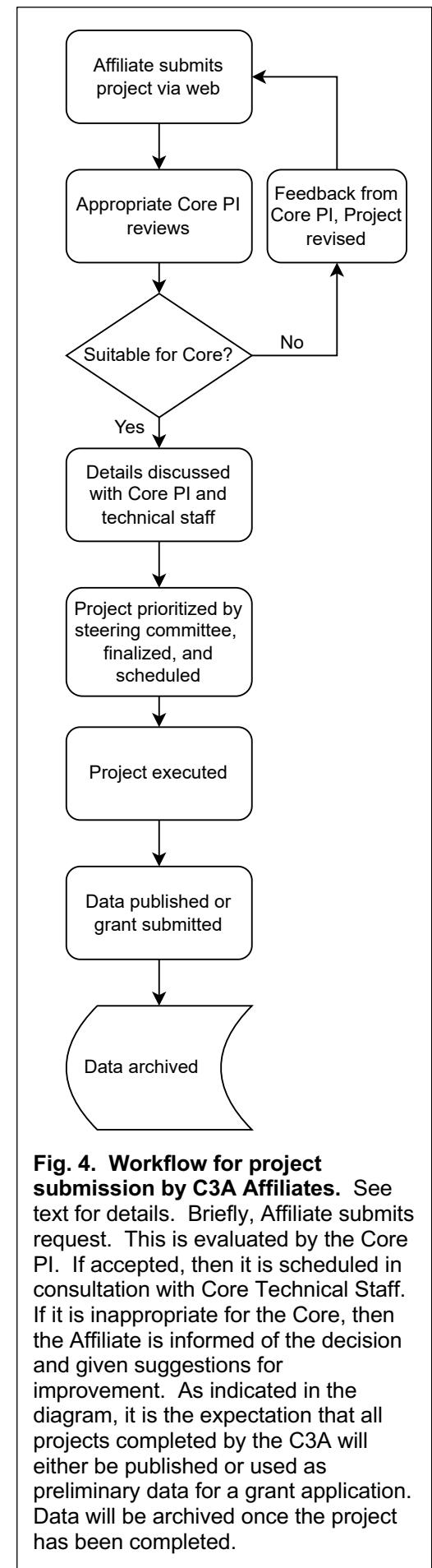


Fig. 4. Workflow for project submission by C3A Affiliates. See text for details. Briefly, Affiliate submits request. This is evaluated by the Core PI. If accepted, then it is scheduled in consultation with Core Technical Staff. If it is inappropriate for the Core, then the Affiliate is informed of the decision and given suggestions for improvement. As indicated in the diagram, it is the expectation that all projects completed by the C3A will either be published or used as preliminary data for a grant application. Data will be archived once the project has been completed.

cores. PI Mackie will devote 20% of his professional effort to these activities.

The C3A will implement the following policy for approving affiliate use of the core resources (illustrated in Fig. 4). Prior to the initiation of a project, the affiliate will submit a short description of their project via a dedicated project portal (accessible to Center Affiliates) on the C3A website. This description will outline the goals of the project, its relevance to and impact on drug addiction research, the hypothesis to be tested based on their preliminary data, the requested experiments, and the preparatory work that needs to be done for performing the experiment. The final item will necessarily vary across cores. The request will be automatically forwarded to the relevant Core PI, the C3A program administrator and Dr. Mackie. This procedure ensures that all use of the cores is documented, well justified, and aligned with the P30's mission. It also gives the Core PI an opportunity for internal quality control before project initiation, and to provide suggestions to the Affiliate regarding appropriate core resource utilization, experimental design, sample size, etc. to ensure rigor and reproducibility. The Core PI will aim to respond to these requests within 48 hours. If the proposed research is suitable for support, the Core PI will refer the investigator to their Core's technical director who will discuss with the Affiliate the best solution to optimize their experimental approach, determine the most appropriate way to proceed, and work to schedule the experiment into the Core's workflow. The nature of this consultation will vary with the experiment and core engaged. For example, use of the BLMC may just require submission of samples to the BLMC after a conversation with Drs. Bradshaw and Johnson. On the other hand, longitudinal two-photon imaging of young mice in an Affiliate-generated mutant mouse line conducted in collaboration with the MSIC will require arranging for the mice to be added to our IACUC protocol and shipped to IUB, for the investigator or a trainee from their lab to come to Bloomington, be trained to perform the surgery, and then be actively engaged in the experiment and the data analysis. Details of these steps are provided in the relevant service core research strategy. Overall, the Center, while recognizing that each investigator has unique needs, gives a high priority to training users in the techniques employed by the C3A (see *Administrative Core* for descriptions of the two courses that will be offered) as opposed to merely performing assays for the Affiliates. Pending projects will be discussed at the monthly steering committee meeting and prioritized based on P30 goals (scientific merit, alignment with P30 goals (SUD-related, etc.), feasibility, availability of core resources, time-sensitivity (e.g., need for preliminary results for a specific grant deadline, etc.).

Communications within the Core and between the Core PIs and technical staff will be facilitated by monthly steering committee meetings that will be attended by the PIs, Core Technical Directors (Drs. Barna, Huang, and Johnson), and the program manager. As appropriate for the task at hand, specific members of the IAB as well as the Center's fiscal officer may attend. These meetings will include discussions on research accomplishments, challenges, future directions, etc. At this meeting Core PIs will review the current and future projects for their core with the group with a goal of ensuring the most efficient

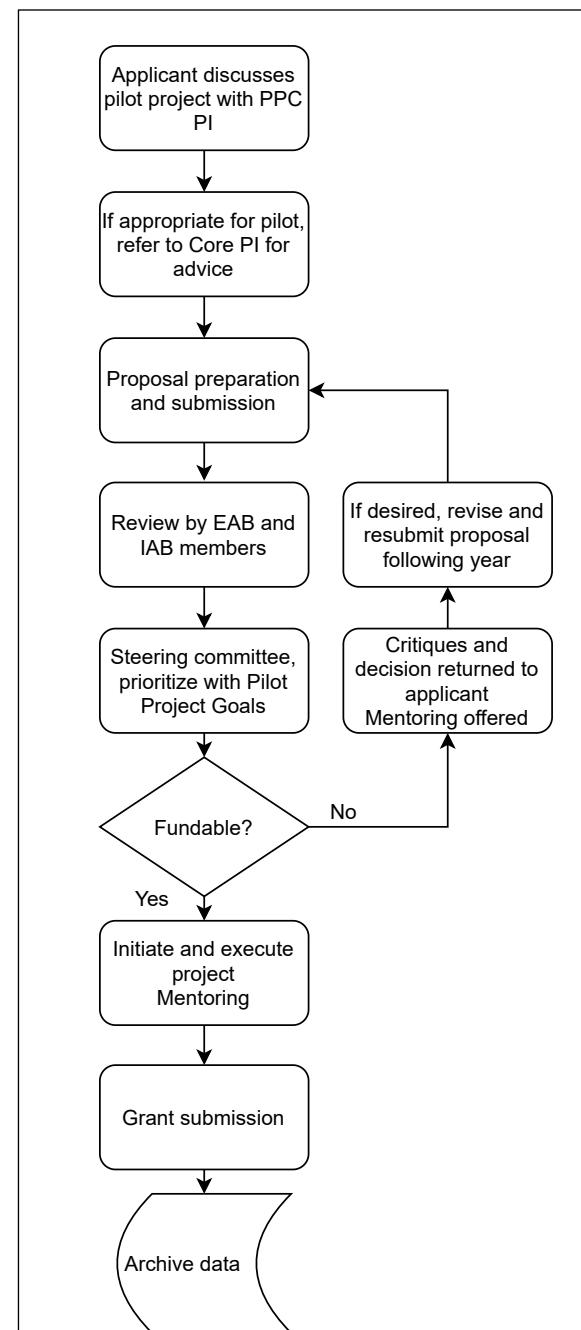


Fig. 5. Process for Pilot Project

submission. See text for details. Briefly, applicants discuss potential projects with the PPC PI, who refers feasible projects to the appropriate core's PI. This PI then works with the applicant to craft a competitive application, which is reviewed by members of the EAB and IAB. Successful applicants are mentored through their project and unsuccessful applicants are offered constructive feedback and mentoring if desired to increase the competitiveness of their subsequent applications.

core utilization. This process will also help ensure that spending by each core is occurring at the rate projected at the beginning of the fiscal year and allows for timely corrections, if necessary. Pilot Project awardees will also present their interim results at these meetings (on a quarterly basis) as well as final accomplishments once their project is completed. The Steering Committee will also provide constructive feedback for future experiments and grant applications to be submitted by Core PIs or Affiliates, particularly for experiments that utilize both cores. Since the offices of the PIs, Core Technical Personnel, and Program Manager and the labs of the PIs and Core Technical Personnel are all in close proximity to one another, we anticipate there will be continuous informal communications among the Core Personnel in addition to the monthly meetings.

While general finances will be discussed at the monthly Steering Committee meetings, key financial decisions (e.g., if a change in the allocation of funds between cores needs to be made or equipment needs to be purchased) will be made by a committee comprised of the five PIs (Bradshaw, Hájos, Katona, Lu, and Mackie), after seeking advice from the IAB and EAB. If a disagreement arises among the PIs that cannot be settled by discussions and compromise, it will be adjudicated by Dr. Jeff Zaleski, chair of the IAB (see his letter of support).

Pilot Project Core

The Pilot Project Core (PPC) oversees and helps develop pilot projects that bring new investigators into the Center, helps encourage young investigators in our Center's and Affiliate's laboratories to embark on careers in substance abuse research, and disseminates the Center's Core technologies to researchers investigating the neurobiology of addiction whose research would benefit from techniques offered by the two cores.

Professor Norbert Hájos, recently recruited to IUB as an Endowed Gill Chair for Neuroscience, will be PI of this core. This is an excellent role for Professor Hájos given his extensive experience in mentoring and proposal reviewing (see PPC Research Strategy for more details). An important component of the PPC process is to provide useful feedback to PPC applicants, including those that are unsuccessful. Our approach follows the approach used by the highly successfully Yale *Neuroproteomics of Addiction P30* as outlined below. (Of note, Dr. Mackie is an affiliate member of this P30 and a reviewer of their pilot projects. In addition, Dr. Wolff serves on the Yale P30 EAB and also reviews their pilot proposals. These experiences with pilot project review will help to inform our P30 review process.). A schematic of the process for Pilot Project Submission is shown in **Fig. 5**.

Before proposal submission, potential applicants are encouraged to communicate with the Core PIs to fully understand Core capabilities and answer questions about experimental design. Submitted proposals will be reviewed by 4-5 reviewers, 2-3 from IUB (not involved in the project) and 2 externally, either by Center affiliates or EAB members, depending on the required expertise. Scoring will follow the current 5 category, 9-point NIH scale and applicants will receive written feedback on the strengths and weaknesses of their applications. Our goal here is to strengthen unfunded applications so they well be competitive in the next round or for other sources of funding. Trainee proposals will be prioritized in the review process and all applicants will be aware of this prior to submission. Each successful applicant will be assigned a Core staff member (e.g., Barna, Huang, or Johnson) who will provide training in the technology used in the application. For awardees entering the drug abuse field, the trainee will be assigned a Center affiliate experienced in drug addiction (e.g., Hohmann, Katona, Mackie, etc.), based on the awardee's interests and needs to provide guidance in appropriate models of substance abuse and experimental design.

Importantly, trainees supported by the PPC will have several opportunities to present their work including research in progress meetings (this will be required) and the EAB meeting. The goal here is to encourage feedback and discussion on the awardee's project, the most profitable future directions the research could go, and to prepare for grant submission. All grantees will be required to submit a final report at the end of the project that will include research accomplishments, presentations/manuscript submissions, and grant applications that were supported by PPC funding. We will encourage past awardees to mentor current awardees as past awardees are keenly aware of the challenges facing scientists at an early career stage and can offer perspectives complementary to those of the established investigators (i.e., Center affiliates). We aim to support 2-4 pilot projects annually, depending on the funds available and the budgets of the individual pilot projects funded.

Education and Training

Education and training are major functions of both cores, though these functions will be implemented in different ways for the two cores. The BLMC will run a summer course that has a goal of attracting undergraduate students from groups historically underrepresented in drug addiction research for in depth, “hands on” laboratory research experience. These students will be hosted on the Bloomington campus for a 8-week mentored summer research and career development experience aligned with an existing IUB program (STEM Summer Scholar’s Institute, SSI, see Deans Deleke’s and Simms’ letter of support). Importantly, we anticipate that about half of these students will be from IU Bloomington. If they are interested in continuing to be involved in research during the academic year, they will be financially supported to continue working in their mentor’s lab, to allow them to build on their summer research experience. This is very important as financial concerns often exclude individuals from underrepresented groups from participating non-course-based laboratory experiences during college [15].

The MSIC will run a 2-week STORM microscopy course four times/year. This course is designed for both trainees and established researchers who would like to learn more about STORM imaging and how they might apply it in their own research. Two slots in this course will be reserved for URM trainees and their expenses covered by P30 funds.

As the Administrative Core will be overseeing these two courses, details of their format and content are discussed in the Research Strategy section of the Administrative Core. An important component of the courses will be to evaluate their effectiveness. The approach is described in the Administrative Core and will include both an immediate assessment of what was learned in the course and a follow up assessment of how taking the course impacted their career path (BLMC course) and whether or not they incorporated techniques learned into their research program (MSIC course) using a structured interview/questionnaire approach [16].

Scientific Focus and Integration of Future Research

Ensure a steady supply of new Affiliates: The C3A will start with about two dozen Affiliates, based on our recruitment efforts. We have settled on this number of inaugural Affiliates based on the number of projects that the two dozen labs will generate that can be realistically handled by the two cores over five years. We expect that there will be an attrition of Affiliates over time due to labs changing their research directions, retirements, etc. Thus, each year we will actively seek out new Affiliates to add to the C3A. New Affiliates will be sought through word-of-mouth, following the literature for relevant studies, inviting successful pilot project awardees, searching NIH Reporter for new NIDA grant awards that might be relevant, and publicizing access to the C3A resources during faculty recruitment to Midwest institutions.

Data Archiving and Dissemination

Several of the experiments that will be conducted by the Cores will generate quite large amounts of data (TB/experiment). This poses challenges in both analysis and archiving. The analysis challenge is experiment specific, with approaches and solutions described in the Research Strategy of the relevant Core. The archiving challenge is common to all experiments generating large amounts of data. We are fortunate to be at IUB with state-of-the-art high-performance computing and a very engaged group of data scientists from the Research Data Services (RDS) team of IU Research Technologies (see letter support from Matt Link, IU Associate Vice President for Research Technologies). As outlined in the Administrative Core, working with our RDS colleagues, we have streamlined the workflow for handling the most data-intensive experiments (multiphoton imaging and STORM microscopy) to improve the workflow for analysis and to have a systematic approach to archiving the data. In this way it will be accessible to C3A Affiliates and other qualified scientists who would like to use it for follow up analyses and also compatible with the NIH Policy for Data Management and Sharing (NOT-OD-21-013).

Website and Twitter account

A website will be established and hosted at IU as the public-facing side of the C3A. Key components of this website will be Center news, contact information for the Center, extensive information on Center resources and mission, how qualified scientist can affiliate with the Center and access its resources, and information (and

application for) the courses offered by the two Cores. There will be pages password protected and accessible only to Affiliates for project submission and data access. We will also post posters presented by Center Affiliates (that might otherwise not be publicly accessible) as well as links to publications supported by the Center and non-copyrighted versions of journal articles that are paywalled.

The Twitter feed (@C3A) will be used to publicize the Center as well as its important findings, publications, events, awards to Center affiliates, and pilot project grant opportunities and deadlines to the general public and scientific community.

Technological and Methodological Goals of the Cores

The Center will continue to develop and apply innovative lipid mass spectrometric techniques to the problem of drug addiction. In the short-term, the BLMC will focus on optimization of lipid extraction, partial purification, and mass spectrometric analytical techniques taking advantage of the significantly enhanced sensitivity of the API 7500. In addition to streamlining traditional analysis with the API 7500, we will couple this to the development of novel data science machine learning algorithms to drive lipid signaling network analysis being developed here at IU. Using the studies outlined in the BLMC Research Strategy **Aim 4**, we can generate lipidomics data from drug treatments in cell-based assays rapidly to begin the work on this network analysis system so that it is available for animal and human-based analysis in a shorter timeframe. The MSIC will continue to develop techniques for longitudinal *in vivo* imaging of very young mice, further improve on the PharmacoSTORM technique, and optimize the protocols of Fluorescent Lifetime Imaging Microscopy (FLIM) for *in vivo* 2P imaging to follow metabolic and structural changes after chronic drug exposure (e.g., chronic THC exposure). See the Research Strategies of the two Cores for more details on these innovations and how they will be implemented.

Impact of the IUB-C3A on Research Accomplishments, Collaborations and Other Outcomes to Provide a Regional and National Resource

It is important to have ways to quantitatively evaluate the success of the C3A. The primary outcome measures that will be used to assess the effectiveness of the C3A are as follows: The number and impact of projects completed, the geographic spread of the Affiliates using Core resources, posters presented, papers published (and their impact), new collaborations established, grants submitted/funded using data from the C3A cores, trainees completing the summer program (and their career paths, as these data become available), and researchers completing the STORM microscopy course and how they use their new knowledge (findings, papers, grants, etc.). These data will be reported annually to the EAB and IAB as well as the NIH (communicated via the RPPR).

Summary

Indiana University Bloomington Center for Cannabis, Cannabinoids and Addiction (IUB C3A) has been conceptualized and is now prepared to bring together established substance abuse researchers from across the Midwest (and beyond) to give them access to techniques to enhance their ongoing research. To do this, the C3A will offer state-of-the-art imaging and lipid mass spectrometric analyses to address key unresolved questions in addiction science. The analysis and integration of these results between the two service cores will provide fundamental insights into the molecular, cellular, and circuit reorganizations that occur during chronic drug use or following prenatal drug exposure. These insights will then serve to generate novel hypotheses and motivate additional research that will leverage knowledge gained through the C3A to test new therapies to treat drug addiction and its sequelae. In addition, the educational and training components of the C3A will help to recruit more individuals into the field of drug addiction research, especially from groups traditionally underrepresented in this field, as well as introduce established researchers to high resolution imaging techniques. Finally, its pilot project program is designed to support high impact exploratory studies, particularly by new investigators. That the goals of the C3A are achievable is ensured by a lean, well-organized administrative structure whose individuals have a proven record of collaboration and accomplishment in drug abuse research.

Table I. Qualifying Research Projects

PI	Effort	Number	End date	Direct Costs	Name
Penner/Mackie	2.4/0.7	R01AT011162 ¹	11/30/25	\$427,554	Modulation of pain mechanisms by cannabis-derived phytochemicals
Lu/Mackie	1.2/0.9	R01DA053746	2/28/27	\$313,076	Mechanisms and treatment of adolescent phytocannabinoid impairment of prefrontal cortex function
Kawata	3	R01NS113950 ²	11/30/25	\$373,377	Subconcussive neurodegenerative progression in adolescent athletes
Salvemini	2	R01CA261979 ³	6/30/27	\$292,023	Fingolimod and Ozanimod for the treatment and prevention of chemobrain
Babalonis	2.4	R01DA054347	5/30/26	\$474,690	Cannabis Modulation of Outcomes Related to Opioid Use Disorder: Opioid Withdrawal, Opioid Abuse Potential and Opioid Safety
Porreca/ Anderson/ Navratilova	1.2	R01NS114888 ⁴	6/30/25	\$317,456	Mechanisms and therapeutic strategies for post-traumatic headache
Hurd	2.4	R01DA051191	3/31/27	\$359,443	Molecular Neurobiology of human opioid use disorder
Hurd	2.0	R01DA055434	5/31/27	\$503,577	Molecular underpinnings of the developmental Effects of Cannabis
Cheer	2.0	R01DA022340	4/30/26	\$274,141	Endogenous cannabinoid control of reward substrates
Kepecs	2.4	R01MH097061 ⁵	3/31/25	\$280,959	Behavioral and neural algorithms for decision confidence
Dey	2.4	R01HD103475 ⁶	5/31/25	\$304,835	Endocannabinoid signaling during early pregnancy
Dani	3.6	R01DA053296	2/28/26	\$325,072	Altered midbrain GABAergic circuitry drives greater cocaine self-administration
Kimbrough	0.4/3	R01AA029985 ⁷	7/31/27	\$225,000	Identifying the relationship between alcohol and Alzheimer's Disease

Comments for non-NIDA qualifying grants and how they relate to drug addiction:

¹ Studies minor cannabinoids present in cannabis.

² Extend to examine impact of cannabis use in human mild traumatic brain injury.

³ Examines role of lipid signaling in "chemobrain".

⁴ Inadequate analgesia/inappropriate use of opioids for headache is a major cause for opioid misuse.

⁵ Poor decision making is central deficit in most addictive disorders.

⁶ Interplay between cannabis use and early pregnancy. Until this past year it was funded by NIDA.

⁷ Many of the principles in this study for alcohol use pertain to the use of other drugs.

Table II Additional Relevant Grant Support

Number	PI	Direct costs \$'s	Name	End date
T32DA024628	Mackie/Hohmann	\$217,020	Integrative predoctoral training in drug abuse research at Indiana University	6/30/25
T32MH103213	Hetrick (Hohmann, Mackie, Co-I's)	\$280,716	Training in Clinical Translational Science: Maximizing the Public Health Impact.	7/31/25
R01DA046196	Mackie/Manzoni	\$241,682	Perinatal cannabinoids delay KCC2 expression and lead to developmental abnormalities	6/30/23
R01DA044999	Morgan/Guindon	\$269,909	Mechanisms of cannabinoid tolerance	7/31/23
R01AA027214	Atwood/Yamamoto	\$321,861	Methamphetamine-Alcohol Interactions and Mechanisms of Augmented Toxicity to Brain and Peripheral Organs	5/31/23
R01MH121454	Hillard/Liu	\$250,000	Circuit-specific actions of endocannabinoids in stress and mood disorders	8/31/24
R00AA027301	Kimbrough	\$160,644	Network wide analysis of brain activity involved in alcohol withdrawal	8/31/23
R44CA241513	Schwimmer/Hohmann	\$750,000	Therapeutic antibodies for treating chemotherapy induced peripheral neuropathic pain	4/30/23
P01DA009158	Hohmann (project 3 PI)	\$155,831	Project 3 - In vivo pharmacology of cannabinoid receptor probes	5/31/27
R01AA026267	Dani	\$299,640	Adolescent Exposure to Stress or Nicotine Increases Rodent Alcohol Self-Administration	7/31/23
R01HD068524	Dey	\$276,141	Molecular signaling in uterine receptivity to implantation	2/28/27
R01NS109381	Dunaevsky	\$300,459	The role of astrocytes in the Fragile X pathogenesis	12/31/23
R21DA056825	Katona	\$154,000	Novel tool development for quantitative PharmacoSTORM super-resolution imaging of the nanoscale distribution of D3 dopamine receptors	7/31/24
R01DA048613	Hurd	\$490,641	Translating CBD treatment for heroin addiction	6/30/24
R01NS086794	Lu	\$379,468	Molecular and genetic studies of NMNAT2 in neuroprotection	3/31/26

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

Yes No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved

Yes No

Is the Project Exempt from Federal regulations?

Yes No

Exemption Number

1 2 3 4 5 6 7 8

Other Requested Information

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Letters Enclosed

External Advisory Board Letters of Support

IUB Administration Letters of Support

Internal Advisory Board Letters of Support

Technical/Method Development Letters of Support

C3A Affiliates Letters of Support

External Advisory Board Letters of Support

W NEUROBIOLOGY OF ADDICTION, PAIN & EMOTION
UNIVERSITY *of* WASHINGTON

Sept 12th, 2022

Ken Mackie, MD
Jack and Linda Gill Chair
Gill Center for Biomolecular Science
Distinguished Professor
Psychological and Brain Sciences
Indiana University, Bloomington, IN

Re: IUB Center for Cannabis, Cannabinoids, and Addiction Center (IUB-C3A)

Dear Ken,

I enjoyed learning the details of your proposed NIDA P30 Center of Excellence, the **IUB Center for Cannabis, Cannabinoids, and Addiction (IUB-C3A)** in our conversation yesterday. It will be my pleasure to serve on your external advisory board.

From my knowledge of the IUB cannabinoid investigators, my own role as the co-director of the University of Washington's, Center for the Neurobiology of Addiction, Pain and Emotion, P30-supported Imaging and Neural circuits Core, and my visit to IU Bloomington a few years ago, I am confident that the center you are putting together will be a great asset to the cannabinoid field, and NIDA's central mission across the country. I also anticipate it will be valuable resource to the broader addiction community, particularly with the cutting-edge imaging techniques (e.g., PharmacOSTORM and its derivatives) Istvan Katona is developing. I look forward to helping to guide the growth and progress of your center and will be happy to provide advice and suggestions in its efficient running over the next five years.

Specifically, as an EAB member, I will provide advice to you on strategic issues facing the center, evaluate your center's performance through its annual reports, and attend an in person (if travel is feasible) review of the Center's activities in its third or fourth year.

I wish you good luck with your exciting proposal.

Sincerely,



Michael R. Bruchas | Ph.D
Professor
Center for the Neurobiology of Addiction, Pain and Emotion
Department of Anesthesiology and Pain Medicine
Department of Pharmacology
Department of Bioengineering
University of Washington, Seattle
www.bruchaslab.org



MARGARET M. McCARTHY, PhD
James and Carolyn Frenkil Dean's Professor and Chair
Director - Program in Neuroscience
Department of Pharmacology

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mmccarthy@som.umaryland.edu

www.medschool.umaryland.edu

August 23rd 2022

Kenneth Mackie, MD
Jack and Linda Gill Chair
Gill Center for Biomolecular Science
Psychological and Brain Sciences
University of Indiana
Bloomington IN

Dear Ken,

Thank you for inviting me to serve on the external advisory board (EAB) of the **IUB Center for Cannabis, Cannabinoids and Addiction (IUB-C3A)** that you are proposing. It would be a pleasure for me to join your center as a member of your EAB.

From my understanding of the IUB cannabinoid community and my visits to IU Bloomington on a couple of occasions, I am confident that the center you are putting together will be a great asset to both cannabinoid researchers and the greater drug abuse community. I am particularly intrigued by the cutting-edge imaging techniques (e.g., PharmacoSTORM and its derivatives) that Istvan Katona is developing. I look forward to helping to guide the growth and development of your center over the next five years. To do this I will be happy to provide advice and suggestions for the efficient running of the Center.

Specifically, as an EAB member, I will provide advice to you on strategic issues facing the center, evaluate your center's performance through its annual reports and conversations with the Core directors, and attend an in person review of the Center's activities in its third or fourth year.

I wish you the best of luck with your proposal.

Sincerely,

A handwritten signature in black ink that reads "Margaret M. McCarthy".

Margaret M. McCarthy, PhD
James and Carolyn Frenkil Dean's Professor and Chair
Director - Program in Neuroscience





Sachin Patel, MD, PhD
Lizzie Gilman Professor and Chair
Department of Psychiatry and Behavioral Sciences
Northwestern University Feinberg School of Medicine
Psychiatrist-in-chief
Norman and Ida Stone Institute of Psychiatry

Arkes Pavilion, Rm 11-117
676 North Saint Clair Street, Chicago, 60611
Email Contact: sachin.patel@northwestern.edu
Admin. Contact: ivy.mosley@northwestern.edu

September 7, 2022

Dear Professors. Lu, Katona, Bradshaw, and Mackie,

I am writing this letter to offer my full support for your efforts to establish the IUB Cannabis and Cannabinoids Center (IUB-C3) as a NIDA Center of Excellence.

It would be my pleasure to serve on your external advisory board (EAB). From my knowledge of the IUB cannabinoid investigators, I am confident that the center you are assembling will be a great asset to the study of cannabinoids and related molecules and their impact on the CNS, particularly the developing CNS. I also anticipate it will be useful to the broader addiction community, particularly with the cutting-edge imaging techniques you are developing. I look forward to helping to guide the growth and progress of your center and will be happy to provide advice and guidance over the next five years.

Specifically, as an EAB member, I will provide advice to you on strategic issues facing the center, critique your annual reports, and attend your in-person (if possible) review of the Center's activities in its third year.
I wish you the best of luck with your proposal.

Sincerely,

A handwritten signature in black ink, appearing to read "Sachin Patel".

Sachin Patel MD PhD



Ivan Soltesz, PhD
James R. Doty Professor of
Neurosurgery and Neurosciences

August 24, 2022

Dear Profs. Lu, Katona, and Mackie,

I am writing this letter to offer my full support for your team's proposal for establishing **an IUB Cannabis and Cannabinoids Center (IUBCC³)**

It would be my pleasure to serve on your external advisory board. From my knowledge of the IUB cannabinoid investigators and my visit to Bloomington a few years ago, I am confident that the center you are assembling will be a great asset to the study of cannabinoids and related molecules and their impact on the CNS, particularly the developing CNS. I look forward to following the progress of your center and will be happy to provide advice and guidance as it grows.

I would also be willing to help you with implementing imaging of endocannabinoids with the recently developed endocannabinoid sensor. As you know, my laboratory has long standing interests in elucidating the molecular mechanisms underlying endocannabinoid signaling in neural functions and cognitive behaviors. Recently we have had great success in using the newly developed endocannabinoid (eCB) sensor, GRAB-eCB2.0 for studying eCB dynamics in the brain of awake behaving mice. *In vivo* two photo (2P) imaging of GRAB-eCB2.0 together with the red Ca²⁺ sensor, jRGECO1, enabled us to characterize the spatiotemporal changes of 2AG signaling in a recent *Neuron* paper.

The 2P Ca²⁺ data from jRGECO1 obtained from awake mouse pups you shared with me are very nice. I am particularly intrigued by the fast changes of cortical network activity at different postnatal ages. It looks like you are well underway with these experiments and I do not foresee you will encounter any difficulties with GRAB-eCB2.0 sensor or with the combination of jRGECO1 and GRAB-eCB2.0 sensors. I am very impressed that you have successfully incorporated *in vivo* calcium imaging in your studies because these are technically sophisticated these experiments. To my knowledge your lab is now one of only a handful of labs worldwide who have been able to successfully record network activity with single

cell resolution using 2P calcium imaging in early postnatal animals. No doubt that your imaging core is going to enable many researchers interested in drug addiction the opportunity to elucidate how early drug exposure perturbs the development of cortical circuits.

We will be happy to assist you if any technical issue arises for imaging the GRAB-eCB2.0 sensor or simultaneous imaging of GRAB-eCB2.0 and jRGECO1 sensors. I look forward to assisting with your lab and seeing the results of your experiments.

Sincerely,



Ivan Soltesz, PhD
Professor
Stanford University



Marina E. Wolf, Professor
Department of Behavioral Neuroscience

tel (503) 494-9797
fax (503) 494-6877

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Portland, OR 97239

August 19, 2022

Ken Mackie, MD
Linda and Jack Gill Chair of Neuroscience
and Distinguished Professor
Dept. of Psychological & Brain Sciences
Indiana University

Dear Ken,

I am writing to express my enthusiasm for serving on the external advisory board (EAB) of the proposed **IUB Center for Cannabis, Cannabinoids and Addiction**. As you know, I have considerable administrative experience in many facets of drug abuse research and academics, including serving on the external advisory board of the Yale Neuroproteomics P30, that I will bring to your EAB.

From my knowledge of the IUB cannabinoid investigators and my visit to IU Bloomington a few years ago, I am confident that the center you are putting together will be a great asset to cannabinoid researchers across the US. I also anticipate it will be a valuable resource to the broader addiction community, particularly with the cutting-edge imaging techniques (e.g., PharmacoSTORM and its derivatives) Istvan Katona is developing. Finally, I am glad that your center includes a significant training component, both for undergraduates not well-represented in drug addiction research as well as for scientists wanting to learn techniques in high resolution optical imaging. I look forward to guiding your center's expansion and progress and will be happy to provide advice and suggestions in its efficient running over the next five years.

Specifically, as an EAB member, I will provide advice to you and your colleagues in the Center on strategic issues facing the Center, evaluate your Center's performance through its annual reports, and attend an in person (if travel is feasible) review of the Center's activities in its third or fourth year.

I wish every success with your proposal.

Sincerely,

A handwritten signature in black ink, appearing to read "Marina E. Wolf".

Marina E. Wolf, Ph.D.
Professor of Behavioral Neuroscience
Oregon Health & Science University

IUB Administration Letters of Support



September 13, 2022

Ken Mackie, MD
Linda and Jack Gill Chair of Neuroscience
and Distinguished Professor
Dept. of Psychological & Brain Sciences
MSBII 120
Indiana University
702 N Walnut Grove Ave
Bloomington, IN 47405-2204

Dear Ken:

This letter is to confirm Indiana University's enthusiastic support for your NIDA P30 application, *IU Bloomington Center for Cannabis, Cannabinoids, and Drug Addiction* (C3A), and our commitment to providing the necessary institutional resources to ensure its success.

That support includes a commitment from my office for \$870,000 in matching funds that will help support the purchase of the equipment (mass spectrometry and imaging) as described in your proposal.

In addition, my office already provides financial and administrative support for core facilities that will benefit the C3A. This includes capital investments in these centers as well as research staff, HR, finance, IT, and related support. Examples of cores supported by OVPR relevant to the C3A include Laboratory Animal Resources and the Light Microscopy Imaging Center. These cores provide animal housing, access to specific equipment, as well technical support and interactions that will be helpful for the C3A principal investigators and affiliates as they and their trainees are doing projects in the C3A.

As you know, research in addictions is of particular interest to Indiana University and Addictions is one of our three Grand Challenges to which we collectively have committed more than \$200 million over the past five years. You have played a leadership role in the design and execution of the Addictions Grand Challenge and all of the PIs contributing to the C3A (Drs. Bradshaw, Katona, Lu, and Mackie) have helped guide this initiative and/or have benefited from funds provided by this Grand Challenge to enhance your research.

Finally, under your leadership and that of Dr. Lu, the Gill Center has become a nucleus of drug addiction research, particularly research focused on cannabis and cannabinoids, and their interactions with other abused drugs. My office, the College of Arts and Sciences, and

other parts of the university are delighted to have contributed financial and other support to the Gill Center and are committed to continuing doing so.

Your P30 Center proposal is a natural outgrowth of these and other initiatives, and on behalf of President Whitten, Provost Shrivastav, and myself, I not only wish you every success with your proposal, but also pledge Indiana University's support to help ensure the C3A's success.

Yours sincerely,



Fred H. Cate
Vice President for Research
Distinguished Professor and
C. Ben Dutton Professor of Law

U
COLLEGE OF
ARTS AND SCIENCES
INDIANA UNIVERSITY
Office of the Executive Dean
Bloomington

September 21, 2022

Dear Ken,

It is a pleasure to write this letter of support for your NIDA P30 application, *IU Bloomington Center for Cannabis, Cannabinoids, and Drug Addiction (C3A)*. The College of Arts and Sciences enthusiastically supports your submission and will provide the necessary resources for it to be successful. Below, I will outline the ways in which the College can help support the C3A to ensure its success.

On the financial side, the College will provide \$435,000 in funds to share the cost of the purchase of the equipment (mass spectrometer, slide scanner, and multi-photon additions to expand our current imaging speed and modalities) outlined in your proposal. This will be matched by an additional \$435,000 in cost-share funds from the IU VP for Research.

Together with direct financial support to purchase equipment, the College provides substantial financial and in-kind support for core facilities that will benefit the C3A. This includes capital investments as well as research staff support. Examples of cores supported by the College relevant to the C3A include Laboratory Animal Resources, the Light Microscopy Imaging Center, and the Neuroscience Core Labs. These cores provide both foundational research support, access to specific equipment, as well technical support and interactions that will be helpful for the C3A principal investigators as well as researchers working in the C3A.

In terms of physical resources, the C3A will be housed in existing space in the MSBII building. This will be in the same footprint as space assigned to the Bradshaw, Katona, Lu, and Mackie labs. The infrastructure for this space is fully adequate for the necessary studies (e.g., fiber optic data connections, environmental control of temperature and humidity, etc.) and the College is committed to ensure its continued suitability.

As you know, addictions research is of particular interest to Indiana University, and combatting the Addictions Crisis in Indiana has been the focus of a \$50,000,000 Indiana University “Grand Challenge”. All of you (Drs. Bradshaw, Katona, Lu, and Mackie) have either helped guide this initiative or have leveraged funds provided by this Grand Challenge to enhance your research. Indeed, The Addictions Grand Challenge was instrumental to the recruitment of Dr. Katona to IU Bloomington.

I am gratified to see that with strong College support the Gill Center has become a nucleus of drug addiction research, particularly research focused on cannabis and cannabinoids, and their interactions with other abused drugs. Your P30 Center proposal is a natural progression of this growth and I wish you all the best of success with your proposal.

Best wishes,



Associate Dean for Natural and Mathematical Sciences and Research



INDIANA UNIVERSITY

OFFICE OF THE VICE PRESIDENT FOR
INFORMATION TECHNOLOGY AND
CHIEF INFORMATION OFFICER

Ken Mackie, M.D.

Director and Gill Chair for Linda and Jack Gill Center for Biomolecular Science
Professor in Dept. of Psychological & Brain Sciences

Dear Dr. Mackie,

It's my pleasure to write this letter strongly supporting your application for a NIDA-P30 center—the IU Bloomington Cannabis and Cannabinoids Center. Research Technologies is committing storage and staff resources to your proposed center. In addition, I am attaching a facilities statement detailing the research cyber infrastructure that is available at Indiana University which includes Big Red 200, the fastest university-owned AI supercomputer. All IU researchers and affiliates have access to the Research Technologies cyberinfrastructure resources on a fair share principle.

The proposal for the center includes budget lines for storage resources across three different types of central storage systems that will be dedicated to the center. Your center will also have access to shared supercomputing clusters including the Big Red 200, Big Red 3, Carbonate and Quartz at no additional cost. Our top high-performance computing user utilizes over 4 million CPU core hours on Big Red 3 in one month. On an equivalent AWS cluster, based on reserved node pricing at a substantial discount compared to on-demand pricing, the average value provided to each of our top 50 Big Red 3 users is about \$100,000 per year in computation alone.

Given the projected data that will be generated by the center and the workflow support available from Research Technologies, your annual HPC usage may reach many 100,000s of CPU core hours. I am looking forward to supporting your center with research computing resources.

Sincerely,

A handwritten signature in black ink that reads "Matt Link".

Matt Link

Associate Vice President, Research Technologies
Center Director, Pervasive Technology Institute
Director, Crisis Technologies Innovation Lab



August 31, 2022

Dr. Heather Bradshaw
Department of Psychological and Brain Sciences
Indiana University
Bloomington, IN 47401

Dear Heather,

We are delighted to partner with the "Center for Cannabis, Cannabinoids, and Addiction" P30 funding mechanism for training opportunities for our STEM Summer Scholars Institute (SSI), which is part of our broader IU – Minority Serving Institution STEM initiative (stem.indiana.edu). Since you have been an SSI mentor since 2008, you understand the impact that this program can have on student's long-term success in STEM fields. Our overarching goals at SSI are to 1) facilitate substantive STEM research opportunities for MSI and IU students, 2) provide increased access for MSI students to IU graduate programs in the STEM disciplines; 3) encourage collaborative research between MSI and IU faculty in the STEM disciplines; 4) enrich the academic opportunities of all the institutions through "visiting scholars" programs, faculty and student exchanges and a faculty research institute. We have partnered with the Groups Scholars and Hudson and Holland programs here at IU in the past with some success to encourage students to apply for SSI; however, this unique opportunity to provide IU students from those programs a more long-term research opportunity beyond SSI is a fantastic way to encourage more students to apply.

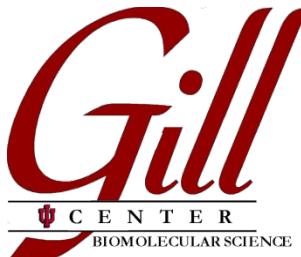
We are particularly grateful that, if funded, the P30 mechanism will provide 5 SSI stipends and the College has agreed to match those 5 for a total of 10 additional stipends/year for the duration of the award. This offer will allow us to continue to expand our program and to provide more students from MSIs and IU a unique opportunity at both research training and professional development.

Thank you for your efforts to support student STEM opportunities and I wish you the best of luck with this proposal.

Sincerely,

David Daleke
Interim Dean and Vice Provost for Graduate Education
Vice Provost for Health Sciences
Professor of Biochemistry & Molecular Biology

Howard Simms
Assistant Dean for Diversity and Inclusion



Hui-Chen Lu Ph.D.
Director and Chair
Linda and Jack Gill Center of Neuroscience
Professor for Dept. of Psychological & Brain Sciences
MSBII 108
Indiana University
702 N Walnut Grove Ave
Bloomington, IN 47405-2204
812-8556-4998 (PH)
812-856-7187 (FAX)

Friday, September 2, 2022

Dear Ken,

As Director of the Jack and Linda Center for Biomolecular Science, it is my pleasure to confirm Gill Center financial support for your P30 application to NIDA, *IU Bloomington Center for Cannabis, Cannabinoids and Addiction (C3A)*. While I broadly support the concept and goals of the C3A, I am particularly pleased that you have integrated a large educational component into the proposal, specifically directed towards undergraduates historically underrepresented in drug addiction research. As we discussed, the Gill Center will provide funding for five \$4,000/summer stipends, up to \$20,000 annually, to support these students in years 1 through 5 of the grant.

The Gill Center is a research center administratively housed in the IUB College of Arts and Sciences and physically housed in the modern MSBII building. The Gill Center was established in 1999 through a generous gift from Jack and Linda Gill. The Center consists of six endowed chairs drawn from Biology, Chemistry, and Psychological and Brain Sciences. The research performed by the Gill Chairs primarily involves neuroscience. Research areas of focus for the Gill Center include mechanisms and treatments for addiction, consequences of the developmental exposure to addictive drugs, treatment of chronic pain, strategies for enhancing neuronal maintenance, etc. Being located on the IUB campus with a large undergraduate population and educated community in the surrounding town, the Gill Center places a high emphasis on teaching, experiential learning, and outreach to the scientific community and public. In this regard, I also see that the goals of your P30 are very well aligned with those of the Gill Center.

The Gill Center has a long history of supporting undergraduate research. We primarily do this by integrating undergraduates into research projects, where they work side-by-side with graduate students, post-docs, technicians, or research faculty. Typically, we have more than twenty undergraduates working in the six Gill laboratories. They are deeply integrated into Gill Center activities (e.g., lab meetings (general and undergraduate student-oriented), seminars, meeting with visiting scientist, participating in social events, etc.) and do well after graduation. I fully anticipate that the summer undergraduate researchers we support with Gill funds will also be integrated into these activities and will similarly thrive with the other students in the Center.

Good luck with your proposal and I look forward to working with you on this project over the next five years.

Sincerely,

A handwritten signature in blue ink that reads "Hui-Chen Lu".

Hui-Chen Lu, Ph.D.

Internal Advisory Board Letters of Support

**LUDDY SCHOOL OF
INFORMATICS, COMPUTING,
AND ENGINEERING**

INDIANA UNIVERSITY

September 15, 2022

Dear Ken, Istvan, Hui-Chen, Norbert, and Heather:

I am writing to express my enthusiasm to serve on the internal advisory board (IAB) of the *IUB Center for Cannabis, Cannabinoids and Addiction (IUB-C3A)* proposal that you are submitting to NIDA as a P30 Center of Excellence. By way of background, I am the Victor H. Yngve Distinguished Professor of Engineering and Information Science in the Department of Intelligent Systems Engineering at the IU Bloomington Luddy School of Informatics, Computing, and Engineering (SICE). My areas of expertise include large-scale data mining, modeling, and visualization in support of efficient decision making.

Particularly relevant for your P30 proposal is my experience in managing large technical consortia. I serve as PI on the IU mapping component of Human BioMolecular Atlas Program (HuBMAP), an NIH initiative that aims to map the human body at single cell resolution, and I co-chair the HuBMAP steering committee. In these roles, I have significant expertise in training the next generation of scholars, setting research priorities, developing modular software, and facilitating team consensus. I look forward to sharing my insights with you as you allocate the resources of your P30 center to maximize the impact of the science you are able to support.

In addition to the above, as a member of your IAB, I will advise you on strategies to address long-range issues facing the center, serve as a second level of review for pilot project proposals, and assist you in other ways that might be needed. Finally, as you know, we are very interested in exploring ways to organically strengthen collaborations between neuroscience and SICE on the IUB campus. I am optimistic that serving on your P30 IAB will naturally lead to a variety of collaborations between our two groups.

Sincerely,



Katy Börner
Victor H. Yngve Distinguished Professor of Engineering and Information Science
Director, Cyberinfrastructure for Network Science Center, <http://cns.iu.edu>
Curator, Mapping Science exhibit, <http://scimaps.org>
Intelligent Systems Engineering
Luddy School of Informatics, Computing, and Engineering, Indiana University
Luddy Hall 4018, 700 N. Woodlawn Ave, Bloomington, IN 47408, USA

Phone: (812) 855-3256 | katy@indiana.edu | Twitter: katycns



DEPARTMENT OF CHEMISTRY
800 E. KIRKWOOD AVE.
BLOOMINGTON, IN 47405-7102
PHONE: 812-855-8259
FAX: 812-855-8300
EMAIL: CLEMMER@INDIANA.EDU

September 8, 2022

Dear Heather,

I am happy to serve as a member of the Internal Advisory Board for the P30 supported IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction. I have over 25 years of expertise in mass spectrometric instrumentation development and analysis including 1) Rapid and sensitive analysis of complex biomolecular mixtures, such as those resulting from enzymatic digestion or combinatorial synthesis; and 2) Highly-resolved measurements of biomolecular structure. This experience will allow me to provide guidance for issues arising with the bioactive lipid mediators mass spectrometric core.

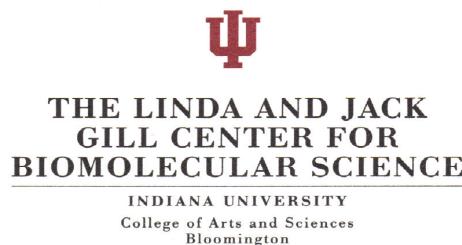
Having a lipidomics mass spectrometric core here with a focus on drugs of abuse research at IU would be a great addition to the institution and I look forward to the opportunity to work with you and the other IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction faculty in the coming years.

Best of luck with the proposal.

Sincerely,

A handwritten signature in blue ink that reads "David E. Clemmer".

David E. Clemmer
Distinguished Professor
Robert & Marjorie Mann Chair



September 5, 2022

Dear Ken, Heather, Hui-Chen and Istvan

It is with great pleasure that I write this letter of support to enthusiastically endorse the revision of your P30 grant application entitled ***IU Bloomington: Center for Cannabis, Cannabinoids and Addiction (IUB-C3A)***. In the event the present proposal is funded, I also herein confirm my willingness to serve on the Internal Advisory Board and as an Affiliate faculty member for this P30 grant. I have been a NIDA-funded researcher for approximately 20 years and have an established track record of collaboration and publication with each of the Core PIs (Bradshaw, Katona, Lu, Mackie). I also hold ongoing collaborative MPI R01 grants with the Project PI (Mackie), and additionally serve as co-Director of a NIDA T32 training grant with Dr. Mackie. My experiences working with the PI in these contexts, and my familiarity with Dr. Mackie's prior administrative roles (member of NIDA council, Director of the Gill Center) also allow me to enthusiastically advocate for Dr. Mackie's exceptional scientific, administrative and organizational skills and his ideal qualifications for serving as both the contact PI of this P30 grant and PI of the administrative core. Thus, there is no question that NIDA resources allocated to this P30 grant led by Dr. Mackie will be fully and maximally leveraged to enhance current and future research impact in a productive fashion. The proposed P30 grant will facilitate scientific advances of researchers, not just at IUB, but also in the Midwest and across the nation, with a particular emphasis on underserved regions in our country where problems of drug addiction are particularly severe. Moreover, there is no question that the present P30 grant will enhance the educational training mission of NIDA, including the mission to enhance diversity, that we have successfully promoted in our T32 grant (Integrative training in drug abuse). Moreover, the large number of laboratories with research programs focused on cannabinoids and drug abuse research at IUB (e.g., Bradshaw, Katona, Hohmann, Kalinovsky, Lu, Mackie, Straiker) and in the institutions of our Affiliates, documents that the present P30 grant will be ideally positioned to amplify existing scientific interactions, promote new scientific interactions and exponentially expand scientific research accomplishments.

Interestingly, my collaborative studies with each of the Core PIs originated when each of the faculty members identified herein were at different institutions from myself. I was recruited to Indiana University as a Linda and Jack Gill Chair of Neuroscience and Professor of Psychological and Brain Sciences in 2010, where Ken Mackie served as Director of the Gill Center. I moved my laboratory to IUB because of Ken's leadership of the Gill Center, and Heather Bradshaw's presence as a tenure track faculty member in the Department of Psychological and Brain Sciences, as well as the presence of towering figures in drug abuse research at IUB such as George Rebec. Interestingly, the neuroscience community at Indiana University has only continued to strengthen its reputation for excellence with the recruitment of Dr. Hui-Chen Lu (as a Linda and Jack Gill Chair and Director of the Gill Center) and Istvan Katona (as the David Naus Family Chair in Addiction in the Department of Psychological and Brain Sciences). This documents the ability of faculty at IUB to work productively in a collaborative fashion. There is no question that the current P30 grant will continue to facilitate research excellence and collaborations between the laboratories of the Core PI and Affiliate Faculty, while also providing important training and mentoring opportunities. By reviewing pilot project grants and applications for use of research cores, our faculty will also benefit from enhanced interactions and more in depth understanding of the research programs of other investigators. This level of scientific interaction will also permit researchers with diverse research approaches and differing perspectives to make particularly innovative contributions to drug abuse and neuroscience research. Members of my laboratory, and myself, would also personally benefit from training and utilization of STORM imaging, two photon microscopy and the highly sensitive liquid chromatograph/mass spectrometry as well formal coursework in cutting edge microscopy and

liquid chromatography mass spectrometry techniques. By strengthening the breadth of research approaches available to my group, and our collaborators, the P30 grant can be expected to enhance success of the next generation of drug abuse researchers as well as the competitiveness of current and future grant applications.

The ability to answer research questions in my existing NIH grants would be specifically enhanced through access to the high performance liquid chromatography mass spectrometry core facilities led by Dr. Bradshaw. Acquisition of more sensitive mass spectrometry equipment will permit us to measure changes in brain and plasma lipid mediators in response to drugs of abuse like oxycodone and morphine in discrete regions of the CNS and periphery. These studies have potential to identify and validate plasma biomarkers that explain sex differences in responsiveness as well as vulnerabilities to drugs of abuse in the presence and absence of pathological pain. Such approaches offer considerable promise for enhancing prospects for successful clinical translation. The resources described herein would be complementary to and expand the impact of my R01 funded work exploring CB2-opioid interactions. My laboratory also recently published a previously unreported link between the gut microbiome and opioid withdrawal (Thomaz et al. (2021) Experimental Neurology); we could also capitalize on our previous findings by exploring changes in the lipidome in the gut-brain axis during opioid dependence and withdrawal. My lab is also funded through an Indiana University Addiction Grand Challenges grant to evaluate the impact of a CB2 agonist on unwanted effects of opioids (i.e. opioid-induced reward, opioid-induced dopamine efflux, opioid-induced respiratory depression). Elucidation of sex differences in phenomena under study would also be enhanced by using the above experimental approaches. We would also be able to take advantage of STORM microscopy with Dr. Katona to study the microstructure of CNS regions involved in opioid addiction, and their changes during withdrawal and cue-induced reinstatement, including in our models using intravenous drug self-administration to study motivation to self-administer drugs of abuse. Similarly, two photon microscopy with Dr. Lu would permit a better understanding of *in vivo* changes in calcium mobilization and neuronal activation in discrete brain regions *in vivo* within the context of the phenomenon described above.

In summary, the resources available through the P30 grant will amplify scientific discoveries and enhance current understandings of drug addiction vertically and exponentially, rather than horizontally and incrementally. Thus, the potential for the **IU Bloomington: Center for Cannabis, Cannabinoids and Addiction (IUB-C3A)** to amplify and accelerate vibrant and impactful research on mechanisms and treatment of drug addiction is beyond question. I am excited to participate in this exciting P30 grant and look forward to our continued research collaborations and interactions.

Sincerely,



Andrea G. Hohmann, Ph.D.
Linda and Jack Gill Chair of Neuroscience and Professor
Department of Psychological and Brain Sciences
Indiana University, Bloomington, IN 47402



DEPARTMENT OF BIOLOGY

INDIANA UNIVERSITY
College of Arts and Sciences
Bloomington

Dear Ken,

I am writing to express my willingness to serve on the internal advisory board (IAB) of the *IUB Center for Cannabis, Cannabinoids and Addiction (IUB-C3A)* program you are proposing as a NIDA P30 Center of Excellence.

In addition to my faculty position in Biology, I developed the IUB Light Microscopy Imaging Center (LMIC) on the IUB campus in 2008 and I have directed the center since 2016. The LMIC provides access and training to high-end light microscopes including various confocal and super-resolution systems. In addition, I serve as the director of the Integrated Freshman Learning Experience (IFLE) for the College of Arts and Sciences. The IFLE honors program brings talented undergraduate students with a stated desire to pursue life-sciences research to the IUB campus for a 6-week laboratory program prior to their first year. The summer program is followed by a 3-part intensive honors course spanning the freshman year focused on Genome/Cell Biology, Biochemistry, and Neuroscience from a research perspective.

I feel that my experience in both roles will allow me to offer constructive advice for establishing and running the proposed center. The implementation of multi-photon and STORM imaging in your center dramatically extends IUB's imaging capabilities and the recruitment of Laszlo Barna to manage the P30 imaging core will be a great addition to the Bloomington campus. In addition, I am extraordinarily pleased that the training aspect of your proposal presents a coordinated plan to recruit underrepresented students into neuroscience labs for the summer and includes funds to keep students in the lab through the academic year. This aspect of the proposal will provide a much needed avenue for students who might otherwise not have the opportunity to gain laboratory experience at this level.

As an IAB member, I will provide advice to you on strategic programmatic issues facing the center, critique your annual reports, and assist you in other ways that might be needed.

I wish you the best of luck with your proposal.

With best regards,
Sidney L. Shaw, MPhil. PhD

A handwritten signature in black ink, appearing to read "Sidney L. Shaw".

Professor of Biology (and Physics)
Technical Director, Light Microscopy Imaging Center
Department of Biology
Indiana University, Bloomington



INDIANA UNIVERSITY

SCHOOL OF EDUCATION
Department of Counseling and
Educational Psychology
Bloomington

September 26, 2022

Dear Dr. Heather Bradshaw,

I am pleased to serve on the internal advisory board (IAB) of the IUB Center for Cannabis, Cannabinoids, and Addiction (IUB-C3A) proposal that you are submitting to NIDA as a P30 Center of Excellence.

My current position as an Assistant Professor in the Department of Counseling & Educational Psychology, with an appointment in Human Development, provides me with a unique set of insights and research tools to address issues of addiction. I received my Ph.D. in Developmental Psychology from Virginia Commonwealth University. Soon after, I completed a postdoctoral fellowship at Indiana University's Center for Research on Race and Ethnicity in Society. Thus, my research investigates human development across the lifespan and the influence of cultural and community contexts.

Specifically, I am an adolescent researcher with a focus on adolescence and emotions in three areas; understanding the emotional development of Black youth, examining how Black youth's social context (family, friends, school) influences emotion expression and emotion regulation, as well as the role emotion expression and emotion regulation play in Black youth's decisions to engage in both risk-related and prosocial behaviors. Within this work, my collaborators and I have examined what makes the developmental age stage of adolescence vulnerable to particular outcomes, including mental health and problem drug use. My research seeks to expand our foundational knowledge of adolescent emotional development and situate that knowledge within a sociocultural context for Black youth. Given my previous research and point of view as a developmental psychologist, I am sure that I will provide a unique perspective to the advisory board by addressing the ways in which each of these aspects of development likely has an intersection with addiction, especially the elements of the proposal that address how drug use during particular developmental stages has an impact on brain development and decision making.

I look forward to collaborating in this capacity with your team.

Sincerely,

A handwritten signature in black ink, appearing to read "Tennisha N. Riley".

Tennisha N. Riley Ph.D.
Assistant Professor of Human Development
Department of Counseling and Educational Psychology
Indiana University School of Education - Bloomington



INDIANA UNIVERSITY

18 SEPTEMBER 2022

Dear Ken:

This letter is to enthusiastically confirm my support for your NIDA P30 application, *IU Bloomington Center for Cannabis, Cannabinoids, and Drug Addiction* (C3A). I strongly endorse your submission and will work to ensure the necessary institutional resources are available for its success.

Thank you for the invitation to participate in this endeavor and I look forward to chairing your Internal Advisory Board (IAB). The IAB's mission is to provide advice to the Center and Core PIs on general directions of the C3A, operational concerns that may arise, and IU Bloomington-specific issues. It will operate at one level above the steering committee (which consists of the five PIs and core technical staff) and will liaise with the external advisory board on strategic issues as indicated. I am confident that my 26 years of experience on the Bloomington campus and 7 years as Associate Vice Provost/Vice Provost for Research in which I oversaw 22 centers at IUB will bring a level of breadth to your vision, in order to help make the center successful and sustainable. I am excited to partner with you in this capacity.

As always, anything I can do to help with the development or management of the center I am happy to contribute.

Sincerely,

Jeffrey M. Zaleski

Jeffrey M. Zaleski

Provost Professor of Chemistry

Technical/Method Development Letters of Support



INDIANA UNIVERSITY

SCHOOL OF MEDICINE
Department of Medicine

September 5, 2022

Hui-Chen Lu, PhD
Director, Gill Center for Biomolecular Science
Jack and Linda Gill Chair, Gill Center for Biomolecular Science
Professor of Psychological and Brain Sciences
Multidisciplinary Science Building, Rm 108
702 North Walnut Grove Ave.
Bloomington, IN 47405-2204

Dear Hui-Chen,

I am writing to express my strong support for your P30 project proposal, the "IU Bloomington Center for Cannabis, Cannabinoids, and Addiction" (C3A). The combined expertise and international reputations in cannabinoid-related research from you, and Drs. Ken Mackie, Heather Bradshaw, Andrea Hohmann, as well as Istvan Katona are incredible. The implementation of the C3A center will undoubtedly benefit the Midwest Addiction research community.

I have a long-standing interest in applying quantitative fluorescence microscopy techniques to study endocytic membrane transport in polarized epithelial cells. Our laboratory has open access to five confocal microscopes and three multi-photon microscopes. In addition to supporting conventional confocal and multiphoton microscopy, one of the confocal systems and one of the multi-photon systems are also equipped with ISS FastFLIM systems, supporting time-resolved fluorescence lifetime imaging microscopy (FLIM). As you know, FLIM is a powerful technique for analyzing metabolism and for measuring FRET and 2pFLIM (based upon multiphoton excitation) extends FLIM analyses into living animals. We have significant experience developing methods of quantitative fluorescence microscopy including novel methods of 3-dimensional, multi-parameter microscopy of living cells (PMID:11208134, 11591819, 16641372) that have helped us to correlate defined steps of endocytic transport with the dynamic behaviors of specific regulators. More recently we have developed novel approaches based upon FLIM and 2pFLIM for characterizing metabolism and signaling in living cells and in living animals (PMID:27685098, 28250053, 32191861).

Dr. Jui-Yen Hang has described her plans to use the genetically-targeted neuron-astrocyte proximity assay (NAPA) to identify the dynamic interactions between astrocytes and neurons). The current version of NAPA has been established with intensity-based confocal microscopy. I agree with you that FLIM will provide a particularly effective and reliable approach for identifying and quantifying astrocyte-neuron interactions. I do not foresee any difficulties in accomplishing the proposed studies but am happy to provide consultation to help you address any technical challenges and to help you develop and interpret quantitative analyses.

I look forward to working with you and wish you good luck with this exciting proposal.

Sincerely yours,

Kenneth W. Dunn, PhD
Professor of Medicine and Biochemistry & Molecular Biology
Director, Indiana Center for Biological Microscopy



Dimitri Yatsenko, PhD

DataJoint
4265 San Felipe St, Suite 1025
Houston, TX 77027-2957

dimitri@datajoint.com

September 16, 2022

Dr. Hui-Chen Lu

*Gill Chair for Linda and Jack Gill Center for Biomolecular Science
Professor in Dept. of Psychological & Brain Sciences*

Multidisciplinary Science Building, Rm 108
Indiana University
702 North Walnt Grove Ave.
Bloomington, IN 47405-2204

Dear Dr. Lu,

I extend my support for your proposal to establish a P30 Core Center of Excellence—the IU Bloomington Research Center for Cannabis, Cannabinoids, and Addiction (C₃A).

DataJoint intends to partner with C₃A to develop and optimize data analysis methods for the imaging of cannabinoid, dopamine, serotonin, and voltage fluorescent sensors. The analysis pipelines will be packaged into reproducible and citable workflows following best FAIR principles. DataJoint will offer services to host and operate cloud-based deployments of the analysis workflows to the C₃A affiliates.

DataJoint works with leading research teams developing computational tools to vastly increase the scale and reproducibility of data analysis in groundbreaking neuroscience studies. DataJoint develops and operates data pipelines for several major data-centric projects such as the Allen Institute's Mindscope, the International Brain Lab, the Moser Group, the Mesoscale Activity Project at the HHMI Janelia Campus, and others. DataJoint develops standardized analysis workflows for the major types of neurophysiology experiments—*DataJoint Elements* (NIH Project U24-NS116470) as open-source software.

DataJoint has been working with your lab since January 2022 to operate a cloud-based data analysis pipeline for calcium imaging of neural activity and video-based behavior analysis. This data pipeline will serve as one of the key starting points for the analyses offered through C₃A.

I wish you success in your application.

Sincerely,

Dimitri Yatsenko, PhD
CEO, DataJoint



One Max Planck Way
P.O. Box 998
Jupiter, FL 33458
www.maxplanckflorida.org



Ryohei Yasuda, Ph.D.
Scientific Director

Ryohei.Yasuda@mpfi.org
+1 561 972 9202 office
+1 561 972 9001 fax

September 2, 2022

Dear Hui-Chen,

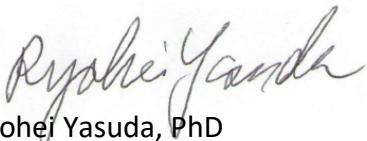
I am writing to enthusiastically support your P30 project proposal, the IU Bloomington Center for Cannabis, Cannabinoids, and Addiction (C3A). During my visit for the 2018 Gill Symposium, I was very impressed by the extensive cannabinoid related research community at IUB and their collaborative nature. The recent additions of Dr. Istvan Katona and Dr. Norbert Hajos into your local scientific community will undoubtedly further expand its scientific impact.

My laboratory aims to elucidate the molecular mechanisms underlying learning-driven neural circuit modifications. To visualize dynamic signaling with high spatial precision and cell subtype-specificity in the brain of awake behaving mice, we pioneered 2-photon fluorescence lifetime imaging microscopy (2pFLIM) and developed many new biosensors optimized for 2pFLIM (Peters et al. *Nature*, 2014, PMID 24805237). Using this technique, we have succeeded in imaging many signaling proteins, including CaMKII, CREB, RhoA, Cdc42 etc., in single dendritic spines undergoing synaptic potentiation. 2pFLIM also enabled us to illustrate the essential role of spatiotemporal regulation in coordinating cellular events in different micro-compartments. Furthermore, we host annual imaging workshop to train scientists on advanced imaging techniques. Both Jui-Yen Huang and Sen Yang from your laboratory have participated in these workshops. I am very proud to see how our training has enabled them to be proficient in several state-of-art imaging techniques, such as FLIM.

I am excited that you are adding FLIM into your 2p imaging repertoire. 2pFLIM will enable you to determine *in vivo* how intracellular biochemical or energy metabolic states are altered by cannabis/cannabinoids or other illicit drugs with high spatiotemporal resolution. There are a growing number of FLIM biosensors being developed. You can also take advantage of the autofluorescence of metabolites such as NAD(P)H and FAD, if you are interested in measuring those, I am happy to consult with you for sensor choice, data analysis, or imaging mechanistic details as you see fit. With our mutual visits to each other's laboratories, you have gotten acquainted with my lab members and Dr. Long Yen, the imaging engineer in our institute. I know that Long will be very happy to provide technical know-how for FLIM and 2P microscopy as well.

I believe your project and the service you are providing with your imaging core will make significant progress in further elucidating how cannabis, cannabinoids, and other drugs of abuse modulate brain function at the molecular levels in the brains of awake animals. This will have important implications for our understanding of the long term impacts of drug addiction and exposure to drugs of abuse. I am thrilled to provide advice and share our expertise and reagents as needed.

Sincerely,



Ryohji Yasuda, PhD
Scientific Director
Max Planck Florida Institute for Neuroscience (MPFI)

C3A Affiliates Letters of Support

I ILLINOIS

Molecular & Integrative Physiology

SCHOOL OF MOLECULAR & CELLULAR BIOLOGY

524 Burrill Hall, MC-114
407 South Goodwin Avenue
Urbana, IL 61801
USA

Dear Heather,

Sept 08, 2022

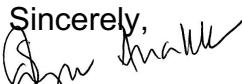
It is great to hear about the P30 proposal for the *IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction*. As you know, my research focus is on bile acids and liver physiology; however, through our recent collaboration, we have pilot data showing that bile acids drive changes in hepatic endogenous cannabinoids in a manner like cannabinoid drugs. This novel area of study is an example of the intersection of bile acids, liver physiology, and drugs of abuse.

Your current ability to measure many small molecule signaling lipids, especially the endocannabinoids and lipoamines, has provided important preliminary data on how bile acid signaling in hepatocytes is changing their regulation and production; however, it is very exciting to hear that the addition of a next-generation mass spectrometer would enable you to have even more sensitivity for measuring oxygenated metabolites like those of cytochrome enzymes that share the same biosynthetic and metabolic pathway as both bile acids, steroids and many drugs of abuse. Being able to measure the changes in these bioactive lipids will broaden our understanding not only of bile acids but of liver function in general. So many drugs of abuse impact on liver physiology and, by extension, bile acid signaling that the intersection of these pathways will be an important component in understanding overall health and disease.

In line with the educational outreach aspect of being an Affiliate member of the Center for Cannabis, Cannabinoids, and Addiction core, I want to highlight my outreach with Beckman open house, American Cancer society relay for life and presenting our work at high schools and Champaign public library. I am happy to be an affiliate of this core and offer my enthusiastic support to develop new projects and research directions through the use of the BLMC of the *IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction* to advance my research questions.

Wishing you the best of luck with your proposal and look forward to our continued collaboration in this new and exciting area of bile acids and cannabinoids.

Sincerely,


Sayee Anakk Ph.D.

Associate Professor,
Department of Molecular and Integrative Physiology,
Division of Nutritional Sciences,
Affiliate Beckman Institute,
Member Cancer Center at Illinois,
The School of Molecular and Cell Biology,
University of Illinois, Urbana-Champaign,
506 S Mathews Ave, Urbana-61801.



INDIANA UNIVERSITY

DEPARTMENT OF PHARMACOLOGY
AND TOXICOLOGY
School of Medicine

September 15, 2022

Gill Center for Biomolecular Science
MSBII 120
702 N Walnut Grove
Bloomington, IN 47405

Dear Hui-Chen, Istvan, Heather, and Ken,

I am delighted to write this letter of support for the P30 project proposal, *IU Bloomington: Cannabis and Cannabinoids Center*. When I heard about this proposal, I was quite excited as it provides Core services that will be of significant utility to advance my research program. I am currently funded through the National Institute on Alcohol Abuse and Alcoholism (NIH/NIAAA R01 AA027214) to explore the role of mu opioid receptors (MORs) on anterior insular cortex (AIC) synaptic terminals in the dorsolateral striatum (DLS) in synaptic plasticity and regulation of alcohol-related behaviors. We have previously determined that synaptic plasticity mediated by MORs at these AIC-DLS synapses is uniquely sensitive (relative to other DLS synapses) to the deleterious effects of alcohol (Muñoz et al., *Nature Communications* 2018). Recent data suggest that these AIC-DLS synapses are also uniquely sensitive to opioids, suggesting a common mechanism of action between alcohol and opioids. Of further interest, especially in relation to this project proposal, we also find that DLS cannabinoid plasticity, just like MOR plasticity, is similarly affected by opioids as it is by alcohol (Atwood et al., *Nature Neuroscience* 2014). Recent work (Haggerty et al., *eLife* 2022) has also demonstrated that these AIC-DLS synapses undergo synaptic plasticity during extensive alcohol consumption that produces gain-of-function adaptations allowing these synapses to dictate ongoing alcohol consumption patterns. Altogether, it is clear that these AIC-DLS synapses have a fascinating bidirectional relationship with alcohol, and likely opioid, consumption: they are especially sensitive to exposure to alcohol and opioids and are also able to regulate consumption of alcohol (and possibly opioids).

We have a number of open questions that we eagerly want to address as the next steps in our project. We want to know what it is about AIC-DLS synapses that makes them unique in relation to other DLS synapses. We also want to know what changes occur in these synapses that leads to their gain-of-function of alcohol drinking control. We have a recently published preprint on a pharmacological assessment of MOR-mediated synaptic plasticity mechanisms at AIC-DLS synapses (Muñoz et al., *BioRxiv* 2022) that has provided some clarity into how MORs induce synaptic depression differently at these synapses relative to other DLS synapses. However, it is not clear how alcohol selectively disrupts this MOR plasticity at these synapses, while leaving MOR plasticity at other synapses intact. Long-term alcohol consumption also alters glutamate release from these synapses, whereas more general glutamate release is relatively unchanged. Stochastic optical reconstruction microscopy (STORM) superresolution imaging could be a key tool

that will help us determine what changes occur specifically within AIC axon terminals within DLS during alcohol consumption that lead to a loss of MOR plasticity and reduced glutamate release. It is incredibly difficult to resolve the localization of MORs in glutamatergic axon terminals in DLS due to the abundant MOR expression on synaptic processes of local neurons intrinsic to the DLS itself. Using STORM to localize MORs specifically within AIC terminals and measuring how their localization changes (or doesn't) following alcohol (or opioid) consumption will be very enlightening. Based on our pharmacological study (Munoz et al., *BioRxiv* 2022), we also have a number of other downstream signaling proteins that could also have differential localization as a result of alcohol or opioid exposure (e.g. HCN1 channels or PKA subunits). STORM could reveal how the localization of these proteins change.

While others previously anatomically detailed that AIC sends projections into DLS (e.g. Hunnicutt et al., *eLife* 2016; Wall et al., *Neuron* 2013), we were the first to identify a functional connection (Munoz et al., *Nature Communications* 2018) between the two brain regions. However, recent preliminary studies in our lab have suggested that AIC inputs may be heterogenous in their targets within DLS. The previous anatomical assessments of AIC inputs to DLS (and a more recent one, Gehrlach et al., *eLife* 2020) have lacked resolution as to the specific subcompartment-specific targets within DLS. The DLS contains both striosome and matrix subcompartments and some cortical areas specifically target one subcompartment over another. In addition, it is unclear if DLS-projecting AIC neurons send collaterals to other brain regions. We could use the Imaging Core's expertise in ScaleS imaging to map the AIC-DLS neurons' subcompartment targets within the DLS as well as whether those neurons have collaterals to other brain regions (and if so, what the tract that those collaterals take is).

Our laboratory has taken a recent interest in whether prenatal opioid exposure produces similar deficits in corticostriatal (e.g. AIC-DLS or other cortical inputs to DLS) function as adult exposure has. To that end we developed a mouse model of prenatal methadone exposure (Grecco et al., *eLife* 2021). That study focused on the motor cortex as we identified numerous sensorimotor dysfunction outcomes in prenatal methadone-exposed offspring. Preliminary studies (see Pilot Project proposal) also identified deficits in the primary somatosensory cortex. We also identified deficits in endocannabinoid signaling at corticostriatal synapses, likely downstream from motor and sensory cortex (manuscript in preparation). It is unclear if the deficits in endocannabinoid signaling are a result of altered CB1 cannabinoid receptor signaling or altered endocannabinoid production (or both). To that end, we could again utilize STORM imaging to identify whether there are changes in the distribution of CB1 receptors and endocannabinoid metabolic enzymes, to go along with planned pharmacological experiments. In addition, we would like to measure the levels of anandamide and 2-arachidonoylglycerol within the DLS of prenatal methadone-exposed mice (and controls) and the Bioactive Lipid Metabolites Core could provide these measures to help us with that project as well.

We would like to build upon our preliminary electrophysiological and anatomical assessments of synaptic function within the primary somatosensory cortex to determine whether there are also deficits in network dynamics as a consequence of those changes

in synapse function with the intent to eventually determine a causative relationship between changes in sensorimotor cortices and the observed changes in sensorimotor behavioral development we previously measured (Grecco et al., *eLife* 2021). We have therefore proposed to work with Dr. Hui-Chen Lu (who helped us to develop and characterize the prenatal methadone exposure model) and the Imaging Core to perform *in vivo* multiphoton imaging of calcium dynamics of primary somatosensory cortex neurons in different cortical layers in awake behaving mice across the span of development where we observed sensorimotor dysfunction.

Altogether, I hope I have made it abundantly clear how enthusiastic I am to utilize the various Cores of the *IU Bloomington: Cannabis and Cannabinoids Center*. While I am primarily an alcohol researcher, my interests have many intersections with the opioid and cannabinoid research fields due to our research into opioid and cannabinoid receptor-mediated plasticity that modulates alcohol-related behaviors. I foresee us utilizing many of the various Core's services, both for our own currently funded work as well as to bolster future projects.

Sincerely,



Brady K Atwood, PhD
Associate Professor
Department of Pharmacology and Toxicology
Stark Neurosciences Research Institute
Indiana University School of Medicine
Indianapolis, IN



University of Kentucky
College of Medicine
Center on Drug & Alcohol Research
845 Angliana Ave
Lexington, KY 40508
Phone: 859-257-1881
Fax: 859-257-5232
babalonis@uky.edu

September 14, 2022

Dear Dr. Bradshaw,

The P30 proposal for the *IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction* would be a great asset to drug addiction research, and I am very happy to support this proposal and join as a core affiliate member and pilot project PI. I am particularly excited about the opportunity to work with you through the Bioactive Lipid Mediators Core and be able to realize projects using human plasma samples that would be difficult and cost-prohibitive without this resource.

As you know, much of my work is aimed at both understanding and optimizing therapeutic interventions for individuals with substance abuse disorders. We have recently received funding (*Cannabis Modulation of Outcomes Related to Opioid Use Disorder: Opioid Withdrawal, Opioid Abuse Potential and Opioid Safety*, 1R01DA054347) and this project would greatly benefit from the addition of lipidomic analyses. We will enroll participants who are physically dependent on opioids into our inpatient hospital research unit and investigate how controlled doses of inhaled cannabis impact opioid withdrawal severity, the abuse potential of opioids and opioid safety (i.e., cannabis modulation of opioid-induced respiratory depression). This line of research would be greatly enhanced by analyzing plasma for endocannabinoids and related lipids; for example, there are no controlled data available on how opioid use disorder or opioid withdrawal affects endocannabinoid tone. Overall, we have so much to learn about the systemic effects of chronic drug use and what cannabinoid therapeutics may or may not be modulating. Being able to incorporate a broad scale lipidomics analysis of plasma samples from these participants at baseline and at multiple time points throughout the study would be extremely beneficial.

In line with the educational outreach aspect of being an Affiliate member of the *Center for Cannabis, Cannabinoids, and Addiction* core, I want to highlight my current outreach activities. I am serving as a Near Peer Mentor for diverse faculty enrolled in a faculty enrichment program (Research Scholars Program) for the second consecutive year. I am also a member of our departmental Diversity, Inclusivity and Equity Council and serve as the chairperson for our White Coats for Black Lives Initiative.

Thank you again for reaching out and for developing this proposal. I fully support this proposal and look forward to utilizing this resource in my future research.

Sincerely,

Shanna Babalonis, Ph.D.

Shanna Babalonis, Ph.D.

see blue.



UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

Joseph F. Cheer, PhD
Professor

Department of Anatomy & Neurobiology
20 Penn Street -HSFI Room 280J
Baltimore, MD 21201
410 706 0112
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09/06/22

Ken Mackie, M.D.

Linda and Jack Gill Chair of Neuroscience
and Distinguished Professor
Dept. of Psychological & Brain Sciences
MSBII 120
Indiana University
702 N Walnut Grove Ave
Bloomington, IN 47405-2204

Dear Ken,

I write this letter in strong support of your P30 project proposal "*IU Bloomington: Cannabis and Cannabinoids Center*." From our conversations about your plans for the Center, I am excited about the possibilities for my current NIDA grants. Namely, for DA045639 (Cannabinoid receptor control of a DRN to VTA pathway and its role in affective states) we are interested in determining if exposure to THC alters serotonin modulation of phasic dopamine release in the VTA. Access to super resolution microscopy with the addition of Dr. Katona's PharmacostORM will greatly advance our understanding of the anatomical details of serotonin inputs onto VTA dopamine neurons. Another one of our projects DA022340 (Endogenous cannabinoid control of reward substrates) would similarly benefit from super resolution imaging (in fact, Dr. Katona is a consultant for this project). An additional important aspect that is currently missing from our grant DA044925 (Neurodevelopmental effects of THC on the VTA dopamine system and behavior), is the ability to accurately measure THC concentrations in different tissues (placenta, lung, liver, brain...) that could be provided by Dr. Bradshaw's expertise.

Additionally, the significant training component in your P30 is encouraging and reassuring. Dr. Katona's imaging techniques hold great promise to address diverse neuroscience questions. The training of scientists to import these imaging approaches (experimental and computational) back to their own labs is a great service to the field. I will certainly encourage some of my students to travel to Bloomington for the STORM class he and Dr. Barna have developed.

With the above, I hope my enthusiasm and support for your proposal center is clearly and unambiguously conveyed, as it holds great promise to advance our programmatic research interests and those of others. I see the Core's services useful for most lines of work in our laboratory.

Best of luck with your timely and significant proposal!

Sincerely,



John A. Dani, Ph.D.
David J. Mahoney Prof. of Neurological Sciences
Chair, Department of Neuroscience
Director, Mahoney Institute for Neurosciences

September 1, 2022

RE: Support for IU Bloomington P30 Center

Dear Ken,

I offer my strongest support for your P30 project proposal, IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction as a Center Affiliate. From your description of the Center, it will be an excellent way to extend several of my ongoing NIH-funded projects.

As you know, my lab studies several drugs of abuse, including ethanol, cocaine, nicotine, and opioids. We use a wide range of techniques from synaptic and cellular neurophysiology to *in vivo* approaches tied to behavioral paradigms, and these techniques are done in combination with neuroanatomical mapping. I was particularly fascinated by Istvan's development of the PharmacoSTORM technique, which allows localization of drug binding sites (e.g., receptors and enzymes) with nanoscale precision. I can readily see application of this technique to several interesting questions we are addressing. For example, we recently have been mapping the underappreciated dopaminergic innervation of the hippocampus from the midbrain ventral tegmental area (VTA). Previously, dopaminergic innervation from the hippocampus was thought to come nearly exclusively from the locus coeruleus as excess dopamine released from noradrenergic neurons. Our recent work shows that there is sparse, but very meaningful, innervation from the VTA.

In addition, our close collaborators in Dr. Mariella De Biasi's lab have been developing a model for THC vaping. We would benefit greatly from having the ability to measure THC and its metabolites in brain tissue and plasma. In associated work, we also are interested in measuring small amounts of lipid mediators, such as endocannabinoids, in specific brain regions, but have had a hard time finding a collaborator who could make these measurements. The inclusion of the Bioactive Lipid Mediators Core seems like a great way for us to get expert help with these experiments.

I was also pleased to learn that your P30 has a significant training component. Istvan's PharmacoSTORM imaging technique holds great promise to address many different neuroscience questions. Certainly, the training of scientists to take these imaging and analytical approaches (that is, experimental and computational) back to their own labs is a great service to the field. I will certainly encourage some of my trainees to travel to Bloomington for the STORM class he and Laszlo Barna have developed.

I hope that I have conveyed my enthusiasm and strong support for your proposal to establish the IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction. I feel it has the potential to advance several of my NIH-funded research projects. I see us being able to use the services of both the imaging and mass spec core, both for our ongoing work as well as to obtain preliminary data for future grant submissions. Finally, it would be a pleasure to interact with you again and work with your group.

Good luck with proposal.

Sincerely,

A handwritten signature in black ink, appearing to read "John A. Dani".

John A. Dani

415 Curie Boulevard | 211 Clinical Research Building | Philadelphia, PA 19104
215-898-8498 | Fax: 215-573-0833 | johndani@pennmedicine.upenn.edu



COLLEGE OF MEDICINE
Department of Neurological Sciences

Istvan Katona, PhD

August 29, 2022

Naus Family Chair of Addiction
Sciences Dept of Psychological & Brain
Sciences Indiana University, MSBII 154

702 N Walnut Grove Ave Bloomington,
IN 47405-2204

Dear Istvan and Ken,

I am writing in strong support of your plans to establish a new Multi-Scale Imaging Core through a P30 grant on substance abuse at Indiana University. The vision of your core, integrating multiple imaging modalities at different scales will provide state-of-the-art opportunities to researchers to investigate molecular, cellular and circuit changes during development as well as in response to disease and treatment. In particular, the new PharmacoSTORM method that your group has developed is exciting and will be extremely useful to those interested in visualizing and quantifying, at nanoscale level, drug-target interaction sites in specific cell types and cell compartments within brain specimens. In my own research, we perform structural and functional multiphoton *in vivo* imaging of synapses and astrocytes in models of neurodevelopmental disorders such as autism and fragile X syndrome. Extending these imaging approaches to the nano-scale imaging of drug-target interactions in those disease models would be of great interest. I would certainly take advantage of the opportunity afforded to affiliated members to collect preliminary data using the PharmacoSTORM approach.

I appreciate the intention of your administrative core to disseminate information and provide training opportunities to researchers in the Midwest. I believe that these opportunities will be utilized by some of the young investigators that are supported by the Cognitive Neuroscience of Development and Aging (CoNDA) Center of Biomedical Research Excellence (COBRE) at UNMC (P20GM130447) that I direct, as well as by my R01 award (R01NS109381) and look forward to seeing this center develop.

Sincerely,

A handwritten signature in blue ink. The signature starts with a vertical line on the left, followed by a loop that contains the letter "D", and then a long horizontal line extending to the right.

Anna Dunaevsky, Ph.D.
Professor and Vice Chair of Basic and Translational
Research Department of Neurological Sciences
Director, Cognitive Neuroscience of Development and Aging
Center



Dear Heather, Andrea and Craig

It will be a pleasure to serve as a consultant on your revised grant application (R01DA056140-01A1) entitled: "Mechanisms of Cannabidiol-induced Analgesic Action". As you know, I have a significant interest in the metabolism of endogenous and phytocannabinoids and your studies on the interactions of the enzyme NAPE-PLD and CBD are critical for our overall understanding of CBD mechanisms of action, especially as it relates to the regulation of endogenous lipids like Anandamide.

My work illustrates that Anandamide and related lipids are metabolized into potent cannabinoid receptor ligands by cytochrome P450s (*Roy J, Watson JE, Hong IS, Fan TM, Das A. Antitumorigenic properties of omega-3 endocannabinoid epoxides. J Med Chem 2018;61:5569-5579 and Arnold WR, Carnevale LN, Xie Z, Baylon JL, Tajkhorshid E, Hu H, Das A. Anti-inflammatory dopamine- and serotonin-based endocannabinoid epoxides reciprocally regulate cannabinoid receptors and the TRPV1 channel. Nat Commun. 2021 Feb 10;12(1):926. doi: 10.1038/s41467-021-20946-6.*) and your studies in this grant complement these findings.

Preliminary data from the Bradshaw lab indicating that both THC and CBD metabolites levels are differentially regulated in males and females and that the effects of CBD on the treatment of pain is also sex-dependent suggests an interconnectedness of these systems that will be important to investigate in the context of metabolic regulation. So, I am pleased to act as an academic consultant on your studies involving monitoring the role cytochrome P450s with chronic CBD treatment in paclitaxel-treated mice.

Best of luck with your resubmission.

Sincerely,

A rectangular box containing a handwritten signature in black ink that reads "Aditi Das".

Sincerely,
Aditi Das, Ph.D.
Associate Professor
School of Chemistry and Biochemistry
Affiliate: Petit Institute for Biosciences and Bioengineering
Georgia Institute of Technology





Georgia Tech College of Sciences
**School of Chemistry
and Biochemistry**



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Center of Reproductive Sciences

S.K. Dey, Ph.D.
Co-Director

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Fax: (513) 803-1160
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Kenneth Mackie
Jack and Linda Gill Chair, Gill Center for Biomolecular Science
Distinguished Professor, Psychological and Brain Sciences

September 4, 2022

Dear Ken,

We are happy to write this letter strongly supporting your application for a NIDA P30 center—the *IU Bloomington Center for Cannabis, Cannabinoids and Addiction for resubmission*. My colleagues and we will greatly benefit from the resources your center will provide, particularly the mass spectrometric determination of endocannabinoids and related lipids and high-end imaging.

Our lab has been studying cannabinoid signaling in female reproduction for more than 2 decades. Using multiple genetically modified mouse models, our work has shown that optimal pregnancy outcomes require tightly toned endocannabinoid signaling meaning either higher or lower endocannabinoid levels result in pregnancy loss in different stages of pregnancy, ranging from preimplantation development to parturition. As you know, we have a long history of collaboration with your division dated back as early as 2000. The Mass Spec center run by you and Heather Bradshaw is among the limited number of mass spec centers that have provided reliable and comprehensive measurements of cannabinoids and other lipids. Our collaboration has been very productive, which is manifested by our joint publications in high-profile journals like JCI, PNAS, JBC, and Biology of Reproduction.

Our own research program has benefited from this expertise in lipid quantification and will continue benefiting from this NIDA P30 center. We have two NIH R01 grants. One of RO1 had been funded by NIDA for than 20 years until the grant was transferred to NICHD last year. Our ongoing project is to study the interaction between cannabinoid receptors and planar cell polarity signaling in mouse placentation. We heavily rely on your group's expertise in endocannabinoid quantification in our ongoing and prospective project in endocannabinoids. The proposed super resolution imaging system will also expand our understanding in mouse placentation. We have developed 3D imaging of mouse uteri in early pregnancy stages. However, due to the limitation of imaging depth, we are still trying to perfect the imaging of implantation sites including placentas in midgestational stages. The proposed STORM imaging system would help us overcome the current barrier.

We have enjoyed and have mutually benefited from our interactions over the past 15+ years. I feel that the P30 center you are proposing builds nicely on the strengths of the program you have assembled at IUB and the Gill Center and I wish you the best of luck with your resubmission.

Sincerely,



Center of Reproductive Sciences

S.K. Dey, Ph.D.
Co-Director

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SK Dey, PhD
Lova Riekert Chair and Professor of Pediatrics
Co-Director, Center of Reproductive Sciences
Division of Developmental Biology
Cincinnati Children's Hospital Medical Center

Xiaofei Sun, PhD
Associate Professor
Center of Reproductive Sciences
Division of Developmental Biology
Cincinnati Children's Hospital Medical Center



Cecilia J. Hillard, Ph.D.
Director

Neuroscience Research Center

Michael W. Lawlor, MD, Ph.D.
Associate Director

September 5, 2022

Dear Istvan, Heather, and Ken,

I am delighted to write this letter of support for your P30 project proposal, *IU Bloomington: Cannabis and Cannabinoids Center*. When Ken first told me about this proposal, I was quite enthusiastic as it provides two different core services that will be very helpful to extend some of my current NIH-funded projects.

For example, in our dual PI project (R01MH121454), Qing-Song and I are looking at the role of endocannabinoids in suppressing input from the medial septum and the nucleus of the diagonal band into the medial habenula. As you know, the medial habenula is important in stress reactivity, aversion, etc., processes closely involved in relapse to drugs of abuse during abstinence. One goal of this grant is to understand the connectivity of these circuits. Access to super resolution microscopy with the addition of Istvan's PharmacoSTORM will greatly advance our understanding of the anatomical details of this connectivity. In addition, we were recently funded to study the impact of cannabidiol (CBD) on the developing nervous system (R21DA051168). These studies involve a careful morphological study of the impact of CBD on the cerebellum, another study that will benefit from super resolution imaging.

My laboratory is also very interested and engaged in exploration of endocannabinoids in the circulation of humans as a potential biomarker for changes in endocannabinoid signaling in the brain. For example, Krista Lisdahl and I are funded by an R21 "add-on" study to the ABCD consortium (R21 DA049109) to examine relationships among circulating endocannabinoids and various cognitive and emotional parameters in adolescents. While my lab can measure the classic endocannabinoids, this project would greatly benefit from the more complete and extensive lipidomic analyses that will be available in your mass spectrometry analysis core, led by Heather.

I am particularly happy to learn that your proposed P30 has a significant training component. The imaging techniques that Istvan has developed hold great promise across many significant neuroscience questions and it will be important to train individuals who will be able to take these techniques (experimental and computational) back to their own labs. I will certainly be sending my students down to Bloomington for his

STORM class and I imagine many of my colleagues will also be interested in doing the same.

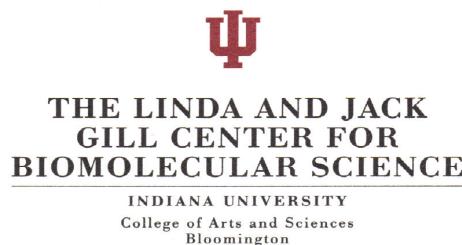
I hope in this brief note I have conveyed my enthusiasm and support for the potential to utilize both the lipid and imaging cores of the *IU Bloomington: Cannabis and Cannabinoids Center* to advance my research projects. I foresee us utilizing many of the various Core's services, both for our own currently funded work as well as to bolster future projects.

I wish you the best of luck with your interesting and important proposal.

Sincerely,

A handwritten signature in black ink, appearing to read "Cecilia J. Hillard".

Cecilia J. Hillard, PhD
G. Frederick Kasten, Jr Chair in Parkinson's Disease Research
Professor of Pharmacology
Director of the Neuroscience Research Center
Associate Dean for Research



September 5, 2022

Dear Ken, Heather, Hui-Chen and Istvan

It is with great pleasure that I write this letter of support to enthusiastically endorse the revision of your P30 grant application entitled ***IU Bloomington: Center for Cannabis, Cannabinoids and Addiction (IUB-C3A)***. In the event the present proposal is funded, I also herein confirm my willingness to serve on the Internal Advisory Board and as an Affiliate faculty member for this P30 grant. I have been a NIDA-funded researcher for approximately 20 years and have an established track record of collaboration and publication with each of the Core PIs (Bradshaw, Katona, Lu, Mackie). I also hold ongoing collaborative MPI R01 grants with the Project PI (Mackie), and additionally serve as co-Director of a NIDA T32 training grant with Dr. Mackie. My experiences working with the PI in these contexts, and my familiarity with Dr. Mackie's prior administrative roles (member of NIDA council, Director of the Gill Center) also allow me to enthusiastically advocate for Dr. Mackie's exceptional scientific, administrative and organizational skills and his ideal qualifications for serving as both the contact PI of this P30 grant and PI of the administrative core. Thus, there is no question that NIDA resources allocated to this P30 grant led by Dr. Mackie will be fully and maximally leveraged to enhance current and future research impact in a productive fashion. The proposed P30 grant will facilitate scientific advances of researchers, not just at IUB, but also in the Midwest and across the nation, with a particular emphasis on underserved regions in our country where problems of drug addiction are particularly severe. Moreover, there is no question that the present P30 grant will enhance the educational training mission of NIDA, including the mission to enhance diversity, that we have successfully promoted in our T32 grant (Integrative training in drug abuse). Moreover, the large number of laboratories with research programs focused on cannabinoids and drug abuse research at IUB (e.g., Bradshaw, Katona, Hohmann, Kalinovsky, Lu, Mackie, Straiker) and in the institutions of our Affiliates, documents that the present P30 grant will be ideally positioned to amplify existing scientific interactions, promote new scientific interactions and exponentially expand scientific research accomplishments.

Interestingly, my collaborative studies with each of the Core PIs originated when each of the faculty members identified herein were at different institutions from myself. I was recruited to Indiana University as a Linda and Jack Gill Chair of Neuroscience and Professor of Psychological and Brain Sciences in 2010, where Ken Mackie served as Director of the Gill Center. I moved my laboratory to IUB because of Ken's leadership of the Gill Center, and Heather Bradshaw's presence as a tenure track faculty member in the Department of Psychological and Brain Sciences, as well as the presence of towering figures in drug abuse research at IUB such as George Rebec. Interestingly, the neuroscience community at Indiana University has only continued to strengthen its reputation for excellence with the recruitment of Dr. Hui-Chen Lu (as a Linda and Jack Gill Chair and Director of the Gill Center) and Istvan Katona (as the David Naus Family Chair in Addiction in the Department of Psychological and Brain Sciences). This documents the ability of faculty at IUB to work productively in a collaborative fashion. There is no question that the current P30 grant will continue to facilitate research excellence and collaborations between the laboratories of the Core PI and Affiliate Faculty, while also providing important training and mentoring opportunities. By reviewing pilot project grants and applications for use of research cores, our faculty will also benefit from enhanced interactions and more in depth understanding of the research programs of other investigators. This level of scientific interaction will also permit researchers with diverse research approaches and differing perspectives to make particularly innovative contributions to drug abuse and neuroscience research. Members of my laboratory, and myself, would also personally benefit from training and utilization of STORM imaging, two photon microscopy and the highly sensitive liquid chromatograph/mass spectrometry as well formal coursework in cutting edge microscopy and

liquid chromatography mass spectrometry techniques. By strengthening the breadth of research approaches available to my group, and our collaborators, the P30 grant can be expected to enhance success of the next generation of drug abuse researchers as well as the competitiveness of current and future grant applications.

The ability to answer research questions in my existing NIH grants would be specifically enhanced through access to the high performance liquid chromatography mass spectrometry core facilities led by Dr. Bradshaw. Acquisition of more sensitive mass spectrometry equipment will permit us to measure changes in brain and plasma lipid mediators in response to drugs of abuse like oxycodone and morphine in discrete regions of the CNS and periphery. These studies have potential to identify and validate plasma biomarkers that explain sex differences in responsiveness as well as vulnerabilities to drugs of abuse in the presence and absence of pathological pain. Such approaches offer considerable promise for enhancing prospects for successful clinical translation. The resources described herein would be complementary to and expand the impact of my R01 funded work exploring CB2-opioid interactions. My laboratory also recently published a previously unreported link between the gut microbiome and opioid withdrawal (Thomaz et al. (2021) Experimental Neurology); we could also capitalize on our previous findings by exploring changes in the lipidome in the gut-brain axis during opioid dependence and withdrawal. My lab is also funded through an Indiana University Addiction Grand Challenges grant to evaluate the impact of a CB2 agonist on unwanted effects of opioids (i.e. opioid-induced reward, opioid-induced dopamine efflux, opioid-induced respiratory depression). Elucidation of sex differences in phenomena under study would also be enhanced by using the above experimental approaches. We would also be able to take advantage of STORM microscopy with Dr. Katona to study the microstructure of CNS regions involved in opioid addiction, and their changes during withdrawal and cue-induced reinstatement, including in our models using intravenous drug self-administration to study motivation to self-administer drugs of abuse. Similarly, two photon microscopy with Dr. Lu would permit a better understanding of *in vivo* changes in calcium mobilization and neuronal activation in discrete brain regions *in vivo* within the context of the phenomenon described above.

In summary, the resources available through the P30 grant will amplify scientific discoveries and enhance current understandings of drug addiction vertically and exponentially, rather than horizontally and incrementally. Thus, the potential for the **IU Bloomington: Center for Cannabis, Cannabinoids and Addiction (IUB-C3A)** to amplify and accelerate vibrant and impactful research on mechanisms and treatment of drug addiction is beyond question. I am excited to participate in this exciting P30 grant and look forward to our continued research collaborations and interactions.

Sincerely,



Andrea G. Hohmann, Ph.D.
Linda and Jack Gill Chair of Neuroscience and Professor
Department of Psychological and Brain Sciences
Indiana University, Bloomington, IN 47402



Icahn
School of
Medicine at
Mount
Sinai

Yasmin L. Hurd

Director, Addiction Institute of Mount Sinai (AIMS)
Ward Coleman Chair in Translational Neuroscience
Professor
Department of Psychiatry,
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Tel : 212-824-9313/4
Email: Yasmin.Hurd@mssm.edu

September 7, 2022

Dear Heather, Ken, and Istvan,

I am happy to write this letter of support for your P30 project proposal, IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction. When Heather first told me about this proposal, I thought this core was a great idea as it provides services that will be beneficial to many of my current NIH-funded projects.

A number of our research projects (e.g., DA048613, Translating CBD Treatment for Heroin Addiction; DA030359 Neurodevelopmental effects of cannabis and its epigenetic regulation) investigate the effects of cannabis exposure (prenatal and adolescence) on neurodevelopmental processes relevant to addiction vulnerability or examines the potential therapeutic effects of cannabidiol (CBD) to modulate behavioral phenotypes of relapse associated with chronic opioid self-administration. Using preclinical animal models, we are able to track the trajectory of early cannabis exposure and to study the endogenous cannabinoid system relevant to opioid use disorder. Moreover, our animal translational studies complement our human research in which biological samples are also examined in regard to the effects of cannabis and alterations of the endogenous cannabinoid system in opioid users. I reached out to Heather last month to begin a collaboration to leverage her lipidomic mass spectrometric techniques to evaluate endocannabinoids and related lipids in relation to our developmental cannabis research paradigms. Those preliminary projects are underway; however, the ability to further advance the mass spectroscopy techniques and sensitivity with the new instrumentation in the mass spec Core will allow us to dissect smaller areas of the CNS for lipidomics analysis to more clearly define potential changes in lipid signaling that are occurring both with chronic opioid exposure and therapeutics.

I am also happy to know that this Core will provide access to super resolution microscopy with the addition of Istvan Katona's PharmacoSTORM. This type of analysis would add significantly to our understanding of microstructure of the CNS regions involved in opioid use, withdrawal, and cessation as well as to developmental effects of cannabis that we have noted not only in the human fetal brain but also in utero environment captured by studying discrete regions of the placenta. I am particularly happy to learn that your proposed P30 has a significant training component. Being able to have hands-on experience with the imaging techniques that Istvan has developed will be a great opportunity for graduate students and post-docs in my lab.

Again, I express my enthusiastic support for the potential to utilize both the lipid and imaging cores of the IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction to advance my research questions.

I wish you the best of luck with your interesting and important proposal.

Sincerely,

A handwritten signature in blue ink that reads "Yasmin".

Yasmin Hurd, PhD
Director, Addiction Institute of Mount Sinai (AIMS)
Ward-Coleman Chair of Translational Neuroscience
Professor of Psychiatry
Professor of Neuroscience
Professor of Pharmacological Sciences



Keisuke Kawata, Ph.D.

Associate Professor

Department of Kinesiology and

Program in Neuroscience - Clinical

Neuroscience Division

School of Public Health-Bloomington

Indiana University

Phone (812) 855-5244

kawata@indiana.edu

September 2, 2022

Dear Ken and Heather,

With this letter, I would like to affirm my enthusiastic support for your NIH-NIDA P30 grant application to bolster the imaging and lipid mass spectrometry capacity for cannabinoid-related research in Indiana and the Midwest. Cannabis research at IU led by you is of the first-class caliber, and as a clinical researcher routinely using neuroimaging and blood biomarker approaches, I understand the importance of employing state-of-the-art technology in biomedical research. The proposed modalities can inform researchers of the neurobiological significance of endogenous and exogenous cannabinoids in the brain. This is a logical and important capacity building to further accelerate the great work that your team has been producing in the past 2 decades. I am particularly interested in applying lipid mass spectrometry to my research of subconcussive neurodegenerative progression. This is timely since several major professional leagues (e.g., NFL, NBA, NHL) have eased their restriction on cannabis use to cope with mental and physical stress, instead of relying on opioids. This trend has begun to influence many amateur sports. Our recent pilot study also suggests that habitual use of cannabis (particularly marijuana) has led to bolstering their neural resiliency to subconcussive head impacts.

These findings justify the need for further exploration into whether cannabis use is prevalent in the amateur adolescent athletic population, where regulation is much looser than, for example, NCAA, and to what extent such cannabis use interacts with subconcussive effects on the brain. As mentioned, I use an array of fluid biomarkers, in addition to imaging, cognition, and functional outcomes in my ongoing NINDS R01 project to evaluate the effect of long-term exposure to subconcussive head impacts in the adolescent population. We currently enrolled about 300 adolescent football players in this longitudinal study, which is the largest study regarding high school subconcussion in the U.S. Per our recent discussion, it is very intriguing and meaningful to study the levels of circulating metabolites and endocannabinoids in response to acute and chronic head impacts. Information related to lipid and cellular metabolites may be a missing piece to address the question, "*Why some people can take more head hits than other? What factors drive the enhancement of neural resiliency to mechanical insult?*". Understanding the role of endocannabinoids in the population that is at risk of developing the early-onset neurodegenerative condition is a clinically significant and much-needed area of research. It is for these reasons; I enthusiastically support your P30 grant application. I look forward to collaborating on many more projects.

Sincerely,

A handwritten signature in black ink that reads "Keisuke Kawata".

Keisuke Kawata, Ph.D.



Washington University in St. Louis

SCHOOL OF MEDICINE

Adam Kepecs, Ph.D.

*Robert J. Terry Professor of
Neuroscience Professor of Psychiatry
BJC Investigator*

September 6, 2022

Dear Istvan and Ken,

I am delighted to write this letter of support for your P30 project proposal, *IU Bloomington: Cannabis and Cannabinoids Center*. I am particularly enthusiastic about your proposal because your Core services would enable us to venture into an entirely new territory that would advance my existing NIH-funded projects (e.g. R01MH097061-08 and R01DA038209-05 in NCE).

As you know, I have relocated to Washington University School of Medicine to be able to interact with a larger community and extend my research into more clinically focused areas. Therefore, I was most excited when you contacted me because your innovative approaches to the study of cannabinoids within your center would open up entirely new avenues for my research.

My lab studies the neural circuit basis of decisions and cognitive functions and how they go awry in mental disorders. We have extensive experience electrophysiology, imaging and optical manipulation in behaving rats and mice. However, we have not yet started with research on cannabinoids. We have a new research program to probe the circuit and behavioral dysfunction of psychosis model mice and the technologies you offer would be very exciting to apply to these. For instance, we would like to test whether and how chronic cannabinoids administration during development alters neural circuits and make them prone to psychotic symptoms, like hallucinations. While we have extensive behavioral expertise using our automated behavioral control systems your behavioral core would be helpful to broadly characterize our animals. We have already identified key circuit nodes for hallucination-like perception, the tail of the striatum (Schmack et al, 2021, Science). PharmacoSTORM technology would be exciting to understand the receptors different cell types, such as D1, D2 and ChAT expressing striatal neurons express and how their numbers or distribution changes with different insults (e.g. genetic models) or upon THC treatment.

I strongly believe that the PharmacoSTORM approach you pioneered will be very useful for the broader life science and medical community. I have discussed this with other colleagues and based on your work we can imagine incredible opportunities in understanding the cell-type-specific targeting of different small molecules. This technique could answer a number of important open questions about drug-receptor interactions underlying behavior that we had been looking to address but the method had been lacking until now.

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Phone: (314) 273-2205 Email: akepecs@wustl.edu

Page 2 of 2

The training component of your P30 would be also particularly useful to us given your nearby location in Bloomington. Students and postdoc from my lab would be able to learn all of these techniques and become power users. This would provide a way to broadly disseminate your technologies as these trained individuals could be able to use these techniques in their own labs.

In sum, your Center and Core services would be an enormous asset to our research program. Your expertise and technologies would open up an important new avenue for us to pursue the role of endocannabinoids and how they can impact neural circuits to heighten the risk of psychotic states. We also foresee using your other core services to augment our existing NIH projects.

Best wishes with your innovative and important application!

Sincerely,



Adam Kepecs, Ph. D.
Robert J Terry Professor of Neuroscience and Psychiatry
Washington University School of Medicine



THE UNIVERSITY *of*
NEW ORLEANS

Dear Heather,

I am very excited to support the P30 project proposal, *IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction*. This would be an amazing opportunity to be able to collaborate and further develop our understanding of how the cannabinoid system is related to the development of autism spectrum disorder (ASD). As you know, my work has shown that the endocannabinoid system is implicated in the underlying pathophysiology of ASD, specifically the social functioning, cognitive function, and emotional responses. This work is now being used to support the investigation into the safety and tolerability of cannabinoid-based interventional therapeutics for this clinical population as well as define the fundamental mechanisms of this disorder. Through collaboration with the *IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction* I would also be supported in expanding the scope my research to address the incidence of features in co-occurring conditions of ASD, such as attention deficit hyperactive disorder and Parkinson's disease.

As a new Assistant Professor at The University of New Orleans (UNO), I am particularly excited about the training opportunities for UNO students in the STEM Summer Scholars Institute program. UNO is a Carnegie R2 institution, thus the university does not have the resources characteristic of R1 institutions, but it serves an important role in the education of STEM students in Louisiana. As the first fully integrated public university in the South, the ethnically diverse student body at UNO would uniquely benefit from participation in this core which will provide students with access to expertise and resources not available within the university or state. Having students have meaningful, hands on experience in the Bioactive Lipids Mediators Core will be a formative experience. I believe this would significantly encourage the number of students and trainees at UNO to pursue careers in biomedical and behavioral sciences.

Being able to participate as an affiliate of the core, will support my study of endocannabinoid signaling in determining of its role in ASD pathophysiology, social learning, and the potential innovation of interventions for neurodivergent populations. Outcomes of this research will advance the primary understanding of the neural mechanisms in social functioning which may be used as a target of engagement for interventions seeking to enhance quality of life and advance of human health.

In line with the educational outreach aspect of being an Affiliate member of the *Center for Cannabis, Cannabinoids, and Addiction* core, I want to highlight my outreach with the Annual Biomedical Research Conference For Minoritized Scientists (ABRCMS) and Black In Neuro. Through these organizations, I actively mentor and support historically excluded and marginalized students interested in a career in the biomedical



THE UNIVERSITY *of*
NEW ORLEANS

sciences. I anticipate many students from these organizations will be excited to learn about the research opportunities available to them through the *Center for Cannabis, Cannabinoids, and Addiction* core.

I am thrilled to be an affiliate of this core and offer my enthusiastic support for the potential develop new projects and research directions with the BLMC of the *IU Bloomington: Center for Cannabis, Cannabinoids, and Addiction* to advance my research questions. I wish you the best of luck with your proposal.

Sincerely,

Debra S. Karhson

Assistant Professor, Dept. of Psychology

The University of New Orleans

Email: dkarhson@uno.edu

Phone: 504-280-6870



MUNROE-MEYER INSTITUTE
for Genetics and Rehabilitation

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September 1, 2022

Istvan Katona, PhD

Naus Family Chair of Addiction Sciences
Dept of Psychological & Brain Sciences
Indiana University, MSBII 154
702 N Walnut Grove Ave
Bloomington, IN 47405-2204

Dear Istvan,

I am very excited about your new PharmacoSTORM method, now in press in Nature Communications. I believe that it will fundamentally change the research landscape in cell-type-specific nanoscale molecular imaging: this method can be as transformational as optogenetics has been in the last few decades. PharmacoSTORM represents a major scientific breakthrough and the further development (and utilization) of this method is virtually unlimited!

Establishing the Multi-Scale Pharmaco-Microscopy core is a critical step for our research endeavors in a Midwest. I can think of dozens of NIH-funded scientists at UNMC who would be eager to utilize PharmacoSTORM - MSPM to further develop their research portfolios. It would allow them to ask scientific questions that they could not address until now using more conventional imaging methodologies.

Related to my personal research, we could finally decipher the mechanism by which cariprazine (and other medications) are inhibiting the DCHR7 enzyme and study the physiological consequences of this inhibition at unparalleled molecular resolution.

So, please understand this letter as my strongest, unconditional endorsement of the new Multi-Scale Pharmaco-Microscopy core, as well as my deep desire to become a user and affiliated member of the Indiana University P30 center grant. I would love to generate preliminary data using PharmacoSTORM for two of my R01 grants which are in the process of competitive renewal, and my research would greatly benefit from super-resolution imaging capabilities.

Keep up the great work, and I am looking forward to our joint scientific endeavors!

The very best,

Károly Mironics, MD, PhD

Director, Munroe-Meyer Institute for Genetics and Rehabilitation, UNMC
Hattie B Munroe Professor of Psychiatry, Biochemistry & Molecular Biology, Pharmacology and Experimental Neuroscience



Department of Biomedical Sciences

September 8, 2022

Dear Dr. Mackie,

It's my pleasure to write this letter strongly supporting your application for a NIDA P30 center—the *IU Bloomington Cannabis and Cannabinoids Center*. My colleagues and I would benefit tremendously from the resources your Center will provide, particularly mass spectrometric determination of endocannabinoids and related lipids and high-end imaging.

By way of introduction, I am an associate professor and the vice chair for research at the Joan C. Edwards School of Medicine at Marshall University. For the last fifteen years, my research has focused on neuropeptide and cannabinoid signaling pathways in drug abuse. In August 2022, I was awarded a diversity mentoring administrative supplement to establish a program for local minority high school students to become involved in biomedical research. My colleagues and I, envision that this supplement funding will be a stepping-stone for a R25 training program at Marshall that would support the full spectrum of career development for minority trainees including high school, undergraduate, graduate school, and postdoctoral training.

A central factor in my decision to move the laboratory to Huntington, WV in 2020 was the opportunity to assemble an innovative and impactful, extramurally funded Addiction and Chronic Pain Research Center in one of the communities most severely affected by the current opioid epidemic. In 2019, the state of West Virginia led the nation in per capita drug overdose deaths (most of those deaths from opioid overdose) and the Huntington-Ashland-Ironton tristate region has been amongst the most severely impacted communities in the already hard-hit region of Appalachia. Currently, a Research Challenge Grant application for seed funding to establish this Addiction and Chronic Pain Research Center is pending with the West Virginia Higher Education Policy Commission. This Research Challenge Grant would provide support for two research cores, a Behavior Research Core, and a Molecular Neuroscience Core, as well as an annual Addiction Research Symposium. These research cores and symposium would be available and open to investigators at Indiana University Bloomington. Dr. Richard Egleton, the Outreach Coordinator for our Center, provides training for clinicians (Marshall Ground Rounds, Evidenced Based Practice Clearing House training) patients (Healthy Connections Program and Maternal Addiction Recovery Center (MARC)), students (social work, psychology, physical therapy, and speech pathology programs), and the regional community (brain week for elementary students, addiction talks for high school students, and foster parent training).

My own research will benefit from the ability of the NIDA P30 Center to perform lipidomic analysis in mutant mouse lines with point mutations in the cannabinoid receptor. Furthermore, we are fortunate to have recently recruited a very strong group of addiction researchers who I feel would also benefit from the resources and training provided by your Center including STORM imaging and correlative confocal microscopy. For example, Dr. Brandon Henderson, who is funded with a R01 from NIDA, is studying nicotinic acetylcholine receptor expression and distribution in transfected cells and brain slices. The impact of his current work would be enhanced by the ability to do STORM imaging and correlative confocal imaging through your Center. Likewise, Drs. Louise (NIH R21 and VA Merit funding) and Chris Risher (NIH R15 funding) routinely use confocal imaging to study the impact of alcohol and opioid exposure on glial-neuronal interactions and would benefit from the high-end imaging capabilities of your proposed Center. Dr. Swarup Mitra, a new assistant professor at Marshall, studies the epigenetic mechanisms of psychostimulant use and will also benefit from enhanced imaging capabilities. However, where I see a particularly important point of synergy is with the NIGMS P20 application that we are preparing for submission. This COBRE will take a comprehensive approach to the

interrelated challenges of addiction and chronic pain. I see your P30 synergizing with our COBRE by providing access to complementary but non-overlapping regional research cores that would provide critical imaging and lipid analysis capabilities to medical school faculty at Marshall including our cohort of junior research project leaders.

I have enjoyed and benefited from our interactions over the past 15 years including making regular trips to catch up and collaborate while attending the annual Gill Symposium. My opinion is that the cluster of cannabinoid researchers assembled at IUB and the Gill Center is one of the most impactful and strongest groups working on this topic in the world, making it the ideal location for the P30 Center you are proposing.

Sincerely,



Daniel J. Morgan, Ph.D.
Associate Professor and Vice Chair
Department of Biomedical Sciences



Sep 20, 2022

RE: NIH Application

Dear Ken,

I am writing to express my enthusiasm regarding your NIH P30 proposal to establish a Center for Cannabis, Cannabinoids and Addiction at Indiana University Bloomington. There is currently a need for centers focused on addiction research in the greater Indiana area and the establishment of this center will provide resources necessary to create a collaborative research environment that pushes scientific advancement forward. I am excited to become involved in this center and make use of the core facilities and pilot resources provided.

As you know, I have a strong background in addiction research, with a focus on alcohol and opioid use disorders. My laboratory is currently funded by an NIH R00 grant from the NIAAA to study neural networks associated with alcohol use disorder. As a junior faculty member, I will personally benefit greatly from the resources provided by this proposal. I will be able to make use of the imaging core for the advanced STORM imaging technologies and the mass spectrometry core to collect data that will help prepare subsequent NIH proposals related to my current R00. One of the focuses of my research has been to identify brain regions that have been underexplored but may be involved in addiction related processes by determining neural network function. A logical follow up to these studies is to use mass spectrometry to examine protein networks within specific brain regions during various stages of drug use, which will be greatly facilitated by the proposed core. Further, the availability of pilot funding will open opportunities for new avenues of research, including a potential focus on cannabis related projects.

Most Sincerely,

A handwritten signature in black ink, appearing to read "Adam Kimbrough".

Adam Kimbrough
Assistant Professor
Purdue University
Purdue Institute for Integrative Neuroscience
Purdue Institute for Inflammation, Immunology, and Infectious Disease
Department of Biomedical Engineering
Department of Basic Medical Sciences



Department of Pharmacology
College of Medicine

1501 N. Campbell Avenue
P.O. Box 245050
Tucson, AZ 85724-5050
(520) 626-6400 Telephone
(520) 626-4182 Fax

August 30, 2022

Istvan Katona, PhD, Ken Mackie, MD
Dept of Psychological & Brain Sciences,
Indiana University
MSBII 154, 702 N Walnut Grove Ave
Bloomington, IN 47405-2204

RE: Letter of Support for the P30 application – “The Center for Cannabis, Cannabinoids, and Addiction”

Dear Istvan and Ken,

I am excited to write in support of your P30 application to create a Center for Cannabis, Cannabinoids and Addiction. In looking over your proposal and recent manuscripts that incorporates the advanced imaging technology including the use of the STORM microscopy to identify location of cannabinoids receptors on neuronal terminals is incredible and thrilling. Furthermore, the ability to utilize these techniques to investigate individual receptor binding and nonlabelled molecules to remove the fluorescence is a method that has revolutionized radioligand binding that is visible. The idea of cannabinoids in the areas of reward and addiction can be more thoroughly investigated utilizing these techniques. These new tools will allow other scientist to advance our understanding of selective receptors in specific brain circuits that may, or may not play a role in addiction. Your ability to utilize this imaging to localize dopamine receptors in a novel area of the frontal cortex is amazing, demonstrating how current medications may have other areas of the CNS of unknown function.

I am very excited to have the opportunity to join the Center as an Affiliate Member. I have an on-going NIH grant (R01-DA056608) that investigates the endogenous cannabinoid activity within the brainstem respiratory centers to help prevent opioid-induced respiratory depression. Your center and its imaging capabilities will greatly benefit our needs to investigate cannabinoid receptors in the preBotzinger complex. As you are aware, there are several faculty here that are excited about your Center and the use of the imaging technology. We have been very fortunate to receive a P30 award to create the Center of Excellence in Addiction Studies at the University of Arizona (Porreca/Vanderah, MPI) and are actively working to expand our efforts in addiction research. We are very interested in having one of our post-doctoral fellows come to Indiana University in Bloomington to receive training on the Pharmacostorm technology so that we could implement this in our research questions.

The Core service that you have proposed would provide a unique methodological resource that is not available elsewhere. We are hoping to incorporate this technology into future planned NIH grants on addiction science. In addition, we would be very excited to develop collaborative research projects with you and with your team.

Thank-you in advance for inviting us to participate in this new and important Center. We look forward to further interactions with you in the very near future.

Sincerely,

A handwritten signature in black ink, appearing to read "Todd W. Vanderah".

Todd W. Vanderah, Ph.D.
Professor & Head of Pharmacology, COM
Joint Appointment with Anesthesiology and Neurology
Co-Director of the MD/PhD Program
Director of the Comprehensive Pain and Addiction Center
Email: vanderah@email.arizona.edu Office phone: (520) 626-7801



September 4, 2022

Dr. Kenneth Mackie
Department of Psychological and Brain Sciences, Indiana University Bloomington

Dear Ken:

I am very pleased to write this letter in support of your application to NIDA for a P30 Center entitled, '*IU Bloomington, Center for Cannabis, Cannabinoids, and Addiction*'. As director of the Purdue Institute for Integrative Neuroscience (PIIN), I can affirm that the goals of your Center mesh very well with the techniques that many of my Purdue neuroscience colleagues are using or hope to use in the near future. PIIN's mission is to enhance the collective research activity and impact of neuroscience groups across the Purdue campus. The Institute consists of more than 140 faculty members and has as its overarching goal to build on existing strengths in engineering and imaging by enabling groundbreaking discoveries related to brain function, CNS disorders, and therapeutics. I would be happy to share information about your center with our large neuroscience community to ensure that PIIN investigators can capitalize on the unique capabilities that you plan to develop.

Of relevance to your proposal, addiction is one of PIIN's high-priority areas in which we have made considerable investments to bring together interdisciplinary research teams. Purdue investigators working in this space are focused on (i) identifying and characterizing biological and environmental risk factors; (ii) developing and characterizing preclinical models; (iii) developing pharmaceutical and behavioral treatments; and (iv) developing biomedical devices. I know that members of our addiction research group will benefit tremendously from access to your Center. Two colleagues having already expressed a strong interest in your Center are Dr. Adam Kimbrough, who studies how addiction alters the brain, leading to motivation for excessive drug intake; and Dr. Julia Chester, whose research is focused on genetic, environmental, and neurobiological factors that influence the development of psychological syndromes including addiction (Dr. Chester also has a long-standing interest in endocannabinoids, e.g. see PMID 31561480).

In addition to the services (lipid mass spectrometry and multiple imaging modes) that your Center will offer, I am pleased that your planned P30 includes a substantial training component that will help to propagate some of the cutting-edge techniques your colleagues have developed. For example, in Hui-Chen's portion of the imaging core, her group will train scientists in the implantation and maintenance of cranial windows in young (P10) mouse pups, the care of these pups, and their behavioral training and multiphoton imaging of developing cortex over subsequent weeks. While we have excellent *in vivo* imaging facilities at Purdue, we do not have anyone skilled at longitudinal imaging in very young mice. Certainly, the possibility for our trainees to acquire these skills and bring them back to Purdue will be an excellent opportunity for the broader PIIN community. Similarly, the course that Istvan is developing around his recently developed "PharmacoSTORM" imaging will be useful for many of our trainees in the pharmacological sciences working on opioids and other drugs relevant to drug addiction.

As you know as the former director of the Gill Center and your role in IU Bloomington's Program in Neuroscience, we are always trying to strengthen the ties among neuroscience experts at the IU Bloomington and Purdue campuses. I believe that your Center, by serving as a regional resource, will have a major impact in fostering these connections. I wish you the best of luck with your proposal and look forward to your Center's implementation.

Sincerely,

Jean-Christophe (Chris) Rochet
Professor and John and Donna Krenicki Director, Purdue Institute for Integrative Neuroscience



Daniela Salvemini, PhD

William Beaumont Professor and Chair
Pharmacology and Physiology
Saint Louis University School of Medicine
1402 S. Grand Blvd
St. Louis, MO 63104
Tel: 314-977-6430

**SAINT LOUIS
UNIVERSITY**

September 1, 2022

Gill Center for Biomolecular Science
MSBII 120
702 N Walnut Grove
Bloomington, IN 47405

Dear Heather, Hui-Chen, Istvan, and Ken,

I am submitting this letter to express my support for your P30 project proposal, *IU Bloomington: Center for Cannabis, Cannabinoids and Addiction*. I write this letter as Professor and Chair of the Department of Pharmacological and Physiological Sciences at Saint Louis University School of Medicine. My colleague, Dr. Andrea Hohmann, who is identified as an Affiliate on this application, communicated to me the prospects that Core services would become available to our research community if the present P30 grant is funded. These Core services could be particularly advantageous for the research programs of multiple scientists and trainees in the Midwest, including here at Saint Louis University School of Medicine. I share research interests with Dr. Hohmann in identifying both non-addicting pain treatments as well as mechanisms to block adverse effects of opioids (e.g. tolerance, hyperalgesia, reward) that limit therapeutic potential of opioids. There is no question that research infrastructure described in your P30 proposal could foster collaborative research efforts between our groups and enhance the research programs of a variety of individuals at different levels of scientific development. I also have extensive prior experience working in industry, including at Metaphore Pharmaceuticals Inc. (1999-2005), where I served previously as the Senior Vice President of Research, and at G.D. Searle (now Pfizer) Discovery Research (1994-1999) where I served as a Research Scientist and Project leader. I thus speak from my dual perspectives of performing impactful research in both academia and industry. I am currently funded through both the National Institute of Neurological Diseases and Stroke (NS111200, NS113257) and the National Institute on Drug Abuse (DA043543).

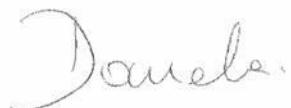
We have a number of research questions that could benefit from the resources made available in this P30 grant. STORM imaging would enable us to show how the localization of targets of interest change in response to opioid treatment or opioid-induced hyperalgesia. The Bioactive Lipid Metabolites Core could help understand how lipid signaling molecules change with our manipulations in both the brain and periphery. Given, my research interests in neuroinflammatory processes that are engaged in the CNS and periphery in pathological states, the approaches employed here are also broadly applicable for my interests in enhancing understanding of peroxynitrite induced nitrosidative stress, ceramide-to-SIP pathway and the adenosine-A3 adenosine receptor signaling pathway among others. My lab is also interested in exploring the contribution of these pathways in the development of opioid-induced hyperalgesia and antinociceptive tolerance known to hamper the effective use of opioids for pain management. Our efforts in these areas have opened a new field of pain research directed toward modulating critical mediators of pain rather than masking symptoms through traditional approaches. Novel chemical entities that specifically target these pathways are also evaluated through our collaborative efforts with the ultimate goal of initiating proof of concept clinical

targeted interventions with currently used analgesics. These large increases in pain relief achievable by targeting multiple synergistic pathways provide a clear path forward for reducing dose and toxicities observed with currently used analgesic drugs.

I am also enthusiastic about the prospects of our trainees being able to apply for lab coursework/training in such approaches as high performance liquid chromatography mass spectrometry, two photon imaging to look at calcium dynamics *in vivo* as well as STORM imaging. Such opportunities can be expected to broaden their training as neuroscientists and enhance their career prospects.

In summary, I am enthusiastic about new opportunities that may become available for our researcher community at Saint Louis University School of Medicine through access to and use of various Cores of the *IU Bloomington: Center for Cannabis, Cannabinoids and Addiction*. It is my sincere hope that this P30 grant, if funded, will facilitate future research interactions between our groups and research programs and propel new scientific discoveries in both our currently funded as well as future research projects.

Sincerely,

A handwritten signature in cursive script that reads "Daniela".

Daniela Salvemini, PhD
William Beaumont Professor and Chair
Department of Pharmacology and Physiology
Director, Henry and Amelia Nasrallah Center for Neuroscience
Saint Louis University School of Medicine
Fellow, Saint Louis Academy of Science
Fellow, National Academy of Innovators

Resource and data sharing plan

Research Resources

The C3A PIs and Affiliates will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the "*Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts*". The C3A PIs and Affiliates acknowledge their willingness to share data and materials with other eligible investigators through academically established means. Data will be shared with collaborators as soon as available, with local colleagues at seminars and talks, and with the scientific community at large by posters and presentations at local, regional, national, and international scientific meetings. In addition, lipidomics and imaging data collected by the two cores will be archived in an organized format and it will be accessible to qualified scientists via the **Administrative Core**. Finally, C3A results will be presented by publication to the widest audience by publication in a timely fashion in reputable journals. Resources used in these publications will be identified by their Research Resource Identification (RRID, Scicrunch) number. Paywalled articles will be made available via the C3A website in a non-copyrighted format and posters presented by C3A PIs, Affiliates, and others using C3A resources will similarly be available. To disseminate the work supported by the C3A more broadly, formal press interviews on findings or publications of broad public interest will be arranged by Indiana University (IU) Communications and more informal communications will be via the C3A website and twitter feed.

Reagent Sharing Plan

Reagents, model organisms, and other materials that may be produced in the course of this work are valuable resources for the scientific community. They will be shared with collaborators as soon as they are available, will be provided to other scientists before publication if the work to be done is different from that we are in the process of publishing, and will be provided to the scientific community upon request after publication. IU agrees to utilize their current material transfer agreement (MTA) capabilities through their offices of Sponsored Research Services, and its intellectual property (IP) protection capabilities through the University Research & Technology Corporation. They can also share these resources through an appropriate license if more formal protection of their IP interests is required. Any reach-through requirements on transferred materials will be addressed within the terms and conditions of IU's MTAs, which follow general U.S. Patent Law principles to govern potential inventorship rights, with ownership following inventorship, and also include general guidelines on good-faith discussions between schools and the recipient of the resources on handling potential subsequent commercial license situations when IP is involved. IU will encourage the investigators to reference any developed resources in its publications, presentations and on its internal web postings so that other researchers are aware that such resources are available.

Data and Code Sharing Plan:

Please see the Data Management Plan on the following pages.

A data management plan for this project was submitted to the National Institutes of Health (NIH) with the information below.



This page represents key information from a data management plan.

Indiana University Bloomington (IUB) Center for Cannabis, Cannabinoids, and Addiction (C3A)

Contributors to this project

Esen Tuna: Data-curation, Indiana University (iu.edu), <https://orcid.org/0000-0001-6585-4195>

Heather Bradshaw: Investigation, Indiana University (iu.edu), <https://orcid.org/0000-0001-7983-5729>

Hui-Chen Lu: Investigation, Indiana University (iu.edu), <https://orcid.org/0000-0002-6628-7177>

Istvan Katona: Investigation, Indiana University (iu.edu), <https://orcid.org/0000-0003-2808-3330>

Ken Mackie: Investigation, Project-administration, Indiana University (iu.edu), <https://orcid.org/0000-0001-8501-6199>

Norbert Hajos: Investigation, Indiana University (iu.edu), <https://orcid.org/0000-0002-4582-2708>

Project details

Research domain: Biological sciences

Project Start: July 01, 2023

Project End: June 30, 2028

Created: September 27, 2022

Modified: September 27, 2022

Ethical issues related to data that this DMP describes?: no

Citation

When citing this DMP use:

Esen Tuna. (2022). "Indiana University Bloomington (IUB) Center for Cannabis, Cannabinoids, and Addiction (C3A) " [Data Management Plan]. DMPHub. <https://doi.org/10.48321/D15028>

When connecting to this DMP to related project outputs (such as datasets) use the ID:

<https://doi.org/10.48321/D15028>

Funding status and sources for this project

Status: Planned

Funder: National Institutes of Health (NIH)

Funding opportunity number: PAR-20-267

Grant:

Project description

This is a project proposal to establish a P30 Core Center of Excellence, the IU Bloomington Research Center for Cannabis, Cannabinoids and Addiction (C3A), at Indiana University, Bloomington. The goal of the center is to provide access to cutting edge imaging and mass spectrometry techniques to researchers ("Center Affiliates") interested in drug addiction and related topics.

While most of our Center Affiliates come from across the Midwest (Indiana, Ohio, Kentucky, West Virginia, Illinois, Missouri, Nebraska, and Wisconsin), we also have scientists from as far away as Hawaii, Pennsylvania, New York, Maryland, and Arizona express interest in using our core resources and participating in our courses. These researchers will be joining the C3A as Affiliates. Building on IU Bloomington's dedicated nucleus of cannabinoid researchers, a major focus of the Center will be on cannabinoids. However, few individuals abuse only a single class of drug (and transition between drugs during their lifetime), and many addictive drugs interact with the endocannabinoid system, so the Center will facilitate research into a broad range of addictive drugs.

Planned outputs

Bioactive Lipid Mediators Core (BLMC) Summer Training Program

Summer training for undergraduate students, particularly those historically under-represented in drug abuse research, with a structured 2-month summer research experience. The BLMC will host this experience and it will integrate with three existing programs at IUB that bring undergraduate underrepresented minority students to the IUB campus for the summer for research experiences.

Up to 10 students who have applied and been selected into the STEM Summer Scholars program (SSI) will be trained in the BMLC. The SSI is a program aimed at providing access to research training for students at minority serving institutions (MSI) as well as underrepresented minorities at Indiana University. The SSI students will participate in an 8-week training program in the BLMC.

Format: Other

Anticipated volume: unspecified

Release timeline: June 30, 2028

Intended repository: Scholarly Database at Indiana University

License for reuse: Creative Commons Attribution Non Commercial No Derivatives 4.0 International

Four Seasons STORM courses teaching ImmunoSTORM and PharmacoSTORM approaches

Single-molecule-based imaging in life sciences, especially in neuroscience is rapidly expanding. In addition to the research mission of the Multi-Scale Imaging Core, the facility has a broad educational mission. The necessary equipment setups for single-molecule localization microscopy (SMLM), the basic know-how on the different approaches (STORM, PALM, PAINT), and the essential data analysis tools are all becoming widely available at imaging core facilities of universities and research institutes throughout the United States.

This 10-day long course will be offered every quarter providing the unique opportunity for the neuroscience community to learn the ImmunoSTORM and PharmacoSTORM workflows from their developers. Each course will have 8 participants with financial support available for 2 under-represented minorities, with applicants selected by a committee of internal and external advisory board members.

Format: Other

Anticipated volume: unspecified

Release timeline: June 30, 2028

Intended repository:

License for reuse: unspecified

Bioactive Lipid Mediators Core Data

Format: Dataset

Anticipated volume: 99 GB

Release timeline: June 30, 2028

Intended repository: DANDI, Scholarly Database at Indiana University

License for reuse: Creative Commons Attribution Non Commercial No Derivatives 4.0 International

Multi-Scale Imaging Core Data

Format: Dataset

Anticipated volume: 899 TB

Release timeline: June 30, 2028

Intended repository: DANDI, Scholarly Database at Indiana University

License for reuse: Creative Commons Attribution Non Commercial No Derivatives 4.0 International

Other works associated with this research project

This product is a service of the University of California Curation Center of the California Digital Library.
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PLAN OVERVIEW

A Data Management Plan created using DMPTool

Title: Indiana University Bloomington (IUB) Center for Cannabis, Cannabinoids, and Addiction (C3A)

Creator: Esen Tuna - **ORCID:**[0000-0001-6585-4195](https://orcid.org/0000-0001-6585-4195)

Affiliation: Indiana University (iu.edu)

DMP ID: <https://doi.org/10.48321/D15028>

Funder: National Institutes of Health (nih.gov)

Funding opportunity number: PAR-20-267

Template: NIH-GEN DMSP (Forthcoming 2023)

Project abstract:

This is a project proposal to establish a P30 Core Center of Excellence, the IU Bloomington Research Center for Cannabis, Cannabinoids and Addiction (C3A), at Indiana University, Bloomington. The goal of the center is to provide access to cutting edge imaging and mass spectrometry techniques to researchers (“Center Affiliates”) interested in drug addiction and related topics.

While most of our Center Affiliates come from across the Midwest (Indiana, Ohio, Kentucky, West Virginia, Illinois, Missouri, Nebraska, and Wisconsin), we also have scientists from as far away as Hawaii, Pennsylvania, New York, Maryland, and Arizona express interest in using our core resources and participating in our courses. These researchers will be joining the C3A as Affiliates. Building on IU Bloomington’s dedicated nucleus of cannabinoid researchers, a major focus of the Center will be on cannabinoids. However, few individuals abuse only a single class of drug (and transition between drugs during their lifetime), and many addictive drugs interact with the endocannabinoid system, so the Center will facilitate research into a broad range of addictive drugs.

Start date: 07-01-2023

End date: 06-30-2028

Last modified: 09-27-2022

INDIANA UNIVERSITY BLOOMINGTON (IUB) CENTER FOR CANNABIS, CANNABINOIDs, AND ADDICTION (C3A)

DATA TYPE

The IUB C3A Cores will generate data from several different instruments with their own specific requirements. The largest volume of data comes mainly from images and video streams acquired from multi-photon, confocal, super resolution (STORM) microscopes. These data need to be efficiently transferred and temporarily stored until they undergo processing for data analysis. The original data needs to be archived regularly and all data will need to be readily accessible to other scientists upon proper authorization.

The Cores are expected to produce about 300 TB of original data per year. The rate of new dataset generation is estimated to be no more than 2 TB per day. The data will be transferred to a spinning disk storage system via Globus or SMB mount point on the local Core's lab systems. Both methods provide encryption in transit. The staging area will be the existing Geode2 storage system within IU's datacenter. Geode2 is a geographically replicated central cyber infrastructure storage system that provide 30 days of data snapshots for data resilience.

Data in Geode2 can be accessed from individual laptops and desktops through IU login. Of relevance for some data (e.g., human plasma samples analyzed in the BLMC), IU's research cyber infrastructure provides for HIPAA alignment.

The data is obtained from multi-photon, confocal, super resolution (STORM) microscopes. This original data will be organized and labeled on Geode2 and transferred to the Scholarly Data Archive (SDA) for preservation and dissemination.

The readout of the STORM and confocal microscopy measurements are digital images. This can include different bit depth images typically 12 or 16 bits. Pixel numbers vary from 256x256 to 2048x2048. STORM microscopy produces 256x256 pixel time lapse images including typically 5000 frames and two channels with around 1.5GB in size while Confocal images file size around 15MB.

The metadata will contain identifiers for its origin, including information for center core, lab, technician, specimen, timestamp, instrument, instrument configuration parameters, and study ID.

The native file format of the Nikon NIS-Elements software used to obtain the original image data is the nd2 file format. nd2 file format can be read by ImageJ which a freely available microscopy software.

RELATED TOOLS, SOFTWARE AND/OR CODE

The following software tools will be used for neuro and behavioral imaging and analysis:

- Signal acquisition: ScanImage, ScanBox, Nikon NIS
- Denosing: [DeepInterpolation](#)
- Cell Segmentation and signal extraction: [CellPose](#), [CalmAn](#), [Sutie2p](#), [EXTRACT](#), [FISSA](#)
- Behavior Analysis: [DeepLabCut](#)
- Online visualizations: [DataJoint SciViz](#), [NWB Widgets](#)

DataJoint Framework will be used for data management, analysis automation, and workflow management ([RRID:SCR_014543](#)). The center will adopt standardized pipeline implementation from DataJoint Elements ([RRID:SCR_012894](#)).

STANDARDS

Our neurophysiology data will be structured and described using the following standards, which have been widely adopted in the neuroscience community:

- DataJoint and DataJoint Elements. The Core will use DataJoint Elements and community-driven standardized workflows for neurophysiology experiments. References [RRID:SCR_014543](#), [RRID:SCR_012894](#).
- NWB and DANDI Archive - raw data and processed data
- [Allen Mouse Common Coordinate Framework \(CCF\)](#)

DATA PRESERVATION, ACCESS, AND ASSOCIATED TIMELINES

The IUScholarWorks and DataCore are institutional repositories provided by Indiana University. Both repositories provide long term access to scientific data and metadata.

The Scholarly Data Share (SDS) is a service provided by Indiana University Research Technologies allowing for long-term storage and access to large (TB scale) research datasets. SDS is built upon disaster-resilient cyber infrastructure of IU replicated in two geographic locations.

DANDI Archive will provide long-term data archival and dissemination to the broad scientific community in a standard data format NWB (Neurodata without Borders).

The Indiana University data repositories (IUScholarWorks, DataCore and Scholarly Data Share) provide metadata and/or persistent identifiers (DOIs), and long-term access. These repositories are supported by Indiana University and datasets are available under Open Access as well as access authorization when data is limited to authorized users.

Data will be available at the end of the project performance period, expected as June 2028.

ACCESS, DISTRIBUTION, OR REUSE CONSIDERATIONS

No HIPAA data is accessed or distributed. Any human samples data used in the project will have no HIPAA identifiers.

No HIPAA data is accessed or distributed. Any human samples data used in the project will have no HIPAA identifiers.

OVERSIGHT OF DATA MANAGEMENT AND SHARING

A data manager (to be hired by the proposed IUB C3A as a full-time Indiana University employee) will be responsible for data collection, management, storage, retention, and dissemination of project data. The data manager is responsible for updating and revising the Data Management and Sharing Plan when necessary.

The data manager will also serve as a data analyst and data workflow developer for data curation, documentation, metadata development and overall data movement and processing in the center.

The project has budgeted \$26,000 for data hosting and sharing costs annually. This includes archival storage and preservation; high performance storage used in processing of the derived data products; redundant and resilient storage for curation of incoming data from instruments.

PLANNED RESEARCH OUTPUTS

SUMMER TRAINING PROGRAM - "BIOACTIVE LIPID MEDIATORS CORE (BLMC) SUMMER TRAINING PROGRAM"

Summer training for undergraduate students, particularly those historically under-represented in drug abuse research, with a structured 2-month summer research experience. The BLMC will host this experience and it will integrate with three existing programs at IUB that bring undergraduate underrepresented minority students to the IUB campus for the summer for research experiences.

Up to 10 students who have applied and been selected into the STEM Summer Scholars program (SSI) will be trained in the BLMC. The SSI is a program aimed at providing access to research training for students at minority serving institutions (MSI) as well as underrepresented minorities at Indiana University. The SSI students will participate in an 8-week training program in the BLMC.

COURSES - "FOUR SEASONS STORM COURSES TEACHING IMMUNOSTORM AND PHARMACOSTORM APPROACHES"

Single-molecule-based imaging in life sciences, especially in neuroscience is rapidly expanding. In addition to the research mission of the Multi-Scale Imaging Core, the facility has a broad educational mission. The necessary equipment setups for single-molecule localization microscopy (SMLM), the basic know-how on the different approaches (STORM, PALM, PAINT), and the essential data analysis tools are all becoming widely available at imaging core facilities of universities and research institutes throughout the United States.

This 10-day long course will be offered every quarter providing the unique opportunity for the neuroscience community to learn the ImmunoSTORM and PharmacoSTORM workflows from their developers. Each course will have 8 participants with financial support available for 2 under-represented minorities, with applicants selected by a committee of internal and external advisory board members.

DATASET - "BIOACTIVE LIPID MEDIATORS CORE DATA"

DATASET - "MULTI-SCALE IMAGING CORE DATA"

PLANNED RESEARCH OUTPUT DETAILS

Research Outputs do not contain sensitive data.

Research Outputs do not contain PII.

Data will be made with “Creative Commons Attribution Non Commercial No Derivatives 4.0 International” license.

Title	Type	Anticipated release date	Initial access level	Intended repository(ies)	Anticipated file size
Bioactive Lipid Mediators Core (BLMC) Summer Training Program	Summer Training Program	2028-06-30	Open	Scholarly Database at Indiana University	
Four Seasons STORM courses teaching ImmunoSTORM	Courses	2028-06-30	Open	Scholarly Database at Indiana University	
Bioactive Lipid Mediators Core Data	Dataset	2028-06-30	Restricted	Scholarly Database at Indiana University DANDI	100 GB
Multi-Scale Imaging Core Data	Dataset	2028-06-30	Restricted	Scholarly Database at Indiana University DANDI	900 TB

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Validation of antibodies:

We have more than thirty-five years-experience in applying antibodies against components of the endocannabinoid and other neurotransmitter systems. Many of the antibodies widely used in the cannabinoid field were generated and validated in the Mackie lab (the PI for administrative core). Antibodies against protein epitopes are generally validated in the following way in our laboratories:

1. If KO is available, then this is our preferred test, using the same application (e.g., if the antibody is to be used for Western blotting, then it is tested for Western blotting using KO tissue, if for ICC, then it is tested in ICC using the same conditions (e.g., fixation and detection)) that will be used. Occasionally we will use shRNA knockdown of protein expression in cultures as an alternative approach.
2. If a KO is not available, then we will use two antibodies directed against different epitopes. A similar staining or detection pattern is taken as strong evidence that the antibodies recognizing the same protein.
3. All antibodies from Mackie Lab will be tested by detection of epitope-tagged protein expressed in HEK or similar cells (overlap of epitope tag and antibody being tested is expected) and block by immunizing protein.
4. When using GFP antibodies, native GFP fluorescence is compared to signal (e.g., fluorescence) from GFP antibody staining of adjacent sections, to ensure qualitative similarity.
5. We check to make sure commercially purchased antibodies have undergone rigorous screening and have an RRID.

Validation of fluorescent small molecules for PharmacoSTORM imaging:

Fluorescent small molecules, the pharmacoprobes will be performed in two independent manners:

1. Using brain slices from knockout mice of the target protein. These slices will be prepared, incubated, imaged and analyzed in parallel with brain slices obtained from wild-type animals.
2. Using ex vivo and in vivo displacement assays. Pretreatment of live mice or acute brain slices with excess concentration of the unlabeled pharmacoprobes readily displaces the subsequent binding of the fluorescent pharmacoprobe.

Validation of transgenic animals used in these experiments:

For tissue-specific Cre transgenic lines, we will cross them to reporter mouse lines such as the Ai9 (tdTomato) (JAX mice Stock No:007909) or mGFP lines (JAX mice Stock No:004077). The distribution of tdTomato or GFP positive cells in the progeny carrying one copy of Cre and one copy of reporter allele will be examined with multiple staining to confirm the brain regions and cell types in which recombination occurs. We have these mouse lines in the IUB vivarium.

Validation of recombinant adeno-associated viral vectors (rAAVs):

We have more than ten years of experience in injecting AAVs into live mice to label or manipulate specific neuronal populations. All of the rAAVs that will be used in this project have been documented with extensive literature and will be acquired from a reputable commercial source such as Addgene. The expression of target genes as well as viral transduction efficacy will be evaluated with immunostaining.

Here are a list of potential rAAVs that we will use in MSIC:

From Addgene (this company has internal quality control procedures including lot numbers and titer measurements): pAAV.Syn.GCaMP6f.WPRE.SV40 (#100837-AAV1), pENN-AAV.hSyn.Cre.WPRE.hGH (#105553-AAV9), pAAV.CAG.Flex.NES-jRGECO1a.WPRE.SV40 (#100852-AAV1), pAAV.CAG.Flex.GCaMP6f.WPRE.SV40 (100835-AAV9) and pAAV.Syn.NES-jRGECO1a.WPRE.SV40 (100854-AAV9)

Validation of novel chemical compounds:

No novel compounds will be used in these studies. All chemicals used will be obtained from commercial companies and accompanied by a certificate of analysis.

**APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)**

5. APPLICANT INFORMATION**UEI***: YH86RTW2YVJ4

Legal Name*: TRUSTEES OF INDIANA UNIVERSITY
 Department:
 Division:
 Street1*: 509 E 3RD ST
 Street2:
 City*: BLOOMINGTON
 County: MONROE
 State*: IN: Indiana
 Province:
 Country*: USA: UNITED STATES
 ZIP / Postal Code*: 474013654

Person to be contacted on matters involving this application

Prefix: First Name*: Middle Name*: Last Name*: Suffix:
 Mr. STEVEN ALLEN MARTIN

Position/Title: ASSOCIATE VP FOR RESEARCH ADMINISTRATION

Street1*: 509 E 3RD ST

Street2:

City*: BLOOMINGTON

County: MONROE

State*: IN: Indiana

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 474013654

Phone Number*: 317-278-3473

Fax Number:

Email: IUAWARD@IU.EDU

7. TYPE OF APPLICANT*

H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type Women Owned Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*

IUB C3A Administrative Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2023	06/30/2028

Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: TRUSTEES OF INDIANA UNIVERSITY

UEI: YH86RTW2YVJ4

Street1*: 702 N WALNUT GROVE AVE

Street2:

City*: BLOOMINGTON

County: MONROE

State*: IN: Indiana

Province:

Country*: USA: UNITED STATES

Zip / Postal Code*: 474052201

Project/Performance Site Congressional District*: IN-009

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* Yes No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes No

If YES, check appropriate exemption number: — 1 — 2 — 3 — 4 — 5 — 6 — 7 — 8

If NO, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* Yes No**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed?

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* Yes No

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* 2022_Abstract_C3A_administrative_core_20220924.pdf**8. Project Narrative*****9. Bibliography & References Cited** 2022_Admin_Core_References_20220924.pdf**10. Facilities & Other Resources** Facilities_OtherResources.pdf**11. Equipment** Equipment.pdf

The Administrative Core will provide oversight and a cohesive framework to maximize the efficiency and impact of the **IU Bloomington Center for Cannabis, Cannabinoids and Addiction (IUB C3A)**. It will be led by Dr. Ken Mackie, a Gill Chair and Distinguished Professor in the Department of Psychological and Brain Sciences at IU Bloomington. Dr. Mackie has more than 30 years of experience in drug abuse research at the University of Washington and Indiana University Bloomington (IUB). During this time his research has been continuously funded by NIDA, NINDS and/or NCCIH, including serving as a project PI for two different NIDA P01's. He has served in several demanding administrative research positions at IUB, including directing the Gill Center for nine years where he oversaw its substantial growth by recruiting four new endowed chairs to IUB and the Gill Center. He and the other four C3A PIs have had longstanding and productive collaborations (and Dr. Mackie played a major role in their recruitments to IUB) and has published more than 60 papers with them over the last twenty years. All these factors suggest that the Administrative Core will function efficiently to achieve the overall goals of the C3A.

The Administrative Core is efficiently structured to accomplish the following specific aims:

- 1. Provide the administrative framework to ensure that the goals of the IUB C3A are met.** In addition to Dr. Mackie, this framework includes the core PIs and key technical staff (together forming the Steering Committee, responsible for governance and day-to-day Center operations), an internal advisory board (IAB), and an external advisory board (EAB). The advisory boards will provide strategic advice to the PIs, help review pilot projects, evaluate Center impact, and offer suggestions for improvement.
- 2. Facilitate communications within the Center, with the greater Drug Abuse community, and the public.** This will be done by organizing monthly center meetings, hosting external speakers, and managing the Center's internet and social media presence. An important activity of the Administrative Core will be archiving and facilitating access to the data sets and other resources generated by the Center.
- 3. Organize the courses offered by the Centers.** The Administrative Core will be responsible for the administration of the two courses that will be offered by the Center. This will be done by publicizing the courses, fielding questions about them and their content, handling registrations, providing advice on travel and lodging, disbursing scholarships for appropriate students, liaising with IUB on integration of the summer BLMC course with existing IUB URM-oriented summer courses, and collecting post-course evaluations and follow up.
- 4. Provide administrative support to improve the Core's efficiency.** Essential administrative activities that are more efficient when centralized (e.g., ordering, fiscal management, compliance, animal management, etc.) will be provided by the Administrative Core.

FACILITIES AND OTHER RESOURCES

Please see the Overall Component for Facilities and Other Resources

EQUIPMENT

Please see the Overall Component for Equipment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Kenneth	Middle Name P.	Last Name*: Mackie	Suffix: M.D.
Position/Title*:	Professor			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 N WALNUT GROVE AVE			
Street2:				
City*:	BLOOMINGTON			
County:	MONROE			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	812-855-2042		Fax Number:	
E-Mail*:	kmackie@indiana.edu			
Credential, e.g., agency login:	KMACKIE			
Project Role*:	Other (Specify)		Other Project Role Category: Center Director	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	KENNETH	P	MACKIE		M.D. PD/PI	305,412.00		1.6	0.8	0.00	0.00	0.00
2.	Norbert		Hajos		PD/PI (Pilot Project Core)	152,775.00		1.2		19,861.00	7,932.00	27,793.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	27,793.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	DATA ANALYST	12.0			86,000.00	34,348.00	120,348.00
1	ADMINISTRATOR	12.0			45,000.00	17,973.00	62,973.00
1	TECHNICIAN	12.0			4,233.00	1,691.00	5,924.00
3	Total Number Other Personnel					Total Other Personnel	189,245.00
						Total Salary, Wages and Fringe Benefits (A+B)	217,038.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

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

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

E. Participant/Trainee Support Costs		Funds Requested (\$)*
1. Tuition/Fees/Health Insurance		
2. Stipends		36,000.00
3. Travel		5,500.00
4. Subsistence		
5. Other:		
Number of Participants/Trainees	Total Participant Trainee Support Costs	41,500.00

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	26,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care Costs	30,000.00
	Total Other Direct Costs
	61,000.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	319,538.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	278,038.00	162,652.00
				Total Indirect Costs
				162,652.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	482,190.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	482,190.00

L. Budget Justification*	File Name: Administrative_core_budget_justification_20220907.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	KENNETH	P	MACKIE		M.D. PD/PI	305,412.00		1.6	0.8	0.00	0.00	0.00
2.	Norbert		Hajos		PD/PI (Pilot Project Core)	152,775.00		1.2		20,258.00	8,091.00	28,349.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	28,349.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	DATA MANAGER	12.0			87,720.00	35,035.00	122,755.00
1	ADMINISTRATOR	12.0			45,900.00	18,332.00	64,232.00
1	TECHNICIAN	12.0			4,318.00	1,725.00	6,043.00
3	Total Number Other Personnel				Total Other Personnel		193,030.00
					Total Salary, Wages and Fringe Benefits (A+B)		221,379.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

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

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

E. Participant/Trainee Support Costs		Funds Requested (\$)*
1. Tuition/Fees/Health Insurance		
2. Stipends		36,000.00
3. Travel		5,500.00
4. Subsistence		
5. Other:		
Number of Participants/Trainees	Total Participant Trainee Support Costs	41,500.00

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	26,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care Costs	30,000.00
Total Other Direct Costs	61,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	323,879.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	282,379.00	165,192.00
				Total Indirect Costs
				165,192.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	489,071.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	489,071.00

L. Budget Justification*	File Name:
	Administrative_core_budget_justification_20220907.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	KENNETH	P	MACKIE		M.D. PD/PI	305,412.00		1.6	0.8	0.00	0.00	0.00
2.	Norbert		Hajos		PD/PI (Pilot Project Core)	152,775.00		1.2		20,663.00	8,253.00	28,916.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	28,916.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	DATA ANALYST	12.0			89,474.00	35,736.00	125,210.00
1	ADMINISTRATOR	12.0			46,818.00	18,699.00	65,517.00
1	TECHNICIAN	12.0			4,245.00	1,695.00	5,940.00
3	Total Number Other Personnel				Total Other Personnel		196,667.00
					Total Salary, Wages and Fringe Benefits (A+B)		225,583.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

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

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

E. Participant/Trainee Support Costs		Funds Requested (\$)*
1. Tuition/Fees/Health Insurance		
2. Stipends		36,000.00
3. Travel		5,500.00
4. Subsistence		
5. Other:		
Number of Participants/Trainees	Total Participant Trainee Support Costs	41,500.00

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	26,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care Costs	30,000.00
	Total Other Direct Costs
	61,000.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	328,083.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	286,583.00	167,651.00
				Total Indirect Costs
				167,651.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	495,734.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	495,734.00

L. Budget Justification*	File Name: Administrative_core_budget_justification_20220907.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2025**End Date*:** 06-30-2026**Budget Period:** 4**A. Senior/Key Person**

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	KENNETH	P	MACKIE		M.D. PD/PI	305,412.00		1.6	0.8	0.00	0.00	0.00
2.	Norbert		Hajos		PD/PI (Pilot Project Core)	152,775.00		1.2		21,076.00	8,418.00	29,494.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	29,494.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	DATA ANALYST	12.0			91,264.00	36,451.00	127,715.00
1	ADMINISTRATOR	12.0			47,754.00	19,073.00	66,827.00
1	TECHNICIAN	12.0			4,330.00	1,729.00	6,059.00
3	Total Number Other Personnel				Total Other Personnel		200,601.00
					Total Salary, Wages and Fringe Benefits (A+B)		230,095.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 4

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

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

E. Participant/Trainee Support Costs		Funds Requested (\$)*
1. Tuition/Fees/Health Insurance		
2. Stipends		36,000.00
3. Travel		5,500.00
4. Subsistence		
5. Other:		
Number of Participants/Trainees	Total Participant Trainee Support Costs	41,500.00

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 4

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	26,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care Costs	30,000.00
Total Other Direct Costs	61,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	332,595.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	291,095.00	170,291.00
				Total Indirect Costs
				170,291.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	502,886.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	502,886.00

L. Budget Justification*	File Name:
	Administrative_core_budget_justification_20220907.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2027**End Date*:** 06-30-2028**Budget Period:** 5**A. Senior/Key Person**

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	KENNETH	P	MACKIE		M.D. PD/PI	305,412.00		1.6	0.8	0.00	0.00	0.00
2.	Norbert		Hajos		PD/PI (Pilot Project Core)	152,775.00		1.2		21,498.00	8,586.00	30,084.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	30,084.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	DATA ANALYST	12.0			93,089.00	37,180.00	130,269.00
1	ADMINISTRATOR	12.0			48,709.00	19,454.00	68,163.00
1	TECHNICIAN	12.0			4,416.00	1,764.00	6,180.00
3	Total Number Other Personnel				Total Other Personnel	204,612.00	
					Total Salary, Wages and Fringe Benefits (A+B)	234,696.00	

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

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

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

E. Participant/Trainee Support Costs		Funds Requested (\$)*
1. Tuition/Fees/Health Insurance		
2. Stipends		36,000.00
3. Travel		5,500.00
4. Subsistence		
5. Other:		
Number of Participants/Trainees	Total Participant Trainee Support Costs	41,500.00

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	26,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care Costs	30,000.00
Total Other Direct Costs	61,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	337,196.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	295,696.00	172,982.00
				Total Indirect Costs
				172,982.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	510,178.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	510,178.00

L. Budget Justification*	File Name:
	Administrative_core_budget_justification_20220907.pdf

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

Budget Justification: Administrative Core

Personnel

Ken Mackie, MD. 1.6 academic months, 0.8 summer months. Gill Chair and Distinguished Professor, Psychological and Brain Sciences, Indiana University, Bloomington. As director of the Gill Center from 2008 to 2017, Ken amassed extensive experience in administration of a research organization. The Gill Center has six endowed chairs working in various aspects of neuroscience or endocrinology. The Gill Center is highly productive, with six Gill Chairs, a dozen research faculty, a dozen post docs, and a dozen graduate students. Its annual externally funding is ~\$2.5 million. In addition, Ken has recruited five endowed chairs in neuroscience/drug abuse to IU Bloomington, 4 of them as Gill Chairs. The fifth, Istvan Katona is tightly affiliated to the Gill Center (as a Naus chair in drug abuse research) with his office and lab adjacent to the offices and labs of the other P30 PIs. Four of the Gill Chairs (Hajos, Hohmann, Lu and Mackie) are associated with this P30 application. In addition, Ken has collaborated extensively over many years with the directors of the two proposed cores: Heather Bradshaw since 2008 (27 papers), Istvan Katona since 1999 (21 papers), and Hui-Chen Lu since 2008 (9 papers). This highlights the excellent working relationship he has with each of the core directors. Ken will devote 1.6 months of effort during the academic year and 0.8 months during the summer. As PI of the administrative core, Dr. Mackie's salary is covered by IU institutional funds, thus no salary is requested.

Norbert Hájos, PhD. Gill Chair and Professor, Psychological and Brain Sciences Indiana University, Bloomington. As PI of the pilot project core, Dr. Hájos will oversee requests for pilot proposals and the pilot review process, and he will also coordinate assignment of trainers and mentors for pilot awardees. He will devote 1.2 academic months to the Pilot Core. Professor Hájos has extensive mentoring experience, has an expansive view of science, and is thus well-suited for this task. He has a considerable experience in organizing review panels as he was charged with developing the review criteria for the Bolyai Scholarship of the Hungarian Academy of Science and for the Neuroscience panel of the Hungarian Scientific Research Fund, which he chaired for 6 years. This experience positions him well to mentor pilot applicants and awardees who are entering the drug abuse field. Dr. Hájos will devote 1.2 academic months to Pilot Core activities supported by the Administrative Core administrative assistant and 1.2 academic months of salary support is requested.

Administrative assistant, TBD. 12 calendar months. A program manager will be hired to oversee key Center-related activities: Major activities will include: Coordination of courses and related activities, scheduling of seminar speakers, their travel, and lodging, arrange for monthly Center meetings, preparation of materials for reports and for the external advisors, handling of animal importation, assessment of equipment utilization and coordination of equipment servicing, point of contact for general C3A questions, general website upkeep, maintaining the C3A Twitter feed, etc.

Data management staff, TBD. 12 calendar months. A Senior System Analyst/Programmer will be recruited from the Research Data Services (RDS) team of IU Research Technologies and will be responsible for building, maintaining, and supporting the described data workflows. The RDS staff includes highly qualified research software engineers who can perform the data management task.

Patricia Franco, PhD. 1 calendar month. Patricia will be responsible for handling compliance issues (IACUC and IBC) related to activities that extend across the cores and that come up with pilot projects. Prior to joining IU, Dr. Franco received her PhD in veterinary medicine in Brazil and has extensive experience with a variety of animal surgeries and animal care. She was involved in large-scale veterinary clinical studies before joining the Lu laboratory. Through her professional experiences, Patricia is highly organized and skilled in communicating and coordinating with large teams.

Fringe benefits:

Fringe benefit rates are set by Indiana University and approved by the Board of Trustees. For the administrative assistant the fringe rate is 39.94% of the requested salary. For the data manager, the fringe rate is 39.94% of the requested salary.

Other expenses:

Seminars: We anticipate hosting six seminars/year bringing in speakers working in research areas relevant to the cores. The average cost per speaker (transportation, lodging and modest honorarium) is estimated to be \$1,000. With six speakers this will be \$6,000.

Animals: Mice and rats will be used by both cores. Ordering, housing, importation, and breeding of therodonts will be coordinated by the administrative core to increase efficiency. Between the BLMC and MSIC we anticipate having of average of approximately 10 rat cages and 50 mouse cages at any one time. At the projected IU Bloomington per diem rates, these will cost \$19,000 annually. In addition, we anticipate spending \$11,000 annually for rodent purchase, quarantine and health screening of mice imported for MSCI two imaging, animal freight charges, etc. The total annual estimated cost for animal-related expenses is \$30,000.

Courses:

MS summer course: We will partner with the existing IU summer courses that bring URM students interested in STEM careers to IUB for the summer. See the Administrative Core for more details on these programs and the MS course. We will select up to 10 students to participate in the MS course. Their living expenses are provided through the IUB parent program and the P30 will provide their summer stipend (\$4,000/student). We have a commitment from the Gill Center (see Gill Center Director Dr. Hui-Chen Lu's letter) to support five of these students, so the total cost to the grant will be \$4,000 x 4 students = \$16,000.

Pharmacostorm course: This course (more details in the administrative core) will be offered free of charge to qualified scientists (selected by Drs. Barna and Katona) through a competitive application process. Two scholarships of ~\$2,500 (more if we have the budget) for each course session will be set aside for investigators from disadvantaged backgrounds, etc., who would be unable to attend without financial support. Thus, with 2 students x 4 courses/year x \$2,500 support = \$20,000.

Page charges/publications: These are requested to cover publications that come from summer intern research, methods development and otherwise innovative research carried out by Center staff that is not supported by external grants. An example would be a concise summer project completed by a summer intern in Heather Bradshaw's MS core or a methods development paper by Dr. Barna in the Imaging core. \$5,000

Scholarly Data Archive (SDA) is the long term tape storage of Indiana University to archive and preserve institutional data. The data generated by the imaging and mass spectrometry cores will be stored in the SDA. The cost of SDA storage is \$20/TB/year, with an average estimated annual need of 900 TB, \$18,000.

Slate is the IU storage to support workflows for the high-performance computer cluster. The annual cost of Slate storage is \$60/TB. We estimate needing an average of 100 TB/year of Slate storage, \$6,000.

Geode2 serves as central storage space for IU research computing users. It is accessible from lab computers and is used for active project storage of data before analysis on the high-performance computing cluster. The annual cost of Geode2 storage is \$200/TB and we estimate using about 10 TB/year, \$2,000.

Indirect Costs:

The indirect cost rate for Indiana University is set by DHHS and was negotiated on 5/22/2019 at 58.5% for research, excluding capital equipment, participant support costs, subawards greater than \$25,000, and graduate student fee remissions.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	144,636.00
Section B, Other Personnel	984,155.00
Total Number Other Personnel	15
Total Salary, Wages and Fringe Benefits (A+B)	1,128,791.00
Section C, Equipment	0.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	207,500.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	180,000.00
3. Travel	27,500.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	305,000.00
1. Materials and Supplies	0.00
2. Publication Costs	25,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	130,000.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other 1	150,000.00
9. Other 2	0.00
10. Other 3	0.00
11. Other 4	0.00
12. Other 5	0.00
13. Other 6	0.00
14. Other 7	0.00
15. Other 8	0.00
16. Other 9	0.00
17. Other 10	0.00
Section G, Direct Costs (A thru F)	1,641,291.00
Section H, Indirect Costs	838,768.00

Section I, Total Direct and Indirect Costs (G + H)	2,480,059.00
Section J, Fee	0.00
Section K, Total Direct and Fee (I + J)	2,480,059.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 09/30/2024

1. Vertebrate Animals Section

Are vertebrate animals euthanized? Yes No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

Yes No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

Yes No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount (\$) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? Yes No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? Yes No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: Yes No

If the answer is "Yes" then please answer the following:

*Previously Reported: Yes No

6. Change of Investigator/Change of Institution Section

Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	2022_Introduction__Administrative_Core_20220924.pdf
Research Plan Section	
2. Specific Aims	2022_Admin_Core_SA_20220924.pdf
3. Research Strategy*	2022_Admin_Core_Research_Strategy_20220924.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	VAS_Admin_core_final.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	LettersOfSupport.pdf
10. Resource Sharing Plan(s)	Resource_DataSharing.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication.pdf
Appendix	
12. Appendix	

Introduction – Administrative Core

We thank the reviewers for their careful reading of our original proposal, thoughtful comments, and enthusiasm for our goals for the C3A. For this Introduction, we will address concerns specific for the Administrative Core.

1. The description of the organizational structure and roles is minimalistic. We have expanded the description and roles of the organization structure as much as possible within the page limits. Basically, the C3A steering committee is the center of the organizational structure. It receives advice from the internal and external advisory boards, it will weigh the needs of the Affiliates, the greater substance use community and its resources when making decisions, and its directives are implemented by the appropriate cores.

2. Inclusion of diversity in the Internal Advisory Board would enrich the success of DEI efforts in the P30. Improving diversity in substance use disorder research is a key goal of the proposal. One example is the summer BLMC specifically targets populations historically under involved in substance abuse research and its integration into existing IUB URM summer programs. We reassessed the membership of our Internal Advisory Board (IAB), which has increased its diversity (see Overall Core for additional details). Briefly, the reconstituted IAB now includes three (of six) women. The research and administrative expertise represented on the board are similarly diverse. On the research side, our IAB members include expertise in mass spectrometry (Clemmer), large network behavior (Borner), Imaging (Shaw), Social determinants of drug use, especially in African American Communities (Riley), potential therapeutic benefits of cannabinoids and endocannabinoids (Hohmann), and organometallics and nanomaterials (Zaleski). On the administrative/applied side our IAB includes co-PI of our NIDA T32 (Hohmann), former vice provost for research (Zaleski), director of the integrated freshman learning experience (Shaw), clinical psychology (Riley), and science communication/visualization (Borner), and IUB former associate dean for Natural and Mathematical Sciences (Clemmer). We feel that the rich mix of personal experiences and perspectives on science and addiction will help us achieve our DEI efforts.

3. While outreach to the P30 community of scientists and mentees is a super and achievable goal, a missed opportunity is to conduct outreach to the regional communities so drastically impacted by the opioid crisis in the Midwest. This is a great venue for mentees to expand their own teaching and mentor expertise. This comment and suggestion strongly resonant with the C3A PIs. Situated in the Midwest, opioid and misuse of other drugs is a major social problem. Our affiliates and mentees are in an excellent position to effectively reach out to these affected populations. Many of our Midwest affiliates are already heavily involved in outreach activities in their towns and cities. These prior experiences are now specifically mentioned in several letters of support. In addition, part of the training of all mentees will be in outreach activities. This training will be tailored to integrate with the specific mentee's community, expertise, and prior experience.

4. Specific role of the director in supervision and mentoring of pilot project applications and class attendees is not very clear. In the revision, we have revised the structure of the Pilot Project Core and recruited a new PI for that core, Gill Professor, Norbert Hájos. Dr. Hájos will provide direct supervision of pilot project mentoring. Each mentee will be paired with the core PI or scientist (this is a planned activity for these individuals and does not require uncompensated time) that best aligns with the mentee's interests and needs. Dr. Hájos will monitor the mentoring process and ensure that it proceeds in a fashion that is most beneficial for the mentee and will intervene (e.g., by assigning a new mentor) if he feels that the mentoring is ineffective or if the mentee or mentor raises a concern.

5. Computing and data management/storage support is not very specifically tailored for these datasets and they're widely varying, project-specific needs. In the revision, we have rewritten and expanded the discussion of data handling in the Administrative Core section and coordination of these activities. Additional details have been added to the relevant sections of each core. To help to accomplish efficient and rigorous data management we have increased the effort of the data scientist to 1.0 FTE. The central principles influencing our data management plan are to make the raw data easily accessible in an intelligible form to qualified scientists (primarily an Administrative Core function) and to streamline and standardize (this is to increase rigor and reproducibility of the C3A's results) data processing done by each core (core-specific due to the very different nature of the data generated by each core). An example of the latter will be to standardize treatment and extraction regimens for tissues that will be sent to the BLMC for analysis so results can be compared across experiments that may be done years apart. We have included a data management and sharing plan in the proposal's appendix. This plan follows the NIH Policy for Data Management (NOT-OD-21-013).

Specific Aims Administrative Core

The Administrative Core provides a cohesive framework to maximize the efficiency, impact, and oversight of the *IU Bloomington Center for Cannabis, Cannabinoids and Addiction (IUB C3A)*. It will be led by Dr. Ken Mackie, a Linda and Jack Gill endowed Chair and Distinguished Professor in the Department of Psychological and Brain Sciences at IU Bloomington. Dr. Mackie has more than 30 years of experience in drug abuse research and his research has been funded continuously by NIDA and/or NINDS, including as a project PI for two different P01's over the past thirty years. Dr. Mackie has amassed considerable administrative experience at IUB and the University of Washington. Particularly, during his nine years as Director of the Gill Center at IUB, he was primarily responsible for the Gill Center growing from two to five endowed Chairs employing more than 40 FTE (tenure track faculty, non-tenure track faculty, post-docs, graduate students, and technicians) researchers with annual external funding of ~\$3,500,00. He was instrumental in developing the Gill Symposium into the largest annual Neuroscience event in Indiana; this symposium attracts >300 attendees to the IUB campus each fall and involves significant scientific and community outreach. In addition, he has extensive organizational and reviewing experience in the field of drug abuse, including initiating (with Nephi Stella) the highly successful Gordon Research Conference on Cannabinoids (now in its tenth year) and serving on multiple drug abuse-oriented review panels, including NIDA Council. Scientifically, he has established many productive collaborations with scientists across the world and published numerous papers with all C3A core PIs and many C3A affiliates. The Administrative Core will ensure the success of the IUB C3A by executing the following specific aims:

Aim 1. Provide the administrative framework for meeting the goals of the IUB C3A. In addition to Dr. Mackie, the central components of the Administrative Center include the core PIs and key technical staff (together forming the Steering Committee, responsible for conduct of daily Center operations), the C3A Program Manager, an internal advisory board (IAB), and an external advisory board (EAB). The advisory boards will provide strategic advice and goal setting to the Center, assist in the review of pilot project applications, and continuously evaluate the Center's impact on drug addiction research and adjust approaches as necessary.

Aim 2. Facilitate communications within the Center, with the greater Drug Abuse community, and the public. Key to an efficient and productive Center are clear lines of communications both up and down the various levels of hierarchy as well as with the scientific community it serves and the public. Clear communications between the Cores and Affiliates will be ensured through monthly center meetings (including the steering committee, researchers using the C3A Cores, and IAB members) and hosting bimonthly external speakers (particularly those that will bring new technologies into the C3A or enhance existing C3A technologies). Communication with the scientific community will be through archiving and making *readily* available the large data sets and other resources generated by the Center. The data management component will be executed by a full time Senior System Analyst/Programmer from the Research Data Services (RDS) team of IU Research Technologies (see letter of support). Finally, communications with the public will be via the Administrative Core-maintained web and social media presence.

Aim 3. Organize and provide logistical support for the courses offered by the Cores. Central to the mission of the C3A is education and training in the technologies used in the Center. This education and training takes two forms. The first is engagement of undergraduate students, particularly those historically under-represented in drug abuse research, in a structured summer research experience. The second is a "hands on" course in STORM microscopy geared towards investigators interested in learning this technique and applying it to their research questions. The Administrative Center will administer both courses. Key aspects of this administration include publicizing the courses, fielding questions about the courses and their content, handling registration, disbursing scholarships to the appropriate students, and conducting follow up assessments of trainee learning and professional development following completion of a course.

Aim 4. Provide key administrative support to improve the Core's efficiency. Essential administrative activities that are more efficiently conducted in a centralized fashion (e.g., ordering, fiscal management, compliance, animal acquisition and allocation, etc.) will be managed by the Administrative Core. The Program Manager, supervised by Dr. Mackie, will carry out/oversse these activities. The goal of this centralization is to allow the Core PIs and technical staff to focus on their Science and not on administrative tasks.

The Administrative Core's Program Manager will be housed in the MSBII building on the IUB campus, adjacent to the IUB C3A labs and the offices of all five C3A PIs. This physical configuration will enhance efficiency and communication between the Administrative Core and the other three C3A Cores.

Research Strategy Administrative Core

A. Significance

The mission of the IU Bloomington Center for Cannabis, Cannabinoids, and Addiction (C3A) is to connect investigators studying high impact problems in drug addiction research with the imaging and mass spectrometric technology that will assist them in efficiently and creatively addressing these problems. When we surveyed the addiction research community during the preparation of this application, we were gratified by the number of researchers, at all career stages and from a range of institutions, who enthusiastically stated the many ways in which the services and educational resources offered by the C3A will greatly advance their research.

The goal of the Administrative Center is to ensure that this promise of creating a high impact Center is met. The Administrative Core will achieve this goal by organizing the C3A's administrative structure, facilitating communications across multiple levels and constituencies, including data, ensure the smooth running of the courses organized by the BLMC and MCIS, and provide centralized administrative activities.

B. Innovation

Innovative aspects of the Administrative Center are many and include: 1) Having the Administrative Core oversee courses, rather than oversight by individual research service cores, thus allowing core scientists to concentrate on their research and "hands on" teaching and ensure training quality with clear standards. 2) Instituting a robust data management plan to ensure data is efficiently handled and analyzed by C3A scientists and readily available to qualified scientists external to C3A Center. 3) Centralizing ordering, animal housing, and compliance to decrease administrative burdens that would otherwise fall on the core scientists.

C. Approach

Aim 1. Provide the administrative framework to ensure that the goals of the IUB C3A are met.

The administrative framework for the C3A Center consists of the Steering Committee, an Internal Advisory Board (IAB), and an External Advisory Board (EAB). The Steering Committee includes the overall PI (Mackie), core PIs (Bradshaw, Katona, and Lu), and key technical staff (Barna, Huang, and Johnson). The Steering Committee will be responsible for conduct of day-to-day Center operations and making tactical decisions involving Center operations. Their intentions will be implemented by the three cores, a process that will be efficient as there is substantial overlap between the members of the Steering Committee and the individual cores. The IAB (Borner, Clemmer, Hohmann, Shaw and Zaleski (chair)) was chosen to leverage the broad range of local scientific and IU Bloomington-specific administrative knowledge. The IAB will advise the PIs and Steering Committee on all issues, but particularly those that are relevant to IUB. The EAB (Bruchas, McCarthy, Patel, Soltesz, and Wolf) was chosen to include international experts in the drug abuse field, individuals with substantial national level service, and those involved in other NIDA P30s, either as core directors or advisory boards. The EAB will provide strategic advice to the Center, review pilot project applications, and evaluate Center impact. The reporting structure and lines of

Table 1.
C3A Communications
• Monthly center meeting (research focused)
• Bimonthly external speakers
• Data Management
• Website and Twitter

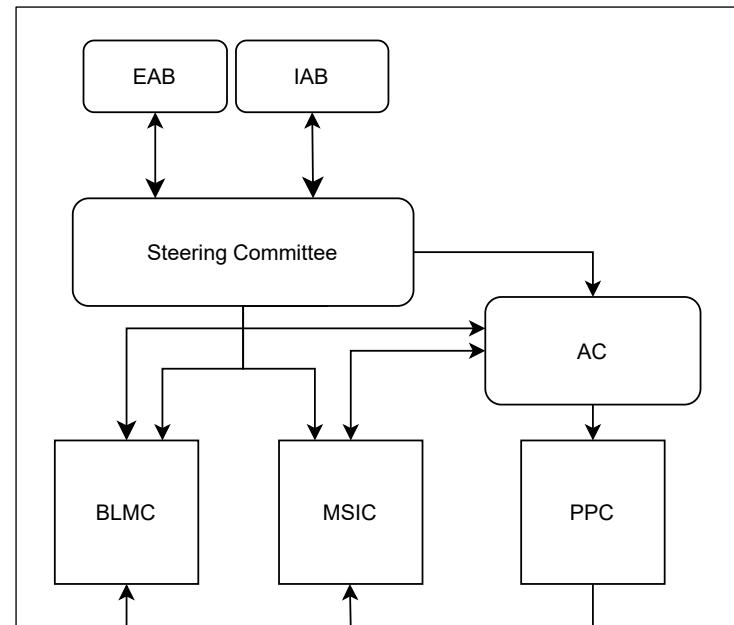


Figure 1. C3A Organizational Structure highlighting primary interactions. The Steering Committee serves as the hub of the C3A administrative organization and consists of the four PIs and the three core technical directors (Barna, Huang, and Johnson). It is advised by the five-member external advisory board (EAB) and the six-member internal advisory board (IAB). It communicates with the administrative core (AC) and the two service cores, the Bioactive Lipid Mediators Core (BLMC) and the MultiScale Imaging Core (MSIC). The Pilot Project Core (PPC) is overseen by the Administrative Core and communicates with the two Service Cores.

communication within the C3A are shown in **Fig. 1**.

The major function of the administrative core is to facilitate communication and coordination with the three other cores to facilitate their function. (See Administrative Core, **Specific Aim 2** for details).

Aim 2. Facilitate communications within the Center, with the greater Drug Abuse community, and the public. Key to an efficiently functioning Center are clear lines of communications both up and down hierarchical levels (**Fig 1**). Communications among users of the Center will be enhanced through monthly center research meetings (distinct from the Steering Committee meetings, but including the Steering Committee, researchers using the C3A Cores, and IAB members) and hosting external speakers (particularly those that will bring new technologies into the Center or enhance existing technologies). The Administrative Core will enhance communications with scientists by archiving and making readily available the large data sets and other resources generated by the Center. To share the science being done in C3A Cores with the public, the Administrative Core will regularly update the Center's internet and social media presence. The efficient management of the large amounts of data generated by the C3A is a major function of the Administrative Core and is described in the following section.

Data Management

The IU Bloomington C3A Cores will generate data from several different instruments each with their own specific attributes. The largest volume of data comes from the images and video streams acquired from multi-photon, confocal, super resolution (STORM) microscopy. These data need to be efficiently transferred and temporarily stored until they undergo processing for data analysis. The original data needs to be archived regularly and all data will need to be readily accessible to other scientists upon proper authorization.

The data management at the IUB Bloomington C3A will follow the general research data life cycle depicted below. The work stated in this section describes the data management activities for collection, processing, analysis, sharing and preservation.

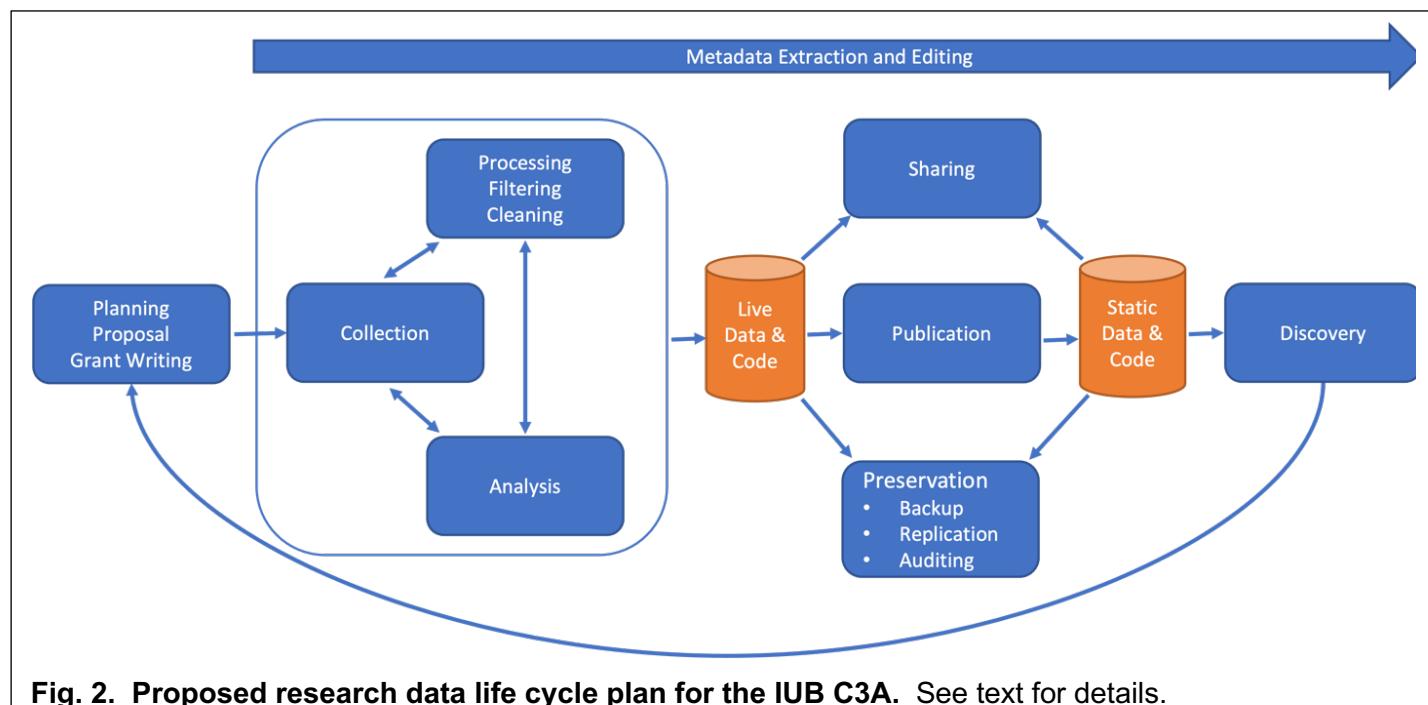


Fig. 2. Proposed research data life cycle plan for the IUB C3A. See text for details.

The Cores are anticipated to produce about 300 TB of original data per year. The rate of new dataset generation is estimated to be no more than 2 TB per day. The data will be transferred to a spinning disk storage system via Globus or SMB mount point on the local Core's lab systems. Both methods provide encryption in transit. The staging area will be the existing Geode2 storage system within IU's datacenter. Geode2 is a geographically replicated central cyber infrastructure storage system that provides 30 days of data snapshots for data resilience. Data in Geode2 can be reached easily from individual laptops and desktops through CAS login.

Of relevance for some data (e.g., human plasma samples analyzed in the BLMC), IU's research cyber infrastructure provides for HIPAA alignment should there be personal health information (PHI) in any dataset.

The data will be organized and labeled on Geode2 and transferred to the Scholarly Data Archive (SDA) for preservation and dissemination.

The Slate storage system will receive a copy the original datasets from Geode2 for processing and analysis. Slate is a high-performance storage system that is designed to support high performance computing (HPC) workflows in the IU data center. The project will have access to the shared HPC clusters: Big Red 200, Big Red 3, Carbonate and Quartz. In addition to the HPC clusters for batch processing, the project staff and the affiliates have access to the Research Desktop (RED) for interactive processing and to Jetstream2. RED is a remote accessible desktop environment to access HPC systems. Jetstream2 is an NSF funded national research cloud operated by IU. Provisioned processes on Jetstream2 can access key resources on the IU network. Research output stored on Slate will be transferred to the tape archive (i.e., SDA) for preservation and sharing with other users.

Over the 5-year life of the project, an estimated total of 1.5 PB data, consisting of original datasets and processed research outputs, is expected to be stored in the tape archive. Project collaborators and the research community will access the tape archived dataset through the Scholarly Data Share (SDS). The SDS [5] is a fully managed service that provides storage, ingestion, and access for curated research data. SDS supports both publicly available as well as private data that requires authentication and authorization for access. Metadata of the original datasets and as well as the derivatives and output will be stored in a relational database for facilitating findable, accessible, interoperable, and reproducible (FAIR) data. This database will be hosted in the RDC, the centrally operated Research Database Complex service. Metadata collected in the RDC will serve as the electronic lab notebook catalog both for live (in process) and static (original or published) data.

A Senior System Analyst/Programmer from Research Data Services (RDS) team of IU Research Technologies will be responsible for building, maintaining, and supporting the described data workflows.

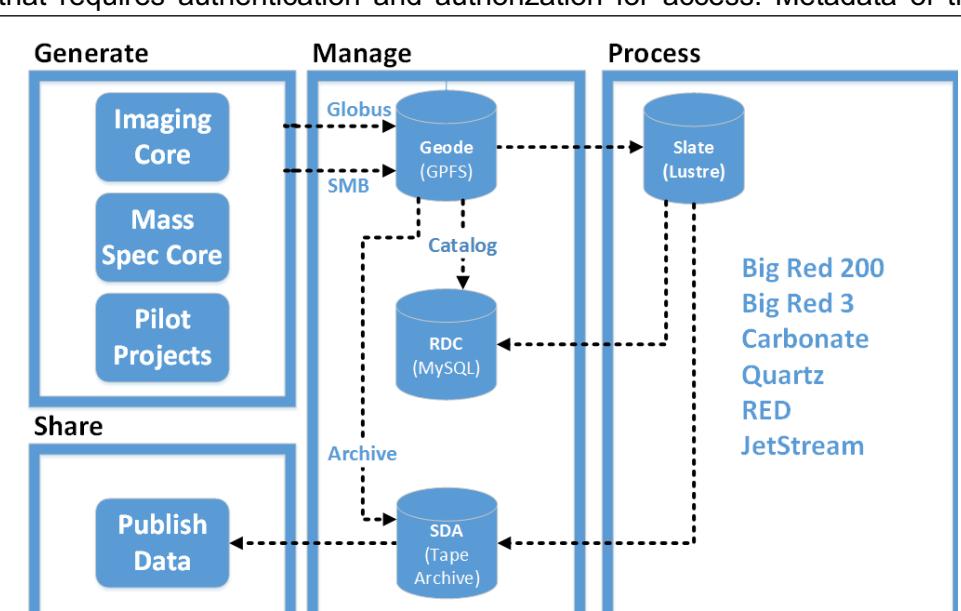


Fig. 3. Schematic of data handling steps for the C3A.

Data Storage and Sharing

Research data will be stored on Geode2, Slate and SDA storage systems (**Fig. 3**). These systems support technical and policy controls to provide HIPAA alignment. SDA is a tape archive that is geographically replicated.

The research data that will be made available to the collaborators and research community will

- Either not contain any PHI if available publicly, or
- Be restricted-access, only available to identified users with access privileges enforced through policy and technical controls.
- Publicly available dataset and private access data will be shared via Scholarly Data Share (SDS) with appropriate authorization if applicable.

A dedicated project personnel (Senior System Analyst, 1.0 FTE, see Budget Justification) for data management will support the capture, transfer, processing, archiving, and sharing of the research data. The data manager is responsible for implementing and executing the data management plan (see DMP document) for the project.

Aim 3. Organize and provide logistical support for the courses offered by the Cores. Central to the mission of the C3A is education and dissemination of the technologies that have been developed by the Cores.

This education and training will take two forms. The first is engagement of undergraduate students, particularly those historically under-represented in drug abuse research, with a structured 2-month summer research experience. The BLMC will host this experience and the experience will integrate with three existing programs at IUB that bring undergraduate underrepresented minority students to the IUB campus for the summer for research experiences. (See letters of support for more details on these programs.) The second is a “hands on” course in STORM microscopy geared towards investigators that would like to learn this super resolution technique and apply it to their research questions. This will be run by the MSIC.

The Administrative Core will be responsible for administering both courses. Key aspects of this administration include publicizing the courses, fielding questions about the courses and their content via the C3A website, coordinating the review of applicants, registrations and waitlists, disbursing scholarships or stipends to the appropriate students, and assisting with travel and housing arrangements.

A. Bioactive Lipid Mediators Core (BLMC) summer training program

Up to 10 students who have applied and been selected into the STEM Summer Scholars program (SSI, see *letter of support for details on the program*) will be trained in the BLMC. The SSI is a program aimed at providing access to research training for students at minority serving institutions (MSI) as well as underrepresented minorities at Indiana University. The SSI students will participate in an 8-week training program in the BLMC. In addition to the professional development activities planned in the SSI program (e.g. weekly GRE prep, weekly SSI data blitz meetings, training in research ethics, mock grad school interviews, cohort building weekend activities, etc.), students will spend 30-hours per week engaged in structured, but independent research activities taking on progressively more responsibility over the 8 weeks. Students will also attend weekly BLMC laboratory meetings and journal club discussions. This course is based on the approaches that the Bradshaw lab has used successfully over the past dozen years to introduce undergraduate students to mass spectrometric analysis and aims to get them quickly involved in “hands” on science. A standard outline for training is as follows:

Weeks 1-2: We will start with a general discussion of separation and analytical techniques, the principles of mass spectrometry and a review of the various types of mass spectrometry. These discussions will focus on the types of problems particularly well-suited to being solved by mass spectrometry, as well as those that are better approached with other analytical techniques. Students will begin their practical experience in mass spectrometry by training and implementation of mass spectrometric analysis of chromatogram quantification using standard curves and Sciex Analyst software. All training will be conducted on previously analyzed data sets. Three workstations with Analyst software are available for offline analysis allowing multiple users to work on identical data sets. Daily progress will be logged through an online course software (Canvas), providing opportunities for daily feedback. Students will spend 2-3 hours/day developing these analytical skills. In addition, students will be trained in cell culture techniques using C6 glioma cell lines, which are particularly amenable to novices.

Weeks 3-5: Students will continue to develop mass spectrometric analytical skills, but time will be reduced to 1-2 hours/day. The primary component of this training period will be to introduce students to lipid extraction and partial purification techniques. Using cell-based assays and cell pellets generated by senior researchers, students will take part in each step of the extraction and purification process. Each student will be responsible for labeling tubes, making solvent dilutions, generating deuterium-labeled spike solutions, weighing tissue prior to analysis, and use of the Preppy apparatus with solid phase extraction columns. The Bradshaw lab is equipped with 4 Preppy stations that run 12 samples each and are used in 2 separate fume hoods. Students are taught in pairs of 2 throughout the training process, thus 4 students can be trained at any one time with 2 mentors. After each extraction training session, samples will be analyzed on the API 3000 to measure recovery of deuterium-labeled spike standards. Students will observe the process of HPLC/MS/MS analysis. Instead of being given the protocols for the various steps involved in mass spectrometric analysis, individual students are required to write detailed protocols of both the extraction procedure and the HPLC/MS/MS procedures from their laboratory notes. We have found this approach results in a much better understanding of the steps involved (i.e., why different steps are being done when) compared to when students just passively read protocols others have prepared. These writeups are completed as weekly assignments with written feedback given by the training mentors, with the goal that the student writeups match with the BLMC protocols.

Weeks 6-8: In the final weeks of training, students will be assigned a drug treatment (THC, CBD, or minor cannabinoid) protocol and use that treatment on C6 glioma cells. This exercise gives both experience in the details of constructing an experiment (e.g., rigor, power analysis, etc.) and putting together the various steps that they have learned in the preceding five weeks into a “real” experiment. Training mentors will maintain backup

cell lines to offer to any students who have had training issues with cell culture and do not have a viable cell line. Students will treat their cell cultures and perform supervised lipid extraction and partial purification on these cell pellets followed by HPLC/MS/MS analysis. Students will then perform quantitative analysis on those data and generate a final report and poster presentation, which will be presented at the final SSI poster session.

B. Four Seasons STORM courses teaching ImmunoSTORM and PharmacoSTORM approaches

In addition to the research mission of the Multi-Scale Imaging Core, the facility has a broad educational mission. Single-molecule-based imaging in life sciences, especially in neuroscience is rapidly expanding. The necessary equipment setups for single-molecule localization microscopy (SMLM), the basic know-how on the different approaches (STORM, PALM, PAINT), and the essential data analysis tools are all becoming widely available at imaging core facilities of universities and research institutes throughout the United States. However, *most labs are only using these approaches with cell culture preparations because of the easier sample preparation*. While cell cultures have certain advantages, nanoscale molecular changes associated with substance use disorders occur in a cell- and compartment-specific manner in the brain. Distinct neuronal types may have differentially altered presynaptic or postsynaptic nanoscale architecture of important signaling molecules underlying maladaptive synaptic plasticity. As a result, functionally meaningful nanoscale molecular measurements must be performed in intact brain circuits requiring the use of brain tissue preparations instead of cell culture assays.

After investigating the nanoscale distribution of cannabinoid signaling molecules (CB₁ receptors, endocannabinoid enzymes) for two decades by using immunogold electron microscopy, Istvan Katona's team switched to apply STORM super-resolution imaging for these types of cell-specific nanoscale molecular imaging experiments. The main reasons were the superior high-yield quantitative data on nanoscale molecular changes in association with behavioral effects evoked by drug exposure, and the use of multi-color imaging that enables the integration of the molecular measurements into the relevant cellular and subcellular contexts. With the help of Dr. László Barna, a physicist-engineer and formerly Director of the Nikon Center of Excellence for Neuronal Imaging (in Budapest, Hungary as part of the Hungarian Academy of Science (KOKI)), they developed a new methodology to use antibody-based immunolabeling in brain tissue preparations for cell- and subcellular compartment-specific nanoscale molecular imaging by correlated confocal and STORM super-resolution imaging (ImmunoSTORM). They demonstrated for the first time that chronic Δ⁹-THC down-regulated CB₁ receptors on identified GABAergic boutons (**Dudok*, Barna* et al. 2015, Nature Neurosci [1]**). The team also developed VividSTORM, the first software tool for correlated data analysis of the different microscope modalities with several still unique analysis features (**Barna et al. 2016, Nature Prot [2]**). Very recently, the team extended their approach into pharmacology and introduced the novel *PharmacoSTORM* method that exploits fluorescent receptor ligands and enzyme substrates for nanoscale pharmacological measurements in a cell- and domain-restricted manner in brain tissue preparations (**Prokop et al. 2021, Nature Comm [3]**).

The major objective of spring STORM, summer STORM, fall STORM and winter STORM courses (4 courses/year) is to offer the **unique opportunity for the neuroscience community to learn the ImmunoSTORM and PharmacoSTORM workflows** from their developers. The 10-day courses will be advertised on a dedicated homepage, Twitter, Facebook, Instagram, the SFN and IBRO homepages. Each course will have 8 participants with financial support available for 2 under-represented minorities. Applicants will be selected by a committee of internal and external advisory board members. On **Day 1**, Dr. Katona will first introduce the essential logic of the different approaches and will present several examples to course participants to illustrate the range of research projects in which the cell-type- and compartment-specific nanoscale molecular measurements represent an unprecedented advantage. Next, Dr. Barna will introduce the basic theoretical concept of single-molecule localization microscopy and provides a detailed account of the most important technological details (tissue quality, imaging buffers, alignment of high-power lasers, optimal data acquisition and tools for data analysis and data visualization). On **Day 2**, the participants will learn sample preparation, prepare acute live slices for PharmacoSTORM labeling and fixed 10 μm free-floating sections for ImmunoSTORM labeling in the Katona lab. On **Day 3**, the group will perform the post-hoc stainings for cellular anatomy, thin section drying and mounting. On **Day 4**, the participants will carry out the first imaging sessions for correlated confocal and STORM imaging. On **Day 5**, Dr. Barna will introduce various data analysis softwares and their specific features for quantitative nanoscale molecular measurements. During the **second week**, the course members will perform the same workflow by preparing their own samples and applying probes brought from their home labs (fluorescent small molecules and antibodies) with guidance from Katona lab personnel. They will perform ImmunoSTORM and PharmacoSTORM imaging as well as data visualization and analysis with the help of Dr. Barna to obtain preliminary data for their own projects. The course participants will receive hand-on manuals and will become long-term members of a Google group of all MSIC users. This group will be an important

interaction platform for the exchange of knowledge, experiences, and information on new tools and troubleshooting ideas under the moderation of Drs. Barna and Katona. A similar workshop will also be part of a larger-scale microscopy course (*BIOC-B 680 Digital Imaging: Light and Microscopy*) offered to IU graduate students.

C. Evaluation of course outcomes

We feel it is critical to evaluate the outcomes of the two courses that will be offered by the C3A to facilitate their continuous improvement. For the BLMC summer experience, the first round of evaluations will be gathered at the end of the course, with subsequent evaluation of the student's success annually. The initial evaluations will focus on key measures of a course-based undergraduate research experience [4]:

1. Collaboration (work with others, help other students, critique other students' work, etc.)
2. Discovery and relevance (do the students feel their work might lead to something new, learn more about doing Science and what doing Science involves, etc.) and
3. Iteration (evolving methods because of specific outcomes, revising written materials, reconciling the data of others with their data, etc.).

In addition, more general data will be sought in terms of student assessment of teaching style, material covered, if they felt appropriately prepared, etc.) will also be obtained. For determining impact, students will be contacted at yearly intervals to determine career trajectories and to solicit open-ended comments on how their summer undergraduate research experience may or may not have affected their career choices and accomplishments. The effectiveness of the seasonal STORM class will be assessed by a questionnaire at the end of the class, and periodic follow-up inquiries to determine if taking the course has positively and materially influenced the student's research, publications, and grant success.

Aim 4. Provide administrative support to improve the Core's efficiency.

Essential administrative activities that are more efficiently conducted in a centralized fashion (e.g., ordering, fiscal management, compliance, animal acquisition and allocation to specific projects, etc.) will be managed by the Administrative Core. Personnel in the Administrative Core include Dr. Mackie, Dr. Hájos, Dr. Franco, and the TBN program assistant.

The C3A will have C3A-specific IACUC and Institutional Biosafety Committee (IBC) blanket protocols. These protocols will be overseen and amended as necessary by Dr. Patricia Franco, who has 10% effort in the Administrative Core. These blanket protocols will allow the efficient importation of mutant mice from Affiliate labs, that then may be used by either Core. (Currently, the BLMC does not have live vertebrate animal use planned, but that may change as experiments evolve and the structure of having the Administrative Core manage their IACUC protocol will be more efficient.) DEA compliance for schedule II-V drugs will be handled by the corresponding core PI DEA registration. Schedule I drugs will be covered under Dr. Mackie's registration. His registration already includes most cannabinoid compounds that affiliates are likely to use in the center. As affiliate projects are being contemplated that would use Schedule I compounds not on Dr. Mackie's registration, these would be added. As adding compounds to a schedule I registration can be a lengthy process, such projects would only be scheduled after appropriate DEA approval has been obtained.

The Program Manager, supervised by Dr. Mackie, will conduct all these activities. This individual will have a full-time appointment in the C3A. The goal of this centralization is to allow the Core PIs and technical staff to focus on their Science more efficiently and not on administrative details. The primary tasks of the Program Assistant are outlined in **Table 2**.

Table 2.

Program Assistant tasks

- Prepare monthly fiscal reports
- Ordering for the Cores
- Coordinate Core equipment service and repairs
- Maintain C3A IACUC protocol
- Maintain C3A rodent colony records
- Maintain C3A Biosafety protocol
- Coordinate rodent transfers for visiting Affiliates (MTAs and shipping)
- Maintain C3A website
- Maintain C3A Twitter feed
- Field questions about C3A courses
- Receive applications for courses and prioritize registrations in consultation with course director.
- Handle stipend disbursement for courses
- Arrange travel, lodging and reimbursement for external speakers (6/year)
- Arrange travel, lodging, and reimbursement for EAB (once every 5 years)

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

Yes No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved

Yes No

Is the Project Exempt from Federal regulations?

Yes No

Exemption Number

1 2 3 4 5 6 7 8

Other Requested Information

Vertebrate Animal Section

Please note that while the Administrative Core will oversee animal ordering and IACUC compliance, the Core will not perform any experiments on vertebrate animals. Those will be done in the Imaging Core and the Pilot Project Core. Please refer to the Imaging Core and Pilot Project Core Vertebrate Animal Sections for details on vertebrate animal use.

References:

1. Dudok, B., L. Barna, M. Ledri, S.I. Szabo, E. Szabadits, B. Pinter, S.G. Woodhams, C.M. Henstridge, G.Y. Balla, R. Nyilas, C. Varga, S.H. Lee, M. Matolcsi, J. Cervenak, I. Kacskovics, M. Watanabe, C. Sagheddu, M. Melis, M. Pistis, I. Soltesz, and I. Katona, *Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling*. Nat Neurosci, 2015. **18**(1): p. 75-86. PMCID:PMC4281300.
2. Barna, L., B. Dudok, V. Miczan, A. Horvath, Z.I. Laszlo, and I. Katona, *Correlated confocal and super-resolution imaging by VividSTORM*. Nat Protoc, 2016. **11**(1): p. 163-83.
3. Prokop S, Á.-B.P., Barti B, Vámosi M, Zöldi M, Barna L, Urban GM, Tóth A, Dudok B, Egyed A, Deng H, Leggio GM, Hunyady L, van der Stelt M, Keserű GM, Katona I *PharmacoSTORM nanoscale pharmacology reveals cariprazine binding on Islands of Calleja granule cells*. Nature Communications, in press.
4. Corwin, L.A., C. Runyon, A. Robinson, and E.L. Dolan, *The Laboratory Course Assessment Survey: A Tool to Measure Three Dimensions of Research-Course Design*. CBE Life Sci Educ, 2015. **14**(4): p. ar37. PMCID:PMC4710398.
5. Chapman, K., Ruan, G., Tuna, E., Walsh, A., and Wernert, E. 2022. Scholarly Data Share: A Model for Sharing Big Data in Academic Research. In Practice and Experience in Advanced Research Computing (PEARC '22). Association for Computing Machinery, Article 2, 1–8.

LETTERS OF SUPPORT

Please see the Overall Component for Letters of Support

RESOURCE AND DATA SHARING PLAN

Please see the Overall Component for the Resource and Data Sharing Plan

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES PLAN

Please see the Overall Component for the Authentication of Key Biological and/or Chemical Resources Plan

**APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)**

5. APPLICANT INFORMATION**UEI***: YH86RTW2YVJ4

Legal Name*: TRUSTEES OF INDIANA UNIVERSITY
 Department:
 Division:
 Street1*: 509 E 3RD ST
 Street2:
 City*: BLOOMINGTON
 County: MONROE
 State*: IN: Indiana
 Province:
 Country*: USA: UNITED STATES
 ZIP / Postal Code*: 474013654

Person to be contacted on matters involving this application

Prefix: First Name*: Middle Name*: Last Name*: Suffix:
 Mr. STEVEN ALLEN MARTIN

Position/Title: ASSOCIATE VP FOR RESEARCH ADMINISTRATION

Street1*: 509 E 3RD ST

Street2:

City*: BLOOMINGTON

County: MONROE

State*: IN: Indiana

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 474013654

Phone Number*: 317-278-3473

Fax Number:

Email: IUAWARD@IU.EDU

7. TYPE OF APPLICANT*

H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type Women Owned Socially and Economically Disadvantaged**11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT***

Multi-Scale Imaging Core (MSIC)

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2023	06/30/2028

Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: TRUSTEES OF INDIANA UNIVERSITY

UEI: YH86RTW2YVJ4

Street1*: 702 North Walnut Grove Ave

Street2:

City*: BLOOMINGTON

County: MONROE

State*: IN: Indiana

Province:

Country*: USA: UNITED STATES

Zip / Postal Code*: 474052204

Project/Performance Site Congressional District*: IN-009

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* Yes No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes No

If YES, check appropriate exemption number: — 1 — 2 — 3 — 4 — 5 — 6 — 7 — 8

If NO, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* Yes No**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an Yes No environmental assessment (EA) or environmental impact statement (EIS) been performed?

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* Yes No

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* Imaging_abstract_20220922-IK-KM.docx.pdf**8. Project Narrative*****9. Bibliography & References Cited** References_MSIC_20220925.pdf**10. Facilities & Other Resources** Facilities_OtherResources.pdf**11. Equipment** Equipment.pdf

SPECIFIC AIMS-Multiscale Imaging Core (MSIC)

Addictive substances trigger **plasticity** at the molecular, cellular and circuit levels that manifest as persistent behavioral changes that may cause **substance use disorders**. Targeting these changes may lead to novel strategies for preventing or treating substance use disorders. However, our knowledge of the molecular changes, the cellular processes and the abnormal circuit activity patterns that underlie various aspects of substance use disorders including compulsion, loss of intake control, withdrawal, and relapse is rather limited. To facilitate a better understanding of the **molecular to circuit level plasticity** accompanying drug abuse, the **C3A multiscale imaging core** will support **center** investigators, **affiliates** from the Midwest and beyond, and **trainees** at different career stages to acquire the conceptual and technical know-how, and to access state-of-the-art equipment for **nanoscale molecular** measurements, for **microscale anatomical** analysis of subcellular and cellular profiles and for **mesoscale physiological** imaging of brain circuits. The **C3A multi-scale imaging core** will provide unprecedented imaging opportunities to examine models of substance use disorders at multiple levels, including: **(1) molecular and cellular level imaging** with internationally unique cell-type- and subcellular compartment-specific correlated STORM super-resolution imaging, and its recently developed PharmacostORM extension for nanoscale pharmacology; **(2) circuit level 2P imaging** to examine selective neural circuits and cell-type-specific dynamic physiological changes among large cell populations.

Aim 1. Determine the cell- and subcellular compartment-specific nanoscale molecular and microscale cellular alterations triggered by chronic exposure to drugs of abuse. By employing fluorescent small molecule-based **PharmacostORM single-molecule nanoscale pharmacology** and antibody-based **ImmunoSTORM super-resolution imaging**, we and C3A-affiliated researchers will determine if chronic drug exposure and/or withdrawal elicit persistently altered nanoscale distribution and abundance of important signaling proteins in the cell types and brain circuits that are most relevant for substance use disorders. By correlating the nanoscale molecular measurements with microscale confocal microscopy data, we will also establish the associated morphological changes in identified subcellular compartments. Particular attention will be devoted to CB₁ cannabinoid and D₃ dopamine receptors that have essential roles in all phases of the addiction cycle and whose antagonists/negative allosteric modulators are among NIDA's ten highest medication development priorities.

Aim 2. Characterize the mesoscale circuit rewiring of long-range glutamatergic, dopaminergic and serotonergic axons induced by developmental or chronic exposure to drugs of abuse. Axon tracts connecting distant brain regions follow irregular trajectories, thus white matter morphology is difficult to evaluate by standard brain section staining. Therefore, we will exploit our experience in **ScaleS methodology** combined with **optimized 2P imaging of the entire mouse brain**. This approach will be used to determine the impact of developmental exposure to THC and other drugs on the integrity and trajectory of identified long-range axons. Because prenatal cannabis exposure modifies human neural circuits and rodent studies found that developing long-range glutamatergic axons are particularly sensitive to THC, we will initially determine the impact of perinatal THC exposure on glutamatergic axons originating from medial prefrontal cortex to various brain regions.

Aim 3. Use *in vitro* and *in vivo* 2P sensor imaging to determine the mesoscale physiological changes in brain circuits elicited by chronic exposure to drugs of abuse. Recent advances in genetically encoded sensors for Ca²⁺, endocannabinoids, and monoamines provide excellent tools to visualize dynamic changes of these signaling molecules in a specific cell-type-specific manner in real-time. By combining our established and comprehensive methodology for **Ca²⁺-imaging** in acute brain slices or awake behaving mice (as young as ten days old) extending from the surgical procedure through the data analysis pipeline with High Performance Computing together with **GRAB-eCB2.1** and **GRAB_{DA} sensor imaging**, we will support center and affiliated scientists to perform longitudinal 2P imaging to examine endocannabinoid, dopamine, and network activity changes in their relevant models of substance use disorders. We will also determine if perinatal THC exposure perturbs the development of endocannabinoid signaling in association with Ca²⁺-spike patterns in the primary somatosensory cortex of awake behaving mouse pups from early postnatal to weaning ages.

Aim 4. Develop *in vivo* protocols for Fluorescence Lifetime Imaging Microscopy (FLIM) in addiction research. Drugs of abuse evoke substantial metabolic changes and perturb astrocyte-neuron interactions. We will use **2P-FLIM imaging** to develop *in vivo* applications using FLIM-based sensors to monitor energy metabolism, signaling cascades, protein-protein interactions and to estimate the proximity between astrocytes and neurons in the substance use disorder models established by local and affiliate researchers of the imaging core.

FACILITIES AND OTHER RESOURCES

Please see the Overall Component for Facilities and Other Resources

EQUIPMENT

Please see the Overall Component for Equipment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: HUI-CHEN	Middle Name	Last Name*: LU	Suffix: Ph.D
Position/Title*:	Linda and Jack Gill Chair of Neuroscience			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 N Walnut Grove Ave			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	812-856-4998		Fax Number:	
E-Mail*:	hclu@indiana.edu			
Credential, e.g., agency login:	HL690781			
Project Role*:	Other (Specify)		Other Project Role Category: Core Co-Lead	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Istvan	Middle Name	Last Name*: Katona	Suffix: Ph.D
Position/Title*:	Naus Family Chair of Addiction Sciences			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 NORTH WALNUT GROVE AVE			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	47405-2204			
Phone Number*:	(812) 855-2012		Fax Number:	
E-Mail*:	ikatona@iu.edu			
Credential, e.g., agency login:	katona			
Project Role*:	Other (Specify)	Other Project Role Category: Core Co-Lead		
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Laszlo	Middle Name	Last Name*: Barna	Suffix: Ph.D
Position/Title*:	Light Microscopy Core Facility Manager			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	Sziagony u. 43.			
Street2:				
City*:	Budapest			
County:				
State*:				
Province:				
Country*:	HUN: HUNGARY			
Zip / Postal Code*:	1083			
Phone Number*:	+36203427793		Fax Number:	
E-Mail*:	larnabaci@gmail.com			
Credential, e.g., agency login:	lbarna			
Project Role*:	Other (Specify)	Other Project Role Category: Core Technical Staff		
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Jui-Yen	Middle Name	Last Name*: Huang	Suffix: Ph.D
Position/Title*:	Assistant Research Scientist			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702 N Walnut Grove Avenue			
Street2:				
City*:	Bloomington			
County:	Monroe			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	8128565869		Fax Number:	
E-Mail*:	juiyuan@iu.edu			
Credential, e.g., agency login:	juiyehuang			
Project Role*: Other (Specify)	Other Project Role Category: Core Technical Staff			
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2023**End Date*:** 06-30-2024**Budget Period:** 1**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 (\$)*
							Months	Months	Months			
1.	Hui-Chen	Lu		Ph.D	PD/PI	196,947.00		1.0		0.00	0.00	0.00
2.	Istvan	Katona		Ph.D	PD/PI	198,841.00		1.2		0.00	0.00	0.00
3.	Jui-Yen	Huang		Ph.D	Associate Scientist	78,115.00	6.0			39,058.00	15,600.00	54,658.00
4.	Laszlo	Barna		Ph.D	Research Scientist	122,400.00	9.0			91,800.00	36,665.00	128,465.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	183,123.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Post Doctoral Associates							
Graduate Students							
Undergraduate Students							
Secretarial/Clerical							
1	Research Technician	12.0			25,750.00	10,285.00	36,035.00
1	Total Number Other Personnel				Total Other Personnel		36,035.00
Total Salary, Wages and Fringe Benefits (A+B)							219,158.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

	Funds Requested (\$)*
1. FLUORESCENCE LIFETIME IMAGING (FLIM) MODULE	13,703.00
2. Visible Add-On	23,768.00
3. Workstations for Data Analysis	2,500.00
4. Epredia 3DHISTECH Panoramic Midi II FL Slide Scanner	67,719.00

Total funds requested for all equipment listed in the attached fileTotal Equipment 107,690.00

Additional Equipment: File Name:

D. Travel

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
 2. Foreign Travel Costs

Total Travel Cost 0.00**E. Participant/Trainee Support Costs**

1. Tuition/Fees/Health Insurance
 2. Stipends
 3. Travel
 4. Subsistence
 5. Other:

Number of Participants/Trainees Total Participant Trainee Support Costs 0.00

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	50,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	7,500.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Service Contracts	57,741.00
	Total Other Direct Costs
	115,241.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	442,089.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.8	334,399.00	195,623.00
				Total Indirect Costs
				195,623.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	637,712.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	637,712.00

L. Budget Justification*	File Name: Imaging_core_budget_justification_20220922.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2024**End Date*:** 06-30-2025**Budget Period:** 2**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 (\$)*
							Months	Months	Months			
1.	Hui-Chen	Lu		Ph.D	PD/PI	196,947.00		1.0		0.00	0.00	0.00
2.	Istvan	Katona		Ph.D	PD/PI	198,841.00		1.2		0.00	0.00	0.00
3.	Jui-Yen	Huang		Ph.D	Associate Scientist	79,677.00	6.0			39,839.00	15,912.00	55,751.00
4.	Laszlo	Barna		Ph.D	Research Scientist	124,848.00	9.0			93,636.00	37,398.00	131,034.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	186,785.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	6.0			26,265.00	10,490.00	36,755.00
1	Total Number Other Personnel					Total Other Personnel	36,755.00
					Total Salary, Wages and Fringe Benefits (A+B)		223,540.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	50,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	7,500.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Service Contracts	57,741.00
	Total Other Direct Costs
	115,241.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	338,781.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	338,781.00	198,187.00
				Total Indirect Costs
				198,187.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	536,968.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	536,968.00

L. Budget Justification*	File Name: Imaging_core_budget_justification_20220922.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2025**End Date*:** 06-30-2026**Budget Period:** 3**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 (\$)*
							Months	Months	Months			
1.	Hui-Chen	Lu		Ph.D	PD/PI	196,947.00		1.0		0.00	0.00	0.00
2.	Istvan	Katona		Ph.D	PD/PI	198,841.00		1.2		0.00	0.00	0.00
3.	Jui-Yen	Huang		Ph.D	Associate Scientist	81,271.00	6.0			40,635.00	16,230.00	56,865.00
4.	Laszlo	Barna		Ph.D	Research Scientist	127,345.00	9.0			95,509.00	38,146.00	133,655.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	190,520.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	6.0			26,790.00	10,700.00	37,490.00
1	Total Number Other Personnel				Total Other Personnel		37,490.00
					Total Salary, Wages and Fringe Benefits (A+B)		228,010.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	50,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	7,500.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Service Contracts	57,741.00
	Total Other Direct Costs
	115,241.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	343,251.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	343,251.00	200,802.00
				Total Indirect Costs
				200,802.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	544,053.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	544,053.00

L. Budget Justification*	File Name: Imaging_core_budget_justification_20220922.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2026**End Date*:** 06-30-2027**Budget Period:** 4**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 (\$)*
							Months	Months	Months			
1.	Hui-Chen	Lu		Ph.D	PD/PI	196,947.00		1.0		0.00	0.00	0.00
2.	Istvan	Katona		Ph.D	PD/PI	198,841.00		1.2		0.00	0.00	0.00
3.	Jui-Yen	Huang		Ph.D	Associate Scientist	82,296.00	6.0			41,448.00	16,554.00	58,002.00
4.	Laszlo	Barna		Ph.D	Research Scientist	129,892.00	9.0			97,419.00	38,909.00	136,328.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	194,330.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	6.0			27,326.00	10,914.00	38,240.00
1	Total Number Other Personnel					Total Other Personnel	38,240.00
					Total Salary, Wages and Fringe Benefits (A+B)		232,570.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 4

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 4

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	50,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	7,500.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Service Contracts	57,741.00
	Total Other Direct Costs
	115,241.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	347,811.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	347,811.00	203,469.00
				Total Indirect Costs
				203,469.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	551,280.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	551,280.00

L. Budget Justification*	File Name: Imaging_core_budget_justification_20220922.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2027**End Date*:** 06-30-2028**Budget Period:** 5**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 (\$)*
							Months	Months	Months			
1.	Hui-Chen	Lu		Ph.D	PD/PI	196,947.00		1.0		0.00	0.00	0.00
2.	Istvan	Katona		Ph.D	PD/PI	198,841.00		1.2		0.00	0.00	0.00
3.	Jui-Yen	Huang		Ph.D	Associate Scientist	84,554.00	6.0			42,277.00	16,885.00	59,162.00
4.	Laszlo	Barna		Ph.D	Research Scientist	132,490.00	9.0			99,367.00	39,687.00	139,054.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	198,216.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
Post Doctoral Associates							
Graduate Students							
Undergraduate Students							
Secretarial/Clerical							
1	Research Technician	6.0			27,873.00	11,132.00	39,005.00
1	Total Number Other Personnel				Total Other Personnel	39,005.00	
Total Salary, Wages and Fringe Benefits (A+B)							237,221.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	50,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	7,500.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Service Contracts	57,741.00
	Total Other Direct Costs
	115,241.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	352,462.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	352,462.00	206,190.00
				Total Indirect Costs
				206,190.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	558,652.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	558,652.00

L. Budget Justification*	File Name: Imaging_core_budget_justification_20220922.pdf

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

BUDGET JUSTIFICATION

Katona Laboratory

A. Senior/Key Key Personnel:

Dr. Istvan Katona, Naus Family Chair of Addiction Sciences, PI (1.2 Academic Month): He will be responsible for the oversight of the Multi-Scale Pharmac-Microscopy (MSPM) facility of the Imaging core. He will be involved in managing the team, will carry out the fiscal management of the MSPM core, and will ensure compliance with all relevant policies. He will organize undergraduate training and spring/summer/fall/winter training programs for external research users at all career levels. He will also be responsible for the public outreach and the dissemination of the Core's scientific achievements. In addition, **he will represent the MSPM core in building up the support network for the external, primarily central Midwest scientists to facilitate their addiction-related research project of the C3A research affiliates.** He will be involved in working with the PIs of the approved pilot grants that use the MSPM Core and will help to plan and optimize the experimental design, data acquisition, data analysis and evaluation of the research outcomes. He will be a member of the IUB C3A internal advisory board. Dr. Katona salary will be covered by institutional funds so no salary support is requested.

Dr. Laszlo Barna, Research Scientist (9 Calendar Months): Dr. Barna is the Founding Director of the Nikon Center of Excellence for Neuronal Imaging at the Institute of Experimental Medicine, Budapest, Hungary. He has led this imaging center for more than ten years. On the management side, he was responsible for the establishment, optimization, mainentance and training of users for the use of eight large microscope systems (confocal, STORM super-resolution, multiphoton, spinning disk, and slide scanners). His imaging center provided key data in the publication of more than 100 scientific papers in high profile journals (Science, Nature Neuroscience, Nature Protocols, Nature Communications, Neuron, Journal of Neuroscience, PLOS Biology, eLIFE etc) and **he has trained more than 150 external and internal researchers for microscopy use and data analysis.** On the innovation side, in collaboration with the engineers of Nikon, he helped to establish the first N-STORM super-resolution system in Europe in 2010-2011. He then developed several hardware and software approaches to be able to perform cell-type-specific STORM super-resolution imaging in complex tissue preparations that was not routinely feasible before. **His innovative accomplishments were published in Nature Neuroscience in 2015 and Nature Protocols in 2016 with his first and co-first authorships.** He also contributed to numerous other projects including innovating the use of fluorescent small molecules for PharmacSTORM nanoscale pharmacology (**Nature Communications, 2021**). He has been an invited speaker at several microscopy conferences and received the Denis Gabor Award in 2018, the most prestigious prize for engineers in Hungary. Dr. Barna spent 5 months at Indiana University Bloomington to help to establish the new MSPM imaging center in 2021. He helped to build and optimize the combined confocal and STORM super-resolution imaging setups, and organized the microscopy use, data acquisition and data analysis training for numerous IU researchers. After becoming familiar with the outstanding research environment in Bloomington, **he decided to permanently move to Bloomington in 2022.** On August 1, 2022, he started working at Indiana University Bloomington as a Senior Research Scientist and as the Director of the MSPM core facility. He acquired several of the PharmacSTORM imaging data presented in this proposal. He will be responsible for all aspects of the theoretical and practical training of all internal and external researchers of the MSPM core (optimization of staining and tissue handling, data acquisition and data analysis, image preparation). In addition, he will organize and teach microscopy courses for undergraduate and graduate students. He will be involved in further innovations on the use of fluorescent pharmacoprobes for super-resolution imaging and will continue to contribute to the development of the respective software features for data visualization and analysis. Importantly, he is also working together closely with the data management team of Indiana University to standardize the format of the data structure and public data dissemination that enables sharing correlated cell-type-specific confocal and STORM super-resolution data across the affiliates of the current P30 application and makes post-hoc data analysis by the broader research community possible with continuously evolving AI-based analytical tools. Salary support for Dr. Barna 9 months FTE will be requested.

Fringe Benefits:

The fringe benefit rates are set by Indiana University and approved by the Board of Trustees. For the Reserach Scientist, the fringe rate is 39.94% of the requested salary.

C. Equipment

Epredia 3DHISTECH Pannoramic MIDI II FL slide scanner (\$67,719 during the first year):

The first step in the Pharmac-Microscopy and general gene expression workflows is an automatic whole-brain analysis of the binding sites of the fluorescent pharmacoprobe at the regional level. This is important, because **this imaging task will identify those brain regions in which the highest abundance of binding sites occur and in which the largest changes in binding density are present in the respective model of substance use disorders for the specific drugs.** This imaging step is essential to focus the subsequent cellular analysis with confocal microscopy and the nanoscale analysis with STORM microscopy on the most important brain regions, cell types and subcellular profiles. In addition, the automatic whole-brain data analysis is also a routine step to establish the precise anatomical sites of virus injections used to interrogate specific cell types to better understand their roles in different aspects of addiction behavior. Moreover, the slide scanner-based data analysis will also be used to detect changes in the cellular activity pattern, distribution of altered anatomical projections and afferent presynaptic or efferent postsynaptic partners upon administration of retrograde or anterograde viruses, respectively. The same slide scanner was successfully used in the first application of Pharmac-STORM imaging (Nature Communications, 2021) in experiments performed by Dr. Laszlo Barna in his imaging center in Budapest, Hungary and he has extensive several years-long experience to run this system. Price includes 5 years of service contract. As IU is cost sharing 75% of the expense, we are only requesting $0.25 * \$270,875 = \$67,719$ from the NIH.

In addition, \$5,000 for a workstation for the analysis of data obtained by the slide scanner is required. This workstation will also be used for training of Pharmac-Microscopy school participants and undergraduate students. This will also be 75% cost shared by IUB, so \$1,250 is being requested for this purchase.

D. Other Direct Costs

1. Materials and Supplies (\$30,000 per year for 5 years):

Internal and external researchers as well as training school participants will use the imaging buffer (Abbelight), Alexa647-conjugated secondary antibodies for Pharmac-STORM staining, primary and secondary antibodies for cellular imaging, high viscosity silicone oil for Pharmac-STORM imaging with a silicone immersion objective, regular staining buffers, nail polish, glassware (slides, coverslips), plasticware (pipette tips, Eppendorf tubes).

4. ADP / Computer Services (\$2,000 per year for 5 years):

To ensure software updates with new modules for data acquisition and data analysis. This is important, because the NIS-Elements software is undergoing a rapid and continuous development for fully automated acquisition pipeline by using JOBS and an AI-based data processing and analysis pipeline (e.g., convert, segment, denoise, enhance and clarify features).

5. Subawards/Consortium/Contractual Costs

None

6. Other Expenses (\$29,596 per year for service contracts for 5 years)

Service Contracts:

70% of \$15,732 (\$11,012) annually for Nikon A1 confocal microscope

70% of \$26,548 (\$18,583) annually for Nikon N-STORM super-resolution microscope

We estimate that these two instruments will be used 70% of the time for Center-related activities, so are requesting 70% support for the three service contracts.

Lu Laboratory

A. Senior/Key Key Personnel:

Dr. Hui-Chen Lu, PI (effort: 1 Academic Month): will be responsible for the oversight of MP core, personnel, training, fiscal management, and research compliance. She will work with C3 research affiliates and PIs of approved pilot grants to optimize experimental design, data collection, data analysis, and summaries of experimental outcomes. She will be a member of the IUB C3 internal advisory board. Dr. Lu's academic year salary is covered by institutional funds, and thus, no salary support is requested.

Dr. Jui-Yen (Edna) Huang, Associate Scientist (6 Calendar Months): Edna is an associate scientist (IUB's equivalent of a research associate professor) in the Lu lab, where she has worked for ~11 years. During this time, she has gained enormous knowledge on neural circuits and has also mastered many techniques, including cranial window installation surgery, Scale-S procedure, high resolution multiphoton imaging and data analysis with machine-learning based analysis pipeline. She generated all the multiphoton imaging data presented in this proposal and has worked with DataJoint and IUB-SICE data scientists to establish data analysis pipelines listed in the proposal. Edna has developed standard operating procedures for Aim2 and Ca²⁺ imaging in Aim3. She has great success in imaging signals from an updated version of eCB sensor and will set up the standard operating protocol for eCB sensor imaging proposed in Aim 3. Edna will train Dr. Franco Sacle S and 2P imaging procedures. She will also provide first-hand detailed training to affiliate scientists in the surgical and imaging procedures as well as on the data analysis (e.g. Imaris and DataJoint pipeline) to ensure the success and scientific rigor of their experiments. She will be responsible for testing new sensors and develop FLIM-FRET imaging procedures for awake behaving mice and ex vivo brain slices proposed in Aim 4.

B. Other Personnel:

Dr. Patricia Frano, Research Technician (6 Calendar Months): She will be responsible for purchasing, mice management, multiphoton scope maintenance, and coordinate the schedules with affiliate scientists to plan their surgical, imaging experiments and data analysis. She will be trained by Dr. Huang for the experimental procedures listed in Aim 2 and conduct Scale-S clearing and multiphoton imaging procedures for affiliate scientists. She will also be responsible to ensure all original data files are deposited into Geode 2 and make them available for C3A affiliates to conduct data analysis using Imaris or other image analysis programs. She will assist C3A affiliates for cranial window installation and conduct post-surgery care of mice and assist affiliates as they get their mice acclimated to the microscope setup, and multiphoton imaging, data storage, and analysis. She will also help harvesting brains for histology. Prior to joining the Lu laboratory, Dr. Franco received her PhD in veterinary medicine in Brazil and has extensive experience with a variety of animal surgeries and animal care. She was involved in large-scale veterinary clinical studies before joining the Lu laboratory. Through her professional experiences, Patricia is highly organized and skilled in communicating and coordinating with large teams.

Fringe Benefits:

The fringe benefit rates are set by Indiana University and approved by the Board of Trustees. For the Associate Scientist, the fringe rate is 39.94% of the requested salary. For the Research Technician, the fringe rate is 39.94% of the requested salary.

C. Equipment

\$54,811.00 for Fluorescence Lifetime Imaging (FLIM) module to add to our existing Nikon A1R MP microscope connected to a Spectra Physics Insight laser (equipment in Lu laboratory). This addition will enable *in vivo* FLIM-FRET imaging with the MP scope to acquire fluorescence lifetime data with minimal tissue damage. Fluorescence lifetime depends on the molecular environment, which renders it feasible for measuring the effects of environmental parameters on molecules. In its conventional configuration, MP imaging acquires the

intensity of fluorescent signals with time and spatial resolution. This expense will be 75% cost shared by IUB, so \$13,703 is being requested from the NIH for this purchase.

\$95,071 for a High Definition (HD) upgrade to the Nikon A1R MP microscope. The HD upgrade will significantly enhance the performance of the current Nikon A1R MP. Specifically, it will increase signal/noise ratios, scanning speeds, and resolution. Furthermore, it enables the acquisition of a higher range of intensities per image. This is especially helpful when viewing dim, subtle details. This expense will be 75% cost shared by IUB, so \$23,768 is being requested from the NIH for this purchase.

\$5,000 for a workstation for data analysis. This will also be used for training summer interns on advanced image data analysis. This expense will be 75% cost shared by IUB, so \$1,250 is being requested from the NIH for this purchase.

D. Other Direct Costs

1. Materials and Supplies (\$20,000 annually for 5 years)

Small equipment and perishable supplies for surgical procedures, adenoassociated viral vectors, antibodies, genotyping, histology, and immunostaining.

4. ADP / Computer Services

GraphPad and Imaris for data analysis (\$5,000 per year for all years). This data analysis software is critical to analyze data from all of the proposed experiments.

5. Subawards/Consortium/Contractual Costs

None

6. Other Expenses (\$28,145 per year for 5 years for service contracts)

Service Contracts:

70% of \$20,680 (\$14,476) for an annual A1RMP service contract from Nikon

70% of \$19,527 (\$13,669) for an annual Dual Beams InSight Laser service contract from Spectra Physics

We estimate that these two instruments will be used 70% of the time for Center-related activities, so are requesting 70% support for the three service contracts.

INDIRECT COSTS

The indirect cost rate for Indiana University is set by DHHS and was negotiated on 5/22/2019 at 58.5% for research, excluding capital equipment, subawards greater than \$25,000, and graduate student fee remissions.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	952,974.00
Section B, Other Personnel	187,525.00
Total Number Other Personnel	5
Total Salary, Wages and Fringe Benefits (A+B)	1,140,499.00
Section C, Equipment	107,690.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	576,205.00
1. Materials and Supplies	250,000.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	37,500.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other 1	288,705.00
9. Other 2	0.00
10. Other 3	0.00
11. Other 4	0.00
12. Other 5	0.00
13. Other 6	0.00
14. Other 7	0.00
15. Other 8	0.00
16. Other 9	0.00
17. Other 10	0.00
Section G, Direct Costs (A thru F)	1,824,394.00
Section H, Indirect Costs	1,004,271.00

Section I, Total Direct and Indirect Costs (G + H)	2,828,665.00
Section J, Fee	0.00
Section K, Total Direct and Fee (I + J)	2,828,665.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 09/30/2024

1. Vertebrate Animals Section

Are vertebrate animals euthanized? Yes No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

Yes No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

Yes No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount (\$) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? Yes No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? Yes No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: Yes No

If the answer is "Yes" then please answer the following:

*Previously Reported: Yes No

6. Change of Investigator/Change of Institution Section

Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	Introduction_MSIC_core_20220925.pdf
Research Plan Section	
2. Specific Aims	Imaging_core_SA_20220923.pdf
3. Research Strategy*	Research_Strategy_only_20220925.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	VAS_MSIC_2022.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	MPI_P30_2022.pdf
8. Consortium/Contractual Arrangements	
9. Letters of Support	LettersOfSupport.pdf
10. Resource Sharing Plan(s)	Resource_DataSharing.pdf
11. Authentication of Key Biological and/or Chemical Resources	Validation_key_resources_0917.pdf
Appendix	
12. Appendix	

Introduction – MSIC Core

We thank the reviewers for their careful reading of our submitted proposal, recognition of our special expertise (“*cross scale nature of the imaging at IUB C3A as applied to SUD research is unique*”), enthusiasm for the proposal and their constructive comments. Below are their major concerns and our replies.

1. Concern that projects require more defined time and resource allocations. We thank the Reviewers for this important comment. In the revised proposal, we expand the details on service supporting C3A investigators, C3A affiliates and the pilot projects. Flexibility in tailoring towards the different needs and resources required for the different projects is considered. Specifically, we added the predicted timelines and the resources committed for specific projects. To provide adequate time to increase the success of the core projects, the MP imaging core will only take one *in vivo* imaging project and accompanying Scale-S project per year. On the other hand, the workflow of the PharmacostORM imaging experiment has been optimized, and pharmacological treatment of *ex vivo* acute brain slices with fluorescent pharmacoprobes enables more rapid and seamless experimental performance than is possible by immunolabeling, increasing throughput for these activities.

2. Question on the involvement of Dr. Laszlo Barna in the multi-scale imaging projects. We apologize for not being specific in the former proposal. Dr. Barna has officially started as a Senior Research Scientist in Bloomington on August 1st, 2022. He has already trained several IU investigators and students as well as started setting up collaboration discussion with external researchers from the substance use disorder field to prepare them for the methodological needs of the entire workflow.

3. Issues with “how training is accomplished, particularly for complicated procedures like imaging” “especially for STORM imaging”. This is an important comment. Accordingly, we improved the design of the training aspects of the P30 grant. In short, two types of training will be offered. IU researchers and P30 Affiliates will spend a longer period in the core. After basic theoretical training, sample preparation, image acquisition, data analysis, and visualization will be performed on-site in Bloomington, enabling sufficient time for the trouble shooting and optimization steps. Two week-long quarterly imaging courses will be offered to applicants who have access to equipment and expert support in their home institution. Many US universities and institutes have already STORM setups in core facilities run by microscopy experts, but these lack substance use disorder background. The training will help the participants to learn the approach workflow and best exploit the capabilities of their home institutes core facilities. Post-training help will be offered for data analysis and visualization.

4. Clarify role of Dr. Lu in the collaborative and training missions of the core. Dr. Lu will mentor IU and affiliate scientists to perform projects involving multiphoton (MP) microscopy. MP imaging is not a component of the Four-Season STORM course. Her responsibility will be to oversee method development and project execution, including experimental design, setting timelines and milestones performed by MP imaging.

5. Questions on details for *in vivo* imaging projects and how they will be implemented. We have outlined financial and logistical aspects in the revised version. The duration and number of Bloomington trips and the level of training/technical support provided by PhD level specialists in the MSIC core are given. Affiliates will analyze MP imaging data such as Ca²⁺ transients with the Cloud-based data analysis pipeline that we have established with DataJoint. The MSIC core will also consult with Affiliates on experimental design, protocol optimization/ troubleshooting, data analysis and interpretation via Zoom meetings during project planning. If a specialized analysis approach is required, DataJoint is willing to engage C3A core affiliates to customize analysis programs as needed. For short-term visits to the C3A/IUB, Bloomington is a college town with lots of short-term housing available. IU and Bloomington team up to provide extensive public transportation around the town.

6. Little synergy between cores. Synergy is a key concept for P30s, and we apologize for lacking clarity in the previous submission. As we describe in the revised version, synaptic endocannabinoid signaling is known to be essential for aspects of all substance use disorders. However, the brain circuit-, cell-type or synapse-type specific mechanistic details in association with distinct aspects of addictive behavior have remained rather elusive. Lipid measurements will define which endocannabinoids show region-specific alterations, and subsequent cell-type- and synapse-specific STORM imaging will uncover how nanoscale redistribution of synthesizing and degrading enzymes underlie the observed changes in lipid levels. This research will then help to focus Scale S and MP imaging to determine the associated anatomical rewiring and consequent physiological changes by measuring endocannabinoid sensor (e.g., eCB2.1 GRAB) signaling and performing calcium imaging of neuronal activity.

SPECIFIC AIMS-Multiscale Imaging Core (MSIC)

Addictive substances trigger **plasticity** at the molecular, cellular and circuit levels that manifest as persistent behavioral changes that may cause **substance use disorders**. Targeting these changes may lead to novel strategies for preventing or treating substance use disorders. However, our knowledge of the molecular changes, the cellular processes and the abnormal circuit activity patterns that underlie various aspects of substance use disorders including compulsion, loss of intake control, withdrawal, and relapse is rather limited. To facilitate a better understanding of the **molecular to circuit level plasticity** accompanying drug abuse, the **C3A multiscale imaging core** will support **center** investigators, **affiliates** from the Midwest and beyond, and **trainees** at different career stages to acquire the conceptual and technical know-how, and to access state-of-the-art equipment for **nanoscale molecular** measurements, for **microscale anatomical** analysis of subcellular and cellular profiles and for **mesoscale physiological** imaging of brain circuits. The **C3A multi-scale imaging core** will provide unprecedented imaging opportunities to examine models of substance use disorders at multiple levels, including: **(1) molecular and cellular level imaging** with internationally unique cell-type- and subcellular compartment-specific correlated STORM super-resolution imaging, and its recently developed PharmacostORM extension for nanoscale pharmacology; **(2) circuit level 2P imaging** to examine selective neural circuits and cell-type-specific dynamic physiological changes among large cell populations.

Aim 1. Determine the cell- and subcellular compartment-specific nanoscale molecular and microscale cellular alterations triggered by chronic exposure to drugs of abuse. By employing fluorescent small molecule-based **PharmacostORM single-molecule nanoscale pharmacology** and antibody-based **ImmunoSTORM super-resolution imaging**, we and C3A-affiliated researchers will determine if chronic drug exposure and/or withdrawal elicit persistently altered nanoscale distribution and abundance of important signaling proteins in the cell types and brain circuits that are most relevant for substance use disorders. By correlating the nanoscale molecular measurements with microscale confocal microscopy data, we will also establish the associated morphological changes in identified subcellular compartments. Particular attention will be devoted to CB₁ cannabinoid and D₃ dopamine receptors that have essential roles in all phases of the addiction cycle and whose antagonists/negative allosteric modulators are among NIDA's ten highest medication development priorities.

Aim 2. Characterize the mesoscale circuit rewiring of long-range glutamatergic, dopaminergic and serotonergic axons induced by developmental or chronic exposure to drugs of abuse. Axon tracts connecting distant brain regions follow irregular trajectories, thus white matter morphology is difficult to evaluate by standard brain section staining. Therefore, we will exploit our experience in **ScaleS methodology** combined with **optimized 2P imaging of the entire mouse brain**. This approach will be used to determine the impact of developmental exposure to THC and other drugs on the integrity and trajectory of identified long-range axons. Because prenatal cannabis exposure modifies human neural circuits and rodent studies found that developing long-range glutamatergic axons are particularly sensitive to THC, we will initially determine the impact of perinatal THC exposure on glutamatergic axons originating from medial prefrontal cortex to various brain regions.

Aim 3. Use *in vitro* and *in vivo* 2P sensor imaging to determine the mesoscale physiological changes in brain circuits elicited by chronic exposure to drugs of abuse. Recent advances in genetically encoded sensors for Ca²⁺, endocannabinoids, and monoamines provide excellent tools to visualize dynamic changes of these signaling molecules in a specific cell-type-specific manner in real-time. By combining our established and comprehensive methodology for **Ca²⁺-imaging** in acute brain slices or awake behaving mice (as young as ten days old) extending from the surgical procedure through the data analysis pipeline with High Performance Computing together with **GRAB-eCB2.1 and GRAB_{DA} sensor imaging**, we will support center and affiliated scientists to perform longitudinal 2P imaging to examine endocannabinoid, dopamine, and network activity changes in their relevant models of substance use disorders. We will also determine if perinatal THC exposure perturbs the development of endocannabinoid signaling in association with Ca²⁺-spike patterns in the primary somatosensory cortex of awake behaving mouse pups from early postnatal to weaning ages.

Aim 4. Develop *in vivo* protocols for Fluorescence Lifetime Imaging Microscopy (FLIM) in addiction research. Drugs of abuse evoke substantial metabolic changes and perturb astrocyte-neuron interactions. We will use **2P-FLIM imaging** to develop *in vivo* applications using FLIM-based sensors to monitor energy metabolism, signaling cascades, protein-protein interactions and to estimate the proximity between astrocytes and neurons in the substance use disorder models established by local and affiliate researchers of the imaging core.

Research Strategy-MSIC core

SIGNIFICANCE: (i) **The basic problem:** More than a billion people worldwide are affected by substance use disorders including smoking tobacco products (22.5% adults in the world), alcohol use disorder (4.9% adults in the world), misuse of cannabis products (3.5% globally), and about 15 million people use opioids, such as heroin [1]. The recent spike in opioid (e.g., fentanyl) overdose deaths together with increased overdose deaths with stimulants and alcohol in association with the COVID pandemic lays clear a tragic trend in the United States [4]. Due to the molecular, cellular and circuit-level complexity of the mechanisms of actions of drugs of abuse, the neurobiological processes culminating in substance use disorders have remained rather elusive. Therefore, current evidence-based treatment approaches have limited efficacy and novel treatment options are needed.

(ii) **Brain plasticity in substance use disorders:** There is substantial evidence that addiction in general, and abused substances in particular trigger dynamic dysregulation in the activity of several brain circuits driving motivational behavior, controlling emotional states, and operating executive functions [11]. Drug exposure evokes substantial changes in the density and functional activity of target proteins and their downstream signaling components [13]; elicits substantial cellular adaptations including synaptic remodeling [14]; and causes long-range rewiring between distant brain circuits. These molecular and cellular changes lead to altered brain circuit activity that perturbs the computational function of specific ensembles of neurons [15,16] and culminating in the feeling of compulsion to take an addictive substance or to perform a certain behavior.

(iii) **Animal models of substance use disorders:** While the MSIC imaging core will support the proposed experiments of Center members and affiliate researchers for any IACUC-accepted behavioral models of substance use disorders, the core team will primarily focus on the impact of perinatal THC exposure. While no model perfectly replicates the human condition of cannabis abuse by pregnant women, the proposed mouse models are associated with hyperdopaminergic activity and impaired cortical function that produce similar behavioral outcomes as those reported in longitudinal human clinical studies [17-21]. This topic is important, because clinical reports of cannabis use to attenuate nausea during pregnancy suggest an increasing trend, with prevalence varying from 3%-35% in North America [22].

INNOVATIONS: Our imaging core is technically and conceptually innovative by: (1) exploiting correlated STORM super-resolution imaging and confocal microscopy to determine how illicit drugs alter the nanoscale distribution of binding sites in a cell-type- and subcellular-compartment-specific manner; (2) applying the ScaleS optical clearing method and 2P imaging to examine the impact of phytocannabinoids on long-range axonal projections in 3D; (3) using longitudinal *in vivo* 2P Ca²⁺ and endogenous cannabinoid (eCB) imaging of awake behaving mouse pups to visualize the establishment of cortical networks during the first postnatal month; and (4) employing 2P-FLIM imaging to visualize astrocyte-synapse interactions.

APPROACH:

Aim 1. Determine the cell- and subcellular compartment-specific nanoscale molecular and microscale cellular alterations triggered by chronic exposure to drugs of abuse.

Rationale: ImmunoSTORM and PharmacoSTORM super-resolution imaging enable researchers to determine nanoscale molecular changes in addiction-related brain circuits. Our team recently developed a novel methodology that combines 1) whole-cell patch-clamp recordings for physiological measurements, 2) confocal microscopy for anatomical investigations, and 3) STORM super-resolution imaging for quantitative nanoscale molecular analysis. Importantly, these three experimental modalities collect physiological, anatomical, and molecular information from the very same

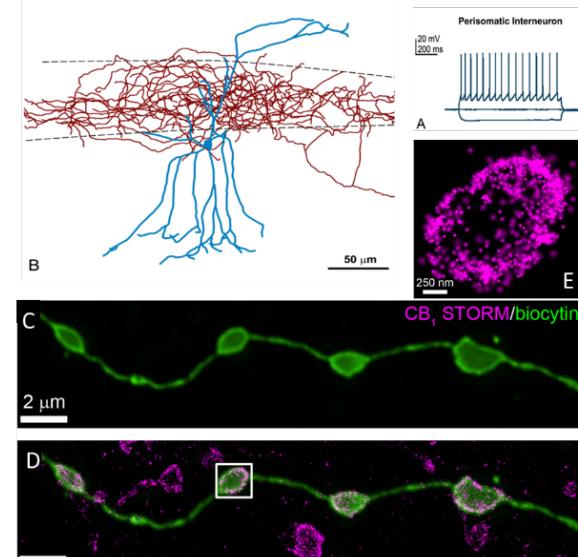


Fig 1 Integrated molecular, cellular and physiological analysis of target neurons by patch-clamp recording, confocal microscopy and ImmunoSTORM super-resolution imaging. (A) Representative voltage traces in response to depolarizing and hyperpolarizing current steps recorded in whole-cell patch-clamp configuration from a GABAergic interneuron in the CA1 subfield of the hippocampus. (B) The neuron was filled with biocytin during recording, and reconstructed with Neurolucida. Dendrites are blue and the axon cloud is red. (C,D) Correlated confocal and STORM super-resolution imaging revealed CB₁-immunolabeling on the identified boutons. (E) The STORM image depicts CB₁ receptors (purple) on the axon terminal shown in the boxed region in D. Images were taken from [2].

identified neuron (Fig 1).

This workflow enabled us to demonstrate that chronic exposure to Δ^9 -THC (10 mg/kg, i.p., twice a day for 6.5 days) causes a robust down-regulation of presynaptic CB₁ cannabinoid receptors in identified GABAergic axon terminals of perisomatic region-targeting basket cells in hippocampal CA1 subfield [23]. This study was the first direct demonstration of molecular tolerance at the level of a

subcellular compartment of a specific neuronal cell type in an intact brain circuit (Fig 2). The strength of this unique approach to generate high yield, statistically powerful nanoscale molecular data in association with a behavioral pharmacological treatment was highlighted by additional results demonstrating that the effect of THC exposure is dose-dependent and reversible [23]. By exploiting the multi-color feature of STORM imaging that is another advantage over electron microscopy, our subsequent study could reveal that prenatal THC exposure altered the molecular ratio of presynaptic CB₁ receptors and downstream effector molecules in the neurotransmitter release machinery of glutamatergic excitatory axon terminals terminating on dopaminergic neurons in the ventral tegmental area [19]. The THC-induced molecular alteration was cell-type-specific and is likely to contribute to the hyperdopaminergic phenotype observed in the THC-treated offspring. In light of the striking molecular and cellular complexity of brain circuits involved in substance use disorders, it is **thus conceivable to hypothesize that substance use disorders are accompanied with molecular changes that occur in a cell-type- and subcellular domain-specific manner.** Indeed, our unpublished preliminary observations uncovered that the same chronic *in vivo* THC treatment that swept away CB₁ receptors from GABAergic axon terminals did not evoke CB₁ downregulation from hippocampal glutamatergic axon terminals. Instead, we noticed a polarized, opposite molecular adaptation in the receptor/effector ratio between GABAergic and glutamatergic axon terminals that implicates a strong shift in the CB₁-mediated control of excitation/inhibition balance in hippocampal circuits upon chronic THC treatment (Fig 3). Taken together, **these observations highlight that plasticity mechanisms induced by drug exposure are associated with cell-type- and compartment-specific nanoscale molecular changes.**

While specific, knockout mice-validated antibodies are available for several target proteins that are relevant for addiction research, other molecular targets remain elusive due to the lack of sensitive and specific antibodies. Autoradiography using radioligands represented a major technique for neuroanatomists and molecular pharmacologists to determine the regional distribution of drug binding in the brain for

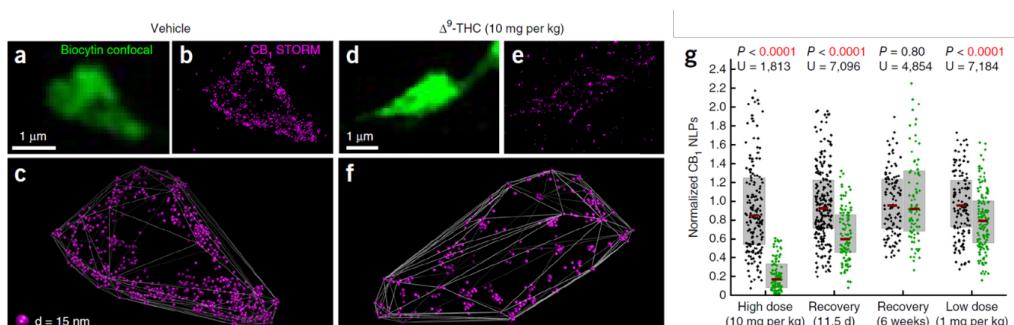


Fig 2 Chronic THC triggers robust downregulation of CB₁ receptors from GABAergic axon terminals. (a,d) Confocal images of axon terminals (green) from mice treated with vehicle or THC. (b,c,e,f) STORM imaging of CB₁ receptors (purple) demonstrates that THC treatment induces loss of CB₁ from the boutons. (g) Quantitative analysis shows that CB₁ receptor downregulation is dose-dependent and reversible. n=185 and 117 axon terminals from 3 vehicle- (black) and 2 THC (green)-treated animals, Mann-Whitney U test. Images were taken from [2].

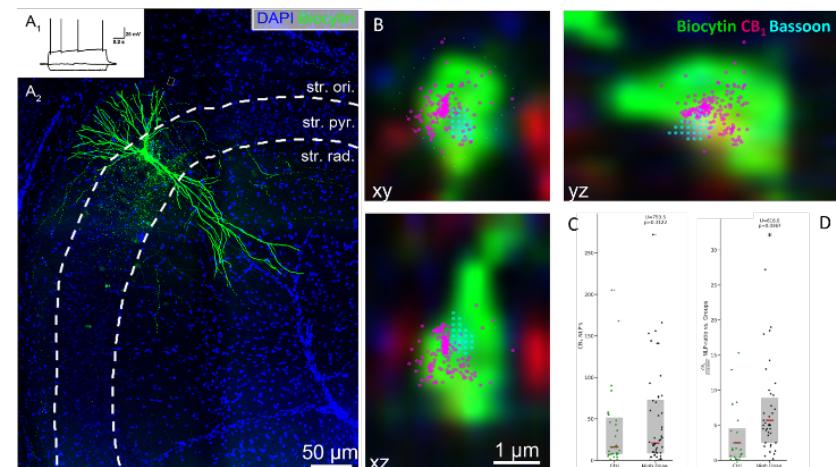


Fig 3 Chronic THC does not change CB₁ receptor numbers on glutamatergic axon terminals, but affects receptor/effector ratio. (A₁) Representative voltage traces in response to depolarizing and hyperpolarizing current steps from a CA3 pyramidal neuron. (A₂) Confocal image of the same CA3 pyramidal neuron. (B) STORM imaging of CB₁ receptors (purple) on excitatory axon terminals (green) from the same CA3 pyramidal neuron. In contrast to the homogeneous nanoscale of CB₁ receptors on GABAergic axon terminals (Fig 1), CB₁ copy number is much lower and always concentrated around the presynaptic active zone visualized by immunostaining for the release machinery protein Bassoon (cyan). (C) Quantitative analysis of CB₁ numbers from mice treated with THC in the same manner as in Fig 2. (D) The CB₁/bassoon ratio is increased upon THC exposure. Because bassoon and voltage-gated calcium channels have stoichiometric abundance in the release machinery, this observation suggests that the same number of CB₁ receptors control more calcium channels on excitatory axon terminals in THC-treated mice.

decades. However, the limited spatial resolution and its incompatibility with immunostaining for cellular profiles prevented its application for cell-type-specific analysis of drug-induced molecular changes. By using the logic of autoradiography and by applying fluorescent small molecules, we recently extended the antibody- and immunolabeling-based ImmunoSTORM methodology to fluorescent pharmacoprobes. By optimizing drug application to live brain slices and then post-hoc immunostaining protocols, we developed a methodology we termed the **PharmacoSTORM** approach [3]. We demonstrated the broad applicability of PharmacoSTORM for ion channels, G protein-coupled receptors and enzymes. Notably, D₃ dopamine receptor antagonists/partial agonists are among the top 10 most wanted drug development candidates in the research portfolio of the National Institute of Drug Abuse to respond to the opioid crisis [24]. The therapeutic usefulness of D₃ receptor antagonists have also been postulated in other substance use disorders [25].

However, there is no specific antibody available for this important GPCR. To better understand the neurobiological mechanisms of D₃ dopamine-mediated signaling in substance use disorders, we developed a fluorescent version of the drug molecule cariprazine. This compound was originally developed against substance use disorders by the pharmaceutical company Richter-Gedeon, and then became FDA-approved and marketed as an antipsychotic and antidepressant called Vraylar [26,27]. In light of the high prevalence of comorbidity of substance use disorders with schizophrenia and depression [28], our unexpected findings that the highest density of fluo-cariprazine binding sites were observed in the brain region called Islands of Calleja has a very important psychiatric significance (Fig 4). By exploiting the nanoscale molecular imaging capacity of the PharmacoSTORM approach together with traditional ligand binding displacement assays and D₃ knockout mice, we could demonstrate for the first time that cariprazine binding sites on D₃ dopamine receptors are concentrated on the axons of granule cells located in the Islands of Calleja (Fig 5). Neither this cell type nor this brain region has been the focus of neuroscience research in the context of substance use disorders or other psychiatric disorders. In light of these new data, it is plausible to hypothesize that D₃ receptors on the granule cell axons in the Islands of Calleja play an important role in the plasticity mechanisms associated with substance use disorder. Therefore, it is important to determine the impacts of the exposure to THC, opioids and other illicit drugs on the nanoscale density of D₃ receptors located on Islands of Calleja granule cells. Moreover, and most importantly in the context of the proposed MSIC imaging core, our unique PharmacoSTORM approach paves the way to study nanoscale molecular changes for the binding of other drugs that are amenable for fluorescent tagging on any other target cell types in addiction-related brain circuits in behavioral models of substance use disorders.

General method: In order to perform **nanoscale molecular analysis**, target proteins can either be labeled by specific antibodies that are validated in the respective knockout mice (ImmunoSTORM approach); or by fluorescent pharmacoprobes that are validated in knockout mice and with displacement assays using unlabeled specific ligands (PharmacoSTORM approach). Knockout-validated antibodies are available for the CB₁ cannabinoid receptor and other molecular components of the endocannabinoid system (ECS) [23,29], for tyrosine hydroxylase, D₁, and D₂ dopamine receptors [30,31], and for several hundred other target proteins that may undergo altered nanoscale distribution in association with drug-induced brain plasticity processes. The toolbox of fluorescently-tagged small molecule ligands is also rapidly expanding, and our recent demonstration of the feasibility of the PharmacoSTORM approach for the quantitative nanoscale visualization of ligand binding is expected to trigger further interest in the generation of fluorescent pharmacoprobes. A key issue is that the pharmacoprobe is best-tagged with a far-red dye such as Alexa-647 or Cy5. While we demonstrated fluo-cariprazine binding to D₃ dopamine receptors with the help of D₃ knockout mice, other tools for PharmacoSTORM imaging are also becoming rapidly available for the dopamine transporter, for nicotinic acetylcholine receptors,

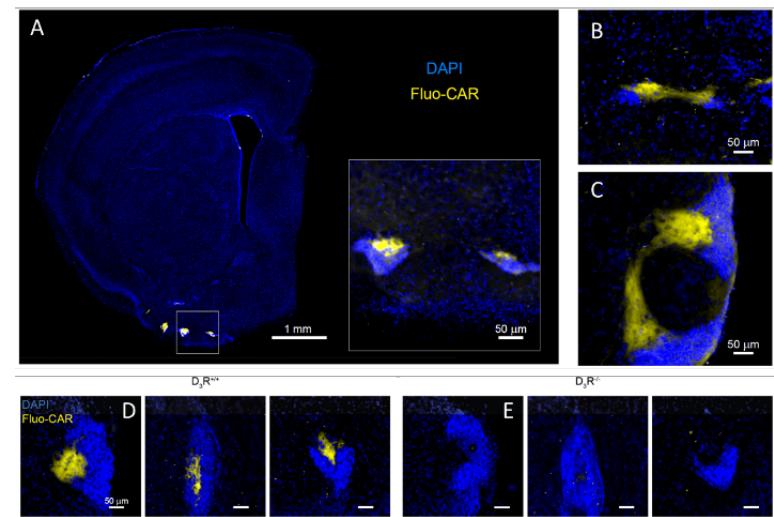


Fig 4 Cariprazine binds to D₃ dopamine receptors on the Islands of Calleja. (A) The highest binding density of fluorescent cariprazine (yellow, 300 nM) is found on the Islands of Calleja in the ventral forebrain. (B,C) While the granule cell bodies (DAPI, blue) are devoid of cariprazine binding, the different cell masses are interlinked via “bridges-like” structures that concentrate cariprazine binding sites. (D,E) Cariprazine binding in the Islands of Calleja is completely absent in D₃ knockout mice [3].

for the CB₂ cannabinoid receptor, and even for opioid receptors [32-35].

In order to carry out **microscale anatomical analysis** that is essential to integrate the molecular measurements into a functionally meaningful cellular and subcellular context, various widely used approaches are available. For example, immunostaining for neurochemical markers and visualization of genetically-targeted fluorescent proteins with cell-type-specific expression are useful for population-level analysis; *in utero* or viral delivery of constructs expressing fluorescent proteins are beneficial for sparse labeling; and targeted single-cell labeling via a patch-clamp electrode in acute brain slices is ideal for correlative physiological, morphological and molecular measurements in an identified neuron. Both the Katona and Lu laboratories have established in their labs the procedures for *in utero* electroporation and stereotaxic injections for gene delivery vectors.

Five brains per treatment for each sex will be used for quantitative analysis to determine the molecular and cellular changes associated with behavioral models of substance use disorders. For ImmunoSTORM-based approaches and population-level investigations, mice will be perfused. Perfused brains can also be shipped from original research sites. For PharmacostORM approach, acute brain slices will be prepared and incubated with the fluorescent pharmacoprobe then fixed and re-sectioned for further immunostaining. For correlative physiological, anatomical, and molecular measurements, acute brain slices will be used to fill individual neurons with biocytin. We have established optimized protocols for immunostaining and pharmacolabeling in brain tissue preparation [36] (**Fig 4**). To determine which brain circuits may exhibit the largest changes in immunofluorescence labeling or in fluorescent pharmacoprobe binding, data acquisition will begin with automatic fluorescent imaging of coronal whole brain slices taken with 3DHISTECH Panoramic MIDI II slide scanner (Epredia), using a Zeiss Plan-Apochromat 20× objective (0.8 NA) as described before [4]. Consecutive sections will be automatically aligned and analyzed with a built-in plugin for feature-based image registration and density measurement in the NIS Elements AR 5.21.01 (Nikon) program. After the regional analysis, the cellular analysis will be performed with Nikon A1-HD25 confocal laser-scanning system built on a Ti-E inverted microscope operated by NIS-Elements AR software 4.50.00 (Nikon). The density, size and staining intensity of the respective cellular profiles will be analyzed by Imaris software. The nanoscale molecular analysis restricted to an identified subcellular profile will then be performed by acquiring STORM super-resolution images and correlated high-power confocal stacks via a CFI Apo TIRF 100× Oil 1.49 NA objective on a Ti-E inverted microscope equipped with an N-STORM system, a Nikon C2 confocal scan head, and an Andor iXon Ultra 897 EMCCD camera. For STORM imaging, the direct STORM (dSTORM) approach will be used in continuous activation mode using 100% 647 nm imaging and 10% 405 nm activation laser power with a far-red STORM filter cube (EX:-DM:660nm EM:670-760nm). PharmacostORM and ImmunoSTORM can even be combined, in this case, ImmunoSTORM targets will be visualized by two-step immunolabeling using CF568-conjugated secondary antibodies, and images will be acquired after PharmacostORM image acquisition in a sequential manner with a HQ Red filter cube (EX:554-568nm DM:575nm EM:557-661nm). We will use a 4X beam-focusing lens and collect 10,000 frames per image in most experiments. The PharmacostORM and ImmunoSTORM images will be first processed and visualized with the N-STORM module in NIS-Elements AR software. The STORM images and the confocal images will be aligned by our open-source VividSTORM software [36] that is used to contour the Region-of-Interest based on the confocal profile, filter out irrelevant molecular localizations, and then measure

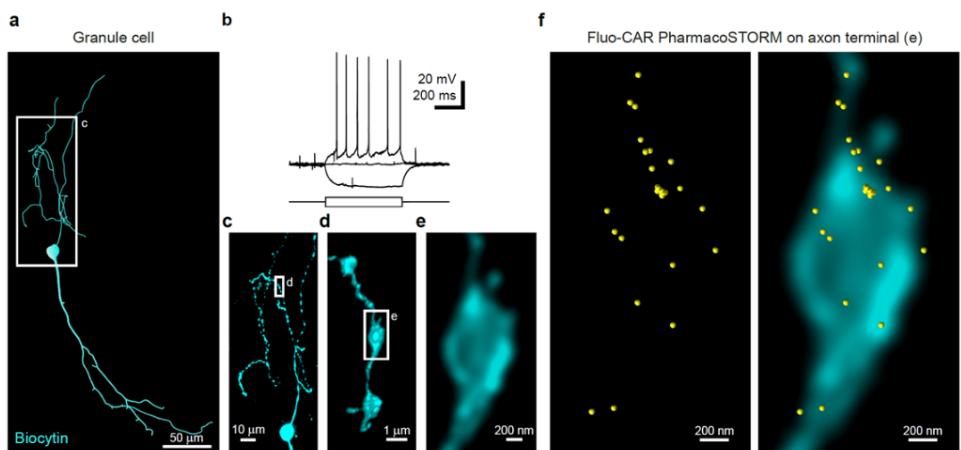


Fig 5 The first example for the nanoscale visualization of the binding sites of a clinically approved medicine on the surface of an identified cell within its native tissue environment. (a) NeuroLucida reconstruction of a representative biocytin-filled granule cell in the Islands of Calleja. (b) Voltage traces in response to +7 pA, 0 pA, -10 pA current steps from resting membrane potential reveal the firing pattern of the same granule cell shown in (a). (c) Maximum intensity z-projection of the confocal image stack of the axon in the boxed area in (a). (d) Volume view of a high-resolution confocal image stack taken from a varicose segment from boxed area in (c). (e) Higher magnification deconvolved confocal image of the boxed area in (d) illustrates a single axon terminal. (f) Correlated confocal and PharmacostORM imaging of the granule cell bouton (cyan) presented in (e), and the corresponding Fluo-CAR binding sites (yellow) along the surface of the axon terminal [3].

the number, density, clustering, nanoscale distance and potential internalization of the STORM localization points representing the nanoscale position of the target protein. The large data sets on the 3D coordinates will be freely available for post-hoc data analysis to measure any nanoscale molecular alterations that is not considered during the first set of data analysis (see Data Management in Administrative Core).

Example project: Use ImmunoSTORM and PharmacoSTORM imaging to determine how the nanoscale distribution of CB₁ cannabinoid receptors and D₃ dopamine receptors are affected by maternal THC exposure. Recent collaborative studies of the laboratories of the other three PI's of the C3A and MSIC core (Drs. Bradshaw, Mackie and Lu) found that perinatal THC elicits substantial changes in endocannabinoid signaling in the prefrontal cortex in a sex-dependent manner (Fig 6). To better understand the multi-scale plasticity processes occurring in the prefrontal cortex, these prior studies will be extended to the nanoscale molecular (Aim 1), the microscale anatomical (Aims 1-2) and to mesoscale physiological (Aims 2-3) investigations. In terms of Aim 1, we will first determine how nanoscale CB₁ cannabinoid receptor distribution is affected on GABAergic axon terminals using the same approach as we have shown before in the hippocampus [23], on glutamatergic axon terminals (Fig 3), and on serotonergic axon terminals [37,38]. In addition, physiological experiments suggested the presence of functional D₃ dopamine receptors on a selected population of prefrontal cortical pyramidal cells that project to the nucleus accumbens [39]. However, their precise anatomical location and density as well as how THC and other drug exposure affects their distribution has remained elusive. This is important, because chronic THC administration is known to elicit weaker prefrontal cortical glutamatergic inputs to the nucleus accumbens [40]. It is possible that increased dopaminergic control of action potential generation on cortico-accumbal projection neurons via elevated D₃ receptor density contributes to this effect [39]. In light of our recent finding that D₃ receptors are highly concentrated in the Islands of Calleja, we will also test this brain region to determine if dopaminergic signaling is also affected in the Islands of Calleja.

Pregnant mice will be treated with vehicle or 3 mg/kg THC from gestation day 5 to P10 as before [17] and below. As described above, the ImmunoSTORM approach will be used to investigate CB₁ receptor distribution, whereas the PharmacoSTORM approach will be used to study D₃ receptors in the vehicle and THC-treated offspring. Because our prior studies found that perinatal THC treatment caused sex-specific alterations in several addiction-related brain areas [17,19], we hypothesize that the nanoscale molecular changes will vary by sex. Therefore, the data obtained from each sex will be compared. Besides the nanoscale molecular measurements, the morphological properties of the identified subcellular profiles will also be analyzed to determine if cellular changes are associated with molecular alterations. This knowledge will be valuable to determine how endocannabinoid signaling is perturbed in the prefrontal cortex of offspring that were perinatal exposed to THC and whether D₃ receptor antagonists could be important tools to balance increased D₃ expression in the prefrontal cortex.

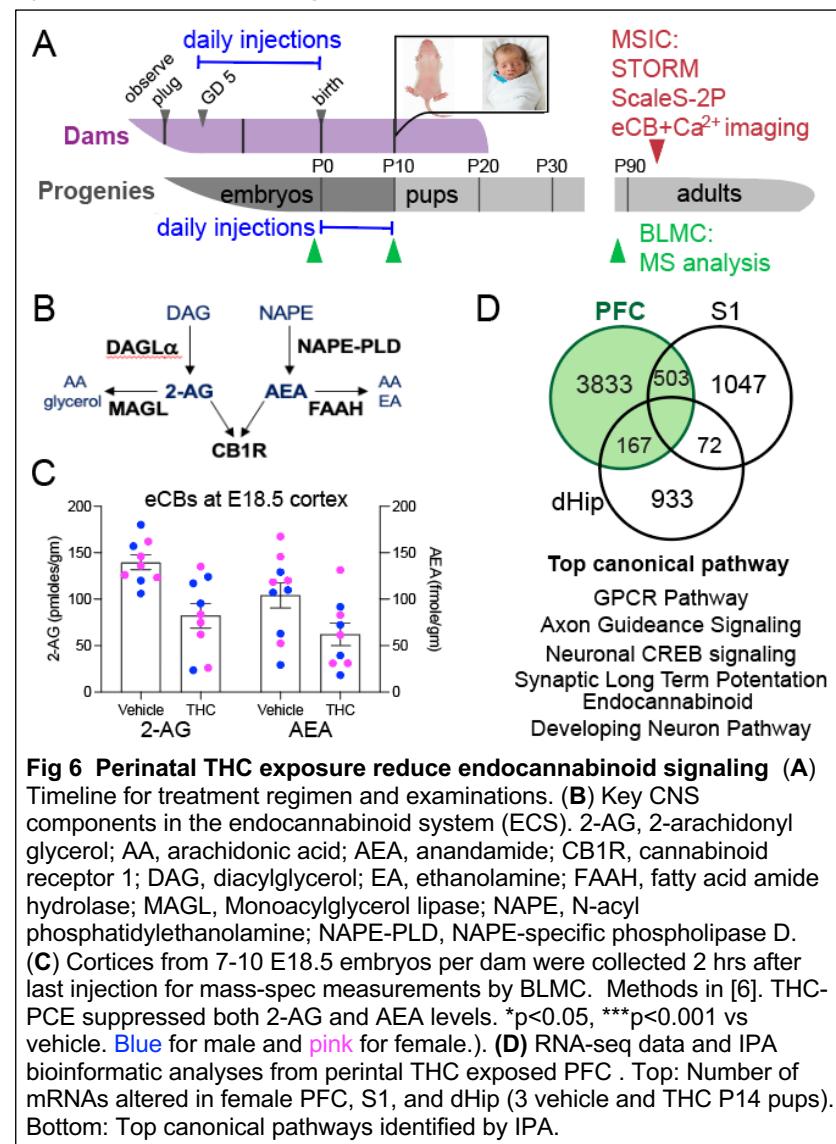


Fig 6 Perinatal THC exposure reduce endocannabinoid signaling (A) Timeline for treatment regimen and examinations. (B) Key CNS components in the endocannabinoid system (ECS). 2-AG, 2-arachidonyl glycerol; AA, arachidonic acid; AEA, anandamide; CB1R, cannabinoid receptor 1; DAG, diacylglycerol; EA, ethanolamine; FAAH, fatty acid amide hydrolase; MAGL, Monoacylglycerol lipase; NAPE, N-acyl phosphatidylethanolamine; NAPE-PLD, NAPE-specific phospholipase D. (C) Cortices from 7-10 E18.5 embryos per dam were collected 2 hrs after last injection for mass-spec measurements by BLMC. Methods in [6]. THC-PCE suppressed both 2-AG and AEA levels. *p<0.05, ***p<0.001 vs vehicle. Blue for male and pink for female.). (D) RNA-seq data and IPA bioinformatic analyses from perinatal THC exposed PFC . Top: Number of mRNAs altered in female PFC, S1, and dHip (3 vehicle and THC P14 pups). Bottom: Top canonical pathways identified by IPA.

Anticipated outcomes, potential problems, alternative approaches, and future directions: We have established

the tissue handling and the staining protocols together with the image acquisition and data analysis approaches for both ImmunoSTORM and PharmacoSTORM super-resolution imaging. We found robust, cell-type-specific changes in CB₁ receptor distribution and receptor-effector ratio in mice treated with THC during perinatal period or adulthood [23] (**Fig 3**). D₃ expression levels are also known to become altered in various substance use disorder models including chronic THC exposure [41-45]. The PharmacoSTORM approach helps to determine the underlying cell types and subcellular compartments that harbor these molecular changes in D₃ levels.

Moreover, the combined nanoscale molecular and microscale cellular analysis will be imperative to distinguish whether the changes in CB₁ receptor or D₃ receptor levels observed at the regional level in prior studies are due to specific alteration of the nanoscale molecular density on selected subcellular profiles, or alternatively, sprouting or pruning of the subcellular profile in association with an ongoing rewiring would explain altered overall binding density. If we don't find changes in the prefrontal cortex or in the Islands of Calleja then other addiction-related brain circuits especially the nucleus accumbens and the ventral tegmental area will also be investigated. *Taken together, the cell-type- and subcellular compartment-specific nanoscale molecular imaging data by using the ImmunoSTORM and PharmacoSTORM approaches will inspire hypothesis-driven research and support cell-type-specific interrogation of selective signaling molecules in brain circuits in order to enable causal exploration of how specific receptors in a given cell type contribute to different aspects of substance use disorders.*

Typical ImmunoSTORM and PharmacoSTORM service & hrs supporting C3A investigators, affiliates and pilot projects: Steps: 1st, conceive a hypothesis with affiliate; design specific labeling tools and strategy, ensure availability of appropriate controls for validation of the labeling tools (40 hours); 2nd, perform the behavioral experiment (variable, done by the affiliate); 3rd, provide detailed protocols and training for investigators for tissue handling and to perform STORM imaging (16 hours); 4th, prepare live or fixed brain samples, pharmacolabeling (4 hours) or immunolabeling (12 hours); 5th, data acquisition by correlated STORM and confocal imaging, five samples from five brains per treatment for each sex generates 100 images for one research project. With the new generation faster STORM setup available in the Katona lab, one correlated confocal and STORM image is taken each 5 minutes (~2 min data acquisition, ~3 min specimen positioning in holder, finding ROI, etc). Hence this step requires about ~8-10 hours completed on the same day for quantitative reproducibility; 6th, data analysis by N-STORM modules and VividSTORM (12 hours); 7th, data summary, deposition of data files and the analysis files into the database to share with scientific community, preparation of report for scientific and logistic feedback to C3A external and internal advisory boards (within two weeks of completion of the project).

Aim 2. Characterize the mesoscale circuit rewiring of long-range glutamatergic, dopaminergic and serotonergic axons induced by chronic exposure to drugs of abuse.

Rationale: Our previous work found that the ECS is required for long-range glutamatergic axonal tracts to fasciculate into axonal bundles of appropriate size [7] and correctly arborize in their target zones [46]. Functional MRI studies suggest that prenatal cannabis exposure modifies neural circuits [47-51]. Rodent studies reveal that developing long-range axons are particularly sensitive to treatment with THC or CB₁ agonists/antagonists [7,46,52-55]. The prefrontal cortex (PFC) is central for executive function, working memory, and the proper execution of social behaviors [56-58]. PFC receives long-range glutamatergic inputs from medial dorsal thalamus (MD), dopaminergic inputs from ventral tegmental area/substantia nigra (VTA/SN), and serotonergic inputs from dorsal raphe nuclei (DRN) [57,59-62]. PFC projects long-range pathways to MD, VTA, DRN, etc. Our preliminary RNAseq and qPCR studies found that perinatal THC exposure alters mRNAs of many axon guidance genes in PFC (data not shown). We hypothesize that perinatal THC causes lasting alterations in long-range axon projections. Recent studies show great promise for circuit-based therapies to reverse behavioral deficits (e.g., [63-65]). Thus, it is important to determine the impact of exposure to THC or other compounds in mouse models of drug abuse/addiction on the integrity and trajectory of selective long-range axons.

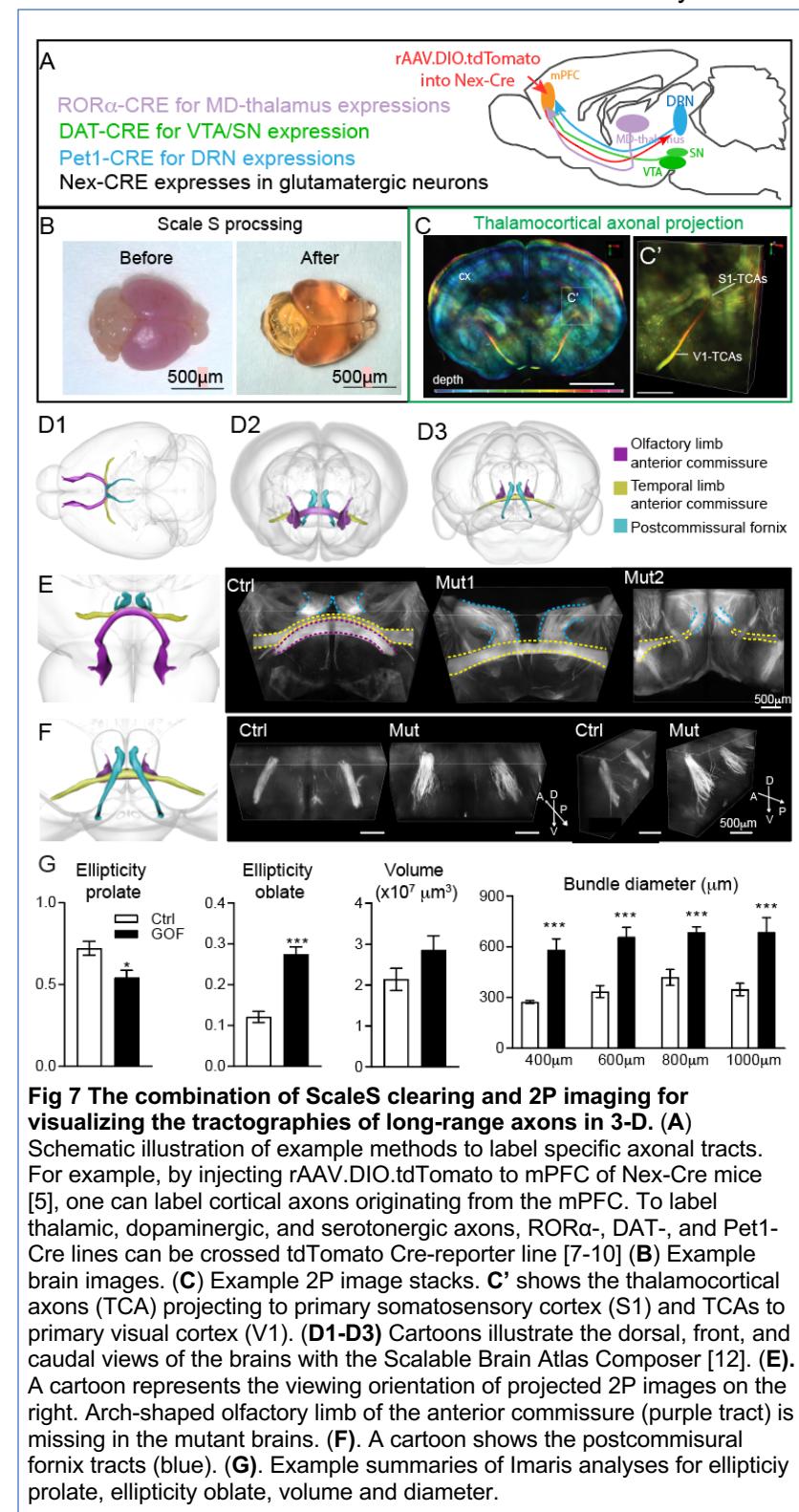
Axon tracts connecting distant brain regions often take irregular and tortuous paths, posing a unique challenge in evaluating white matter morphological characteristics by standard brain section staining. By combining ScaleS tissue clearing methods [66] and **two-photon (2P)** microscope imaging, we can visualize fluorescence labeled long-range axonal trajectories in 3D and quantitatively evaluate their integrity and trajectory in brains (**Fig 7**).

General method: Selected axonal tracts can be genetically labeled with fluorescence by employing stereotaxic-aided AAV injections or by generating transgenic mice (**Fig 7A**). For example, injecting AAV-CAG-DIO-tdTomato into PFC of P60 NEX-Cre mice [5], expressing Cre only in post-mitotic glutamatergic neurons, will label PFC glutamatergic outputs with tdTomato. Thalamic, dopaminergic, and serotonergic projections can be visualized with tdTomato by crossing tdTomato Cre-reporter line, e.g., Ai14 JAX mice, to ROR α -, DAT-, and Pet1-Cre lines,

respectively [7-10]. Four brains per treatment per sex will be quantitatively analyzed to determine axonal trajectories and innervation patterns. We have established ScaleS methodology to preserve fluorescence and optimized 2P imaging of entire brains (**Fig 7**; [67]). Specifically, after ScaleS clearing, brains will be sliced into 1 mm-thick coronal brain sections and mounted in 100 mm diameter Petri dishes filled with fresh ScaleS4(D25) solution, then imaged with 2P microscope (Nikon A1R MP⁺ equipped with an InSight DeepSee infrared pulsed laser from Spectra-Physics) at 1 μm intervals in the z-axis with a 10X/NA 0.5 Multi-Immersion Clarity objective lens (Nikon). Depending on the image area, imaging takes 15 ~ 20h/slice. Axonal projections will be reconstructed by the 3-D Surface module of Imaris v9.2 (Bitplane Inc.). Measurements such as volume, lengths, ellipticity prolate, and ellipticity oblate can be extracted for selected axonal projections in defined brain regions. The comprehensive and large data sets generated here will be shared with the scientific community to further explore 3D trajectories (see Data Management in Administrative Core).

Example project: Use ScaleS and 2P imaging to examine how long-range serotonergic axons are affected by maternal THC exposure. Our recent work show that perinatal THC exposure renders fluoxetine (a commonly used SSRI for treating anxiety and depression) unable to enhance coping behavior [17]. Serotonergic long-range projections originating from DRN play critical roles in stress-coping, but are vulnerable to environmental insults during development [21,60-62]. The PFC receives long-range serotonergic inputs from DRN (**Fig 7A**). We hypothesize that perinatal THC exposure reduces DRN to PFC serotonergic projections and thus results in fluoxetine resistance in adult progenies. 2P microscopy will be used to examine the DRN-mPFC axons in optically cleared brains using the ScaleS methodology [66]. Specifically, Pet1-Cre mice will be mated with tdTomato Cre-reporter mice [5,7-10] and the pregnant female mice will be treated with vehicle or 3 mg/kg THC from gestation day 5 till their pups reach P10 following the paradigm described [17]. The brains of the progenies of appropriate genotype (Cre:tdTomato) will be harvested at P90 and cleared with ScaleS solution for 3D 2P imaging. Volume, diameter, length, axonal bundle trajectories will be analyzed using Imaris to examine how maternal THC affect long-range serotonergic axons projecting into PFC. This knowledge will be valuable to develop future experiments to determine whether this circuit deficit accounts for decreased responsiveness to SSRIs and if carefully tuning their excitability restores normal behaviors.

Anticipated outcomes, potential problems, alternative approaches, and future directions: We have established colonies of the Pet1-Cre and tdTomato reporter mice. Serotonergic Pet1⁺ neurons projecting from DRN release



serotonin in mPFC [61,62]. We found that adult progenies with perinatal THC exposure are resistant to fluoxetine treatment in the FST. Reduced Pet1-tdTomato axons in mPFC will suggest reduced serotonin inputs account for fluoxetine resistance after perinatal THC exposure. This finding will motivate future experiments to test whether enhancing DRN serotonergic neuron excitability restores fluoxetine sensitivity. If we don't find changes in DRN to mPFC projections, we will examine whether reduced glutamatergic inputs from PFC to DRN could account for less serotonin release (and thus decreased sensitivity to SSRIs) from DRN-PFC axons using Nex-Cre x tdTomato reporter mice. Overall, data from ScaleS-2P imaging will enable us to propose circuit-based hypotheses for the behavioral deficits and their rescue in future studies.

Typical ScaleS-2P workflow supporting C3A investigators, affiliates and pilot projects: One project will be served per year. Steps: 1st, form testable hypothesis with investigators, design strategy to label the circuit of interest, and troubleshoot experimental challenges (40hrs); 2nd, Confirm the labeling strategy with data acquired by the investigators using immunostaining and standard imaging (12hrs); 3rd, PIs ship 8-10 labeled brains to C3A Imaging Core to conduct ScaleS clearing and 2P imaging (120hrs); 5th, deposit original 2P image data into database (8hrs); 6th provide training/access to Imaris to conduct data analysis (16hrs); 7th, Summarize results and upload all Imaris analysis files into database (Administrative Core).

Aim 3. Use *in vitro* and *in vivo* 2P sensor imaging to determine the mesoscale physiological changes in brain circuits elicited by chronic exposure to drugs of abuse.

Rationale: Recent advances in high resolution 2P imaging and genetically encoded fluorescence biosensors for calcium [68,69], endocannabinoids [70], dopamine [71,72], serotonin [73], etc., provide unprecedented opportunities to visualize dynamic changes of neural activity, neurotransmitters, neuromodulators, and/or signal transduction in selected cell types in real time [74-76]. This functional imaging can be conducted with *ex vivo* brain slices or in awake behaving animals (Figs 8,9,11). The recent studies by Farrell et al [70] used the newly developed endocannabinoid (eCB) sensor, GRAB-eCB2.0, to examine eCB dynamics in the brains of awake behaving mice. Specifically, with 2P imaging they simultaneously detected signals from GRAB-eCB2.0 and the red Ca²⁺ sensor, jRGECO1a, to characterize eCB spatiotemporal changes in relationship to neural activity. Using this approach, they demonstrated that seizures result in excessive 2-AG, which fuels a prolonged vasoconstrictive, stroke-like event. Currently, we have demonstrated the feasibility and developed imaging protocols for *in vivo* 2P imaging with jRGECO1a and GRAB-eCB2.1 (an updated version of eCB2.0 enabling significantly better signal-to-noise ratios, even in mice expressing CB₁ receptors; Fig 11).

In vivo sensor imaging experiments require an advanced microscope equipped with powerful lasers and sensitive detectors, expertise in delicate surgical procedures, and a sophisticated data analysis pipeline. Here we aim to enable qualified researchers to conduct *ex vivo* or *in vivo* 2P imaging to examine dynamic activity with the sensor of their choice.

General method/equipment: For 2P imaging, the core contains a Nikon A1R MP+ multi-photon microscope (Nikon Instruments Inc.) equipped with an InSight DeepSee infrared pulsed laser (Spectra-Physics Inc), which provides broad and high power across a tuning range at the multiphoton imaging wavelengths of 680 and 1300 nm. Its second line output is at 1045 nm. Dual line lasers allow simultaneous two color excitation and thus multiplex imaging. This A1RMP⁺ is set up with both resonant and galvano scanners and can detect emissions over 400-750 nm. Thus, the investigators can acquire images at a high imaging rate and/or high anatomical resolution for their experimental need. The core also has a surgery suite with two surgery stations set up for conducting surgical procedures with young pups and adult mice. *Example experimental procedure for early postnatal Ca²⁺ and eCB imaging in the primary somatosensory cortex (see below for more details):* (1) A mixture of 100 nl AAV-hSyn-eCB2.1 and 100 nl AAV-Syn.NES-jRGECO1a (Addgene) will be stereotactically injected into the primary somatosensory

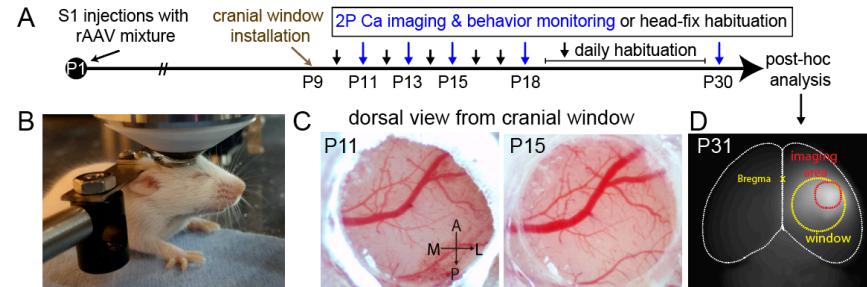


Fig 8 (A) Experimental procedures. To reduce stress-induced neural activity, pups will be habituated on the imaging platform for >10 min prior to imaging, and for 30 min per day on non-imaging days after cranial window implantation. **(B)** P13 pup with cranial window installed and attached to headframe while walking on the light-weight plate. **(C)** Images show vasculature through the cranial window 2 or 6 days post-installation. A, anterior; P, posterior; M, midline; L, lateral. **(D)** Fluorescence image show jRGECO1a expression in S1. Circles mark the locations for cranial window and 2P imaging region.

(S1) cortex of P1 pups as described [77] (**Fig 8A**). (2) Cranial windows will be implanted at P9. (3) After 2 days of recovery, pups will be imaged in a head-fixed configuration as they move on top of a warm mobile-disk (**Fig 8B**). eCB and Ca^{2+} signals will be excited at 1000 nm and emission signals will be acquired with a 25x 1.1 NA water immersion lens on a Nikon A1R MP multi-photon microscope via the resonant scanning mode. eCB and Ca^{2+} signals will be separated with 506-534 (laser 940 nm) and 563-588 nm (laser 1040 nm) filter units. Pup behaviors will be recorded using an IR camera (30 Hz, 1920x1080 pixels) with an 850 nm IR LED light source simultaneously during 2P imaging. The same brain regions will be imaged between P11 and P30, registered via the patterns of vasculature and labeled neurons (**Fig 8C**). Cortical layer (L) 2/3 vs L4 neurons will be imaged by focusing at $\sim 150 \mu\text{m}$ or $\sim 250 \mu\text{m}$ below the pia, respectively [78]. (4) Imaging data registered to electronic lab notebooks will be uploaded into a Cloud-based pipeline (all codes have been shared in GitHub; pipeline will be shared with the scientific community in the near future). All the data analysis files will also be uploaded to database (see Administrative Core).

Our data analysis pipeline assembled by DataJoint consists of the following steps (**Fig 9**): (1) Use the Suite2P toolbox [79] to process raw Ca^{2+} data for motion correction and to identify regions of interest (ROIs); (2) Manually inspect ROIs with defined criteria; (3) Employ the Fast Imaging Signal Separation Analysis (FISSA) toolbox for neuropil correction [80]; (4) Calculate dF/F0 and modified Z scores; (5) Analyze behavior videos (acquired simultaneously with 2P imaging) with DeepLabCut toolbox [82,83] to determine when pups are in *quiet wakefulness* (*stationary*) or *locomotion* states; (6) Ca^{2+} events are analyzed for spike amplitude (peak z-scores), frequency, event duration, and the total integrated Ca^{2+} influx, using methods developed in the Lu Lab. We perform pairwise correlations and synchrony to analyze the joint network activity and effective functional connectivity [84] (see **Fig 10** for example analyses). The entire analyses have been packaged and containerized into a reproducible and citable data pipeline for cloud-based deployment using the DataJoint software framework (see Supportive letter from DataJoint). In this framework, the data are shared through a structured database with web interfaces for data ingest (electronic lab notebooks), curation, and visualization and automated analysis for rigor and reproducibility. This will allow the C3A core to offer a scalable cloud-based deployment for access by the CA3 affiliates, collaborators, and the broader neuroscience community.

Example Project: Longitudinal 2P eCB and Ca^{2+} imaging in S1 L2-4 neurons in the same animals to reveal eCB dynamics in relationship to neural network activity during early postnatal ages and determine the impacts of perinatal THC exposure on eCB signaling and neural networks. Mouse S1 cortex is a useful model to study the development of cortical circuits and determine how perinatal exposure to cannabis or other illicit drugs alter their development. Neurons in mouse S1 cortex receive topographic projections from peripheral sensory organs such as

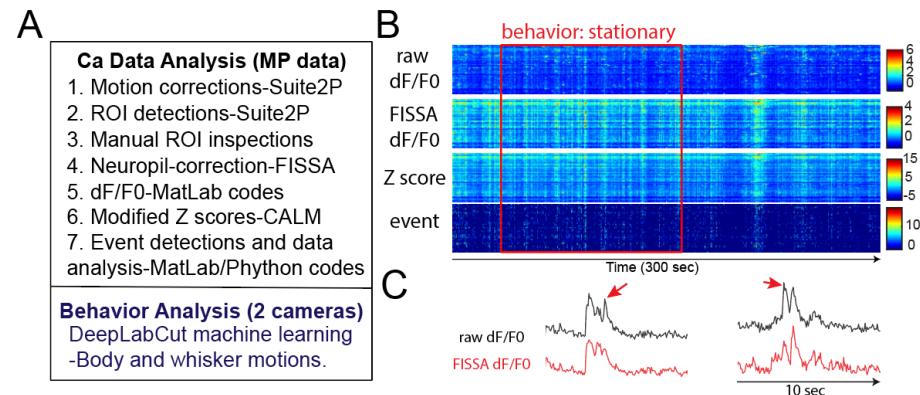


Fig 9 (A) Data analysis pipeline. (B) Example roaster plots after different steps of data analysis. (C) Traces from two representative ROIs show dF/F0 before and after FISSA.

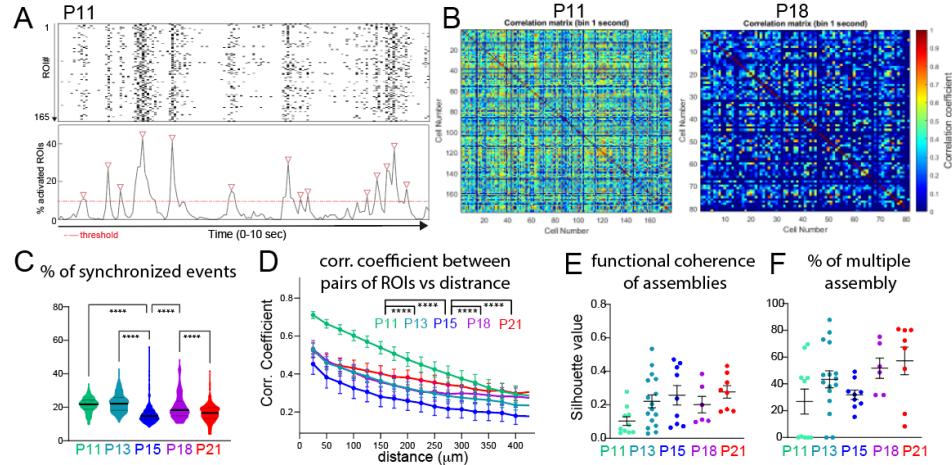


Fig 10 Preliminary *in vivo* 2P Ca^{2+} imaging data show spontaneous Ca^{2+} network synchrony in L4 at P11/15/18/21 from same mice. (A) Upper panel, a representative raster plot of detected calcium events from L4 neurons; lower panel shows the % of active ROIs as a function of time. Red dashed line, the threshold ($p=0.05$) for significant synchronization. (B) Correlation matrices displaying the correlation coefficients between individual ROI pairs for P11 and P18 from the same mouse. (C) Violin plots summarize the percentage of ROIs engaged in synchrony peaks. (D) Plotted correlation coefficients as a function of distance for ROI pairs. (E) Unsupervised K-means algorithm used to cluster functional neuronal assemblies. The higher silhouette value indicates greater similarity. (F) The percentage of neurons engaged in multiple functional assemblies.

the whiskers to create maps in the brain of the physical world [85-92]. Our extensive understanding of the organization and synaptic function of excitatory (E) and inhibitory (I) circuits in barrel columns makes this a great model system for understanding circuit development central to sensory processing, sensorimotor integration, and how abnormal environmental factors disrupt them (e.g. [93-96]). The ECS is required for proper development of cortical somatosensory circuits [7,46] and E/I balance [97]. Exogenous THC exposure disrupts S1 circuit formation and sensory processing [46,98].

The 2nd-3rd postnatal weeks are a critical time when S1 synaptogenesis, synaptic stabilization and refinement occur simultaneously to achieve optimal connectivity [87,93,99]. We will conduct 2P imaging to examine eCB and Ca²⁺ signals in awake behaving pups from P11 to P21 with our established surgical procedure and imaging paradigm (described above). We will first characterize the developmental changes in naïve CD-1 pups and then repeat with pups of both sexes after perinatal THC or vehicle exposure (see above and [17]). We aim to determine whether perinatal THC treatment perturbs the development of population eCB and/or Ca²⁺ events. We will characterize eCB signaling in developing S1 cortex for frequency and duration of individual events and the network relationships among neurons in different cortical layers and ages. Second, we will analyze Ca²⁺ events to quantitatively compare THC- vs vehicle-treated groups for the age-dependent changes in inter-event intervals (invert to frequencies), amplitudes, pair-wise correlation coefficient, and synchrony. Third, we will determine the relationship between eCB and Ca²⁺ signals. Such data will allow us to determine how perinatal THC exposure impacts developmental trajectories and whether there are sex-dependent differences.

Anticipated outcomes, potential problems, alternative approaches, and future directions: Our preliminary studies found by MS conducted by the BLMC that perinatal THC exposure reduces eCB in progenies' brains (Fig 6C) and the number of thalamic axons reaching S1 (data not shown). We hypothesize that cannabis exposure disrupts the ECS and establishment of cortical sensory circuits. We expect to see reduced frequency and duration of eCB signals in S1 cortical neurons of perinatally THC exposed pups. Such 2P eCB imaging will allow us to determine whether there are cortical layer specific changes and if such changes are transient or lasting. In preliminary experiments we were able to detect eCB signals at baseline (Fig 11). After 16 mg/kg JZL 184 (MAGL inhibitor), eCB signals were significant augmented and prolonged while inhibiting CB1R (10mg/kg SR141716) abolished almost all eCB signals. The 10-90% rise and half decay times of spontaneous eCB events were ~9.8 and 7.8 sec, respectively (Fig 11E,F). 60 mins JZL exposure significantly increased peak eCB amplitudes and decay times of eCB events while not affecting the rise time. These exciting preliminary observations suggest that the eCB2.1 sensor is a valuable tool to elucidate eCB dynamics in awake behaving CB1 wildtype mice. We don't anticipate technical difficulties to examine astrocyte eCBs with astrocyte-expressing eCB AAV driven by human GfaABC1D promoter [100].

Our preliminary Ca²⁺ data analyses with naïve pups reveal developmental changes in network properties in L2-4 in a layer-specific manner (L4 data are shown in Fig 10 as example). The degrees of correlation between ROI pairs in L4 for P11 and P18 from the same mouse are strikingly different (Fig 10B). Surprisingly, we observed a transient reduction in the percentage of neurons participating in synchronized Ca²⁺ events in L4 neurons at P15, which bounced back at P18 and then was reduced again at P21 (Fig 10C). At P11, the correlation coefficients are much higher for pairs of neurons in close proximity. However, such distance-dependent correlations are greatly reduced at P13, just 2 days later (Fig 10D). At P21, there is no significant

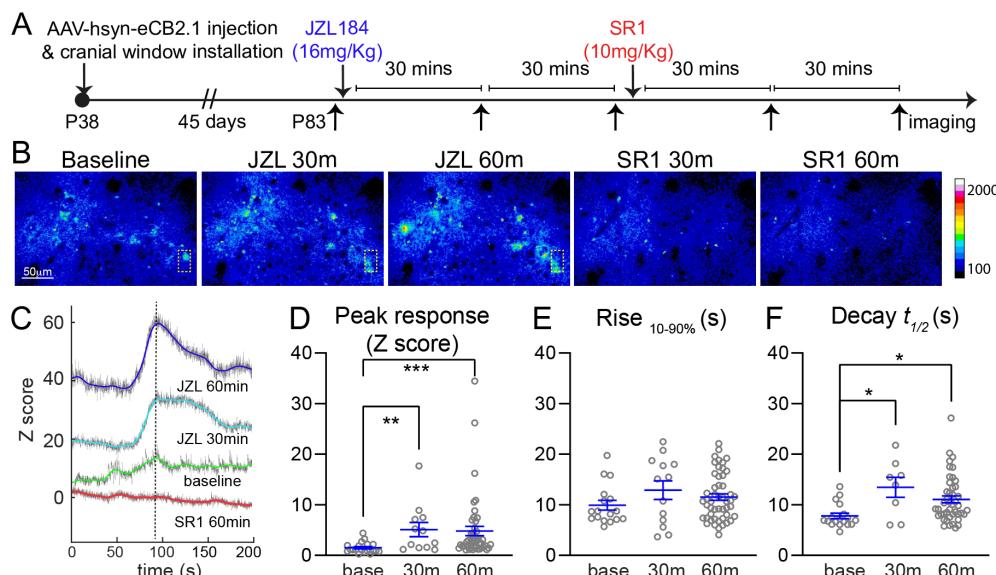


Fig 11 Example eCB dynamics in the S1 cortex of awaking behaving adult C57BL6 wildtype mouse. **(A)** Timeline for the experimental procedures. 5min per imaging session. **(B)** Example eCB signals from different imaging sessions of same mouse. Dashed boxes, ROI for quantifications showed in C. **(C)** The representative normalized eCB2.1 signal. **(D,E)** Summary for peak responses, rise and decay kinetics (mean±SEM) from 12 ROIs.

relationship between distance and correlation coefficient. The unsupervised K-means algorithm was used to cluster functional neuronal assemblies in an unbiased manner (**Fig 10E-F**). We found a developmental increase in silhouette value, which suggests greater similarity with age (**Fig 10E**). The percentage of neurons engaged in multiple functional assemblies increased from P11-P21 (**Fig 10F**). Our preliminary data suggest GABAergic maturation during this time critically guides the development of the S1 neural network (data not shown). Reducing GABAergic neuron excitability using chemogenetics (a DREADD approach) increased the percentage of synchronized neurons. THC exposure perturbs GABAergic maturation [101]. *Thus, we expect perinatal THC exposure will alter synchronous operation in neuronal networks.*

Typical 2P service & hours supporting C3A investigators, affiliates and pilot projects: We will provide required tools, equipment, and comprehensive training for surgical, imaging, data upload and Cloud-based data analysis using the pipeline established by DataJoint in collaboration with the Lu lab (see Supportive Letter). 1-2 projects will be served per yr. Steps: 1st, form testable hypothesis with investigators, design experimental strategies and consult/troubleshoot to carry out region/cell type-specific sensor labeling in PI's own lab (80-120hrs); 2nd, confirm desired sensor expression with PI's own experimental data evaluated with standard imaging (20hrs); 3rd, the core will receive the experimental mice and conduct rAAV injections if needed; 4th, 1st six-week trip, the visiting scientist will receive hands-on training with detailed protocols to conduct surgical installation of the cranial window (80hrs) and 2P imaging (24hrs); 5th, trained scientist will install cranial window with assistance (20hrs) and then install cranial windows independently; 6th, 2nd 6 week trip, the visiting scientist will be trained the 2nd time for 2P imaging and data analysis (40hrs) and receive ~160 hrs of 2P time; 6th, all image data will be deposited and analyzed with DataJoint cloud-based data analysis pipeline; 7th, ensuring appropriate data deposition, discussion, and interpretation (40 hrs). Estimated time to complete one project: ~6-12 months. The core will continue to optimize imaging protocols and data analysis for eCB2.1, eCB-AEA, eCB-2AG sensors.

Aim 4. Develop *in vivo* protocols for Fluorescence Lifetime Imaging Microscopy (FLIM) in addiction research. Neurons and astrocytes are tightly coupled for both metabolism and synaptic transmission [102-104]. Astrocytic processes, together with presynaptic and postsynaptic neuronal compartments, form tripartite complexes [102,105,106]. The degree of astrocytic coverage of synapses influences energy homeostasis and synaptic function [103,107,108]. Neuroinflammation has been observed after chronic exposure to drugs of abuse [109-111]. The Mackie lab found adolescent THC exposure led to sustained increase in the number of reactive astrocytes in adult male mPFC (DA053746), consistent with previous observations [112,113]. Upon activation, astrocytes undergo significant morphologic and metabolic changes and alter their interactions with neurons. CB₁ receptors are present in astrocytes and regulate the tripartite synapse [114,115]. Jimenez-Blasco et al. (2020) [116] found that activation of mitochondrial associated CB₁ (mtCB₁) in astrocytes impairs glucose metabolism and lactate production in the brain, altering neuronal function and social interaction behaviors. Preliminary data from Lu lab found that postnatal THC alters astrocyte morphology. Unpublished RNA seq data from the Mackie lab demonstrates that perinatal THC treatment affected expression of many genes, playing critical roles in establishing and maintaining the tripartite synapse [117] in adult mPFC. Taken together, data from C3A core investigators suggest that chronic THC exposure results in defective neuron-astrocyte interactions.

FLIM measures the amount of time a fluorophore spends in the excited state [74,76,118], different from conventional measurements of fluorescence intensity. When analyte binding to a biosensor changes the decay rate from the excited to the ground state of the fluorophore, this is detected as a change of lifetime. Because the lifetime of fluorescence is independent of fluorescence intensity, there is no need to normalize for expression levels or to correct for photobleaching. Bypassing the requirement for ratiometric imaging (required for Förster resonance energy transfer (FRET) imaging) allows multiplex imaging of different

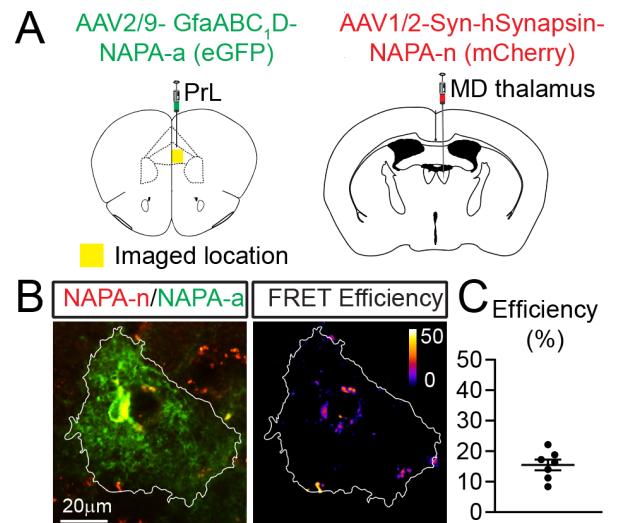


Fig 12 (A) Schematic diagram showing the viral injection positions for astrocyte-synapse proximity assay on thalamocortical projections by using the first-generation NAPA sensors. PrL: prefrontal. MD thalamus: medial dorsal thalamus. **(B)** Representative images of NAPA-n and NAPA-a and FRET efficiency. The white outline represents the estimated edge of astrocyte territory based on NAPA-a expression. **(C)** Quantification of FRET efficiency from 7 astrocytes. Mean ± SEM.

biosensors. A growing number of FLIM-based sensors have been developed to probe energy metabolism, signaling cascades, and protein-protein interactions. Using 2p to conduct FLIM imaging will enable us to determine how intracellular biochemical or energy metabolic states are altered by cannabis/cannabinoids or abused drugs with high spatiotemporal resolution in living brains. Additionally, 2pFLIM is great in capturing autofluorescence signals from cellular components to investigate dynamic physiological changes in live cells and tissues [119], such as redox state with autofluorescence from nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) and flavin adenine dinucleotide (FAD) [120]. We aim to establish 2pFLIM protocols for acute brain slices and awake behaving mice to measure energy metabolism and the interactions between synapses and astrocytic processes.

General method: We will start 2pFLIM experiments with acute adult mouse mPFC brain slices for ex vivo live imaging using a Nikon A1R MP+ with a 25x (NA1.1) water immersion lens. For biosensors generating specific and robust signals in brain slices, we will custom-make rAAVs expressing selective biosensors for testing. Several methods will be optimized to visualize energy metabolism: (A) Examining cytosolic NADH-NAD(+) redox state with NADH/NAD FLIM sensor [121,122]; (B) monitoring glucose changes with SF-iGluSnFR, a FRET sensor (C) measuring NAD(P)H autofluorescence; (D) imaging ATP and H₂O₂ with TFP, a newly developed FLIM sensor for ATP/H₂O₂ [123].

Next, the genetically-targeted neuron-astrocyte proximity assay (NAPA) will be employed to determine dynamic interactions between astrocytes and neurons. NAPA is an imaging-based neuron-astrocyte proximity assay utilizing FRET between astrocyte processes (labeled by NAPA-a, eGFP) and presynaptic terminals (labeled by NAPA-n, mCherry) [124]. This method has been established with intensity-based confocal microscopy. However, several drawbacks challenge its use in intact brain [125]. We will employ 2pFLIM for NAPA to better quantify the FRET efficiency by comparing the fluorescence lifetime of the donor in the presence or the absence of acceptor. 2pFLIM will allow us to distinguish interacting vs noninteracting donors and obtain independent information about the percentage of interactions to estimate their separation. Specifically, we will analyze NAPA-a and NAPA-n colocalization area, FRET area, and FRET efficiency by using Imaris and ImageJ, guided by published literature [124,126-128]. Specifically, the ratio of the acceptor- to the donor-labeled proteins influences FRET efficiency in the intermolecular FRET measurement from independently expressed proteins. The astrocytic territory volume will be further quantified by using Imaris surface module as described in the literature [124,126,129]. Brain slices or brains will be fixed for post-hoc analysis with immunostaining to confirm expression of NAPA-n and NAPA-a in neurons and astrocytes.

Anticipated outcomes, interpretations, limitations, and pitfalls. The Lu lab has had great success with NAD/NADH, ATP/ADP biosensor imaging of cortical neurons for research projects supported by NINDS NS086794 (data not shown). Despite the absence of a FLIM module, we successfully tested the NAPA assay with 2p-FRET imaging and acquired sufficient FRET efficiency to estimate astrocyte-synapse proximity between mPFC astrocytes and axons originating from the mediodorsal thalamus (**Fig 12**), an important connection for affective behaviors [130,131]. We will consult with Dr. Ryohei Yasuda (a pioneer of FLIM imaging) and Dr. Kenneth Dunn (see supportive letters) for data analysis and technical challenges arising (see supporting letters).

Summary: The MSIC will support **Center investigators, affiliates, and trainees** spanning a range of career stages to learn the conceptual and technical know-how of these imaging techniques, and to provide access to state-of-the-art equipment for **nanoscale molecular** measurements, for **microscale anatomical** analysis of subcellular and cellular profiles and signaling, and for **mesoscale physiological** imaging of brain circuits. We anticipate that defining the molecular, cellular, and physiological consequences of chronic exposure to drugs of abuse in a correlated manner will lead to significant advances in the mechanisms underlying substance use disorders and will aid the development of more specific and effective treatment strategies.

Workflow: Drs. Katona and Lu will work with steering committee to select projects based on scientific value, projected impact on the addiction field, and feasibility. Specific training plans, timelines for various experimental steps, and milestones will be jointly established together with PIs of accepted proposals and Drs. Barna and Huang, the two technical experts of the MSIC. Selected trainees will go through all the necessary compliance-related training and be added to C3A approved IBC/IACUC protocols. All MSIC members will be involved in designing experimental approaches and data interpretation with C3A investigators, affiliates, and trainees. Drs. Barna, Huang, and Franco will train them for surgery, imaging and data analysis required for their projects. Dr. Franco will schedule the visits, conduct Scale-S cleaning and imaging procedure, order/transfer mice, conduct rAAV injections and assist in surgery/imaging. Upon complication of the proposed projects/training, PIs are expected to submit a detailed scientific report to Drs Katona, Lu, Mackie, and the advisory board members.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

Yes No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved

Yes No

Is the Project Exempt from Federal regulations?

Yes No

Exemption Number

1 2 3 4 5 6 7 8

Other Requested Information

VERTEBRATE ANIMALS

Mice will be housed and used in the Multiple Science Building-II (MSB-II) at Indiana University Bloomington. All experiments will be conducted in compliance with the NIH guide (as appropriate) and following institutional approval. The Animal Welfare Assurance number for IU Bloomington is D16-00587.

We estimate the animal numbers based on the nature of experimental procedures and time required for individual projects.

Here are the estimated experimental numbers for each Aim:

Aim1-STORM: 5 animals per sex per group for 2 groups per project. We estimate for 10 projects per year.

Aim1-Teaching: 3 mice per trainee and there will be 8 trainees per course for 4 courses per year.

Aim2-Scale S: 8-10 animals for 1 project per year ((~3 weeks 2P time per yr).

Aim3-ex vivo experiments: 4 animals per sex per group for 2 groups and 1-2 projects per year (12 weeks 2P time per yr).

Aim3-in vivo experiments for pilot projects: 6 animals per sex per group for 2 groups and 1-2 project per year (~12weeks 2P time per yr).

Aim4-FLIM: 8 animals per sex per group for 2 groups and 1 project per year (6 weeks 2P time per yr).

1. Description of Procedures

Species: Mice

Strains: CD1, C57BL6, or FVB/C57BL6 mixed

Age: early postnatal ages through adult (3-5 months) for testing or adult (3-10 months) for breeding

Sex: Male and Female

Number: 3500 mice (to provide ~2000 mice for five years of proposed experiments)

A total of ~2000 mice will be used over 5 years for experimental and teaching procedures. These mice will be bred in MSBII. The total number of mice required for all aspects of the study is estimated to be ~3500 mice. This number includes breeders, isolated mice for line maintenance, littermates of the incorrect genotype for a specific study, sickness/death, experimental failure, sacrificed to maintain similar number of pups per dam, etc. ~50% mice will be transferred into IUB from C3A affiliates or approved PIs for MultiScale Imaging Core-approved projects a few weeks before experimental procedure. Thus, those mice won't require breeding.

Wildtype and transgenic strains for proposed example experiments:

1. CD-1 wild-type mice
2. C57BL/6
3. Pet1-Cre:TdTomato mice
4. Other transgenic mice selected for the approved projects. The service nature of the Multi-Scale Imaging Core makes it impossible to list out the transgenic mice that will be employed for selected projects over five years.

Mice will be treated as outlined in the experimental plan and then anesthetized prior to brain slice preparation or perfusion following approved protocols. Housing will be standard grouped housing.

This number of experimental mice (~2000) reflects using an average of 35 mice per month for 12 months per year for 5 years. Animals will be bred in MSB-II following IACUC-approved breeding protocols to generate mice for adolescent phytocannabinoid treatment. CD1 mice will be purchased from Charles River and C57BL/6 mice from JAX to replenish our breeding stocks.

Below is a brief description of animal procedures. Please note that these methods are provided here in response to the PHS398 instructions which request *"Provide a concise description of the proposed procedures to be used that involve live vertebrate animals in the work outlined in the "Research Strategy" attachment. The description must include sufficient detail to allow evaluation of the procedures."* These procedures are also presented in the experimental plan and the references cited within and no new details are presented here.

Tissue harvesting. Brains will be harvested after utilizing an AVMA-approved euthanasia technique.

Administration of substances:

Vehicle, THC, and other compounds will be given by subcutaneous (s.c.) or intraperitoneal (i.p.) injection in a volume of 5-10 μ l/g body weight as approved by our animal care and use committee.

Sterotaxic AAV injections:

P30-60 mice will be anesthetized and prepared for their aseptic surgical procedure. After stereotaxic determination of the coordinates of the medial dorsal thalamus, a single burr hole per injection site will be drilled in the skull while taking care not to damage the dura. Using an automated syringe pump and a 30 gauge needle mounted on the stereotaxic frame, the needle will be advanced to the appropriate coordinates and the desired amount of rAAV-containing solution (< 1 μ l per injection) will be injected at a rate of 100 nL/min as described (1,2).

Newborn AAV injection procedure

For intraventricular (icv) injection, sterile saline containing recombinant AAVs will be injected freehand into both the left and right lateral ventricles of P0/P1 pups which have been anesthetized with hypothermia (5, 6). ~0.2-1 μ l volume will be injected into each ventricle via a Hamilton syringe. After injections, pups will be placed on heating pad until they regain normal color and resume movement before being returned to their moms. For stereotaxic AAV injection, pups or young adult mice will be anesthetized and head-fixed on animal holder . A single burr hole will be drilled in the skull allowing the entrance of pulled-glass capillary or 30 gauge needle connecting to an automated syringe pump mounted on the stereotaxic frame to advance to S1 area according to the coordinates. ~0.2-1 μ l rAAV will be injected at a rate of 100 nL/min as described.

Cranial window installation

This procedure is conducted as described (3,4). Briefly, an anesthetized mouse will be placed in an animal holder, secured with ear bars and kept warm by using a commercial mouse heating pad with temperature controller. After cutting open the scalp over the top of the skull, a ~4 mm in diameter hole will be drilled through the skull with a micro-drill. Next, a sterile glass cover slip will be placed on top of the dura mater. A drop of cyanocrylate-based glue will be applied to secure the cover slip. A customized head frame is placed on top of the cranial window and fixed in place with dental cement.

Two-photon imaging with an awake behaving mouse

To reduce stress during imaging, mice will be handled or trained multiple days to habituate to head-restraint and walking on an air-supported floating platform. Briefly, the mouse is taken from its home cage and gently restrained to secure the head bar on top of the mouse's head into the head holder on top of the stage connected to the Two-photon microscope while allowing it to move freely on the floating platform.

2. Justification for the Use of Animals

The use of animals (mice) is necessary for this project because the project seeks to elucidate the mechanisms underlying neural circuit formation and how it may be disrupted in the intact brain and the subsequent behavioral implications . Mice are chosen because of the similarities between mouse and human neurodevelopment (5,6) and the availability of numerous transgenic lines, which allows us to elucidate underlying molecular mechanisms. Mice are also the standard experimental animal for neural circuit mapping, brain slice electrophysiology, neuroanatomy, pharmacology, and immunohistochemistry. No existing computer simulations are sophisticated enough to address the questions to be studied in this proposal.

Breeding colonies for the mice that we will use for these studies have already been established in the MSBII animal facility at Indiana University Bloomington. The number of mice used will be the minimum necessary to validate the experimental observations and are guided by our extensive experience with these types of studies (Dr. Katona and Dr. Lu collectively have more than 40 years of experience working with mice). As we are conducting these experiments, we will continuously monitor our results and adjust numbers of animals used as

appropriate. We will also perform interim power analyses to verify sample sizes once experiments are underway and as guided by initial effect sizes and observed variance.

3. Procedures to Minimize Pain and Distress

All procedures are consistent with the NIH Guide for the Care and Use of Laboratory Animals and follow the guidelines of the International Association for the Study of Pain. In all cases, mice will receive ad lib access to food and water in their home cages. Moreover, mice in our study will not experience undue pain, injury, or discomfort. For electrophysiology or anatomical studies, prior to decapitation, the animal will be deeply anesthetized with isoflurane administered via inhalation. The mouse will be visually monitored during this procedure and when unconscious, the depth of anesthesia will be assessed by the absence of a response to noxious stimulation (pinching of a paw).

If unexpected health findings are observed, we will consult with one of IUB's veterinarians.

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6. Lewis DA, Levitt P. Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci*. 2002. **25**: p.409-32.

MULTIPLE PI LEADERSHIP PLAN

A multiple PD/PI approach is being used for the Multi-Scale Imaging Core (MSIC) to adequately reflect the complementary skills and unique contributions made by Dr. Katona (many years of experience in super resolution imaging and in elucidating the anatomical localization of various components of the endocannabinoid system as well as expertise in addiction biology) and Dr. Lu (extensive research experience and expertise in neural circuit development, how sensory/environmental factors like cannabis/cannabinoids impact both the anatomy and function of neural circuits, and *in vivo* multiphoton imaging). As outlined in the proposal, several collaborative projects are planned to take advantage of our distinct but complementary strengths. The idea for the present project builds upon our shared interests in the fundamental mechanisms underlying addictive behaviors and how these mechanisms are modified by environmental factors such as long-term use of cannabis or opioids. This mutual interest is expected to manifest in many fruitful collaborations. This is important, because in light of the emerging trends in interdisciplinary neuroscience research, it is expected that most proposals will require multi-scale imaging modalities, incorporating both Drs. Katona's and Lu's expertise, to address fundamental questions in addiction biology.

Drs. Katona and Lu will jointly review the research proposals submitted to the C3A Multi-Scale Imaging Core that propose imaging experiments and will consult with the PIs of these proposals on experimental design and/or training logistics. We anticipate that many proposals will require multi-scale imaging modalities to expand insights into addiction biology. Together with Drs. Barna and Huang, training plans, timelines for various experimental steps, and milestones will be jointly established. They will also coordinate to ensure trainees will receive the necessary compliance-related training and are added to the approved IBC/IACUC protocols before arriving at IUB. Dr. Katona will be primarily responsible for **Aim 1**, while Dr. Lu will take charge of **Aims 2-4**. The two PI's will share the same Administrative Core program assistant. This individual will facilitate purchase of supplies/animals and oversee the core's fiscal management. Dr. Mackie, the C3A lead PI will ultimately oversee the core and its integration with the other C3A core. He also serves as the contact PI for submission of progress reports to the NIH and all formal communications with the NIH.

The Katona and Lu offices and labs are on the same floor and are adjacent to each other. This facilitates efficient communication. In addition, they will hold weekly, primarily face-to-face discussions, on the planning and execution of the C3A-supported affiliate and pilot projects. In particular, they will communicate their thoughts on experimental design, training plans, data analyses and scientific discoveries. Both PI's will share their research findings through their currently ongoing bi-weekly joint lab meetings. Authorship will be decided based on the relative contributions of personnel to a particular study. Personnel from the Katona and Lu and labs already extensively interact with connected, shared laboratory space/equipment, a common biweekly lab meeting, adjacent office space, as well as through biweekly seminars with outside speakers and a monthly journal club organized around the themes of neurodevelopment, drugs of abuse, molecular pharmacology, pain, etc.

Intellectual property

The Technology Transfer Offices at Indiana University, Bloomington will be responsible for preparing and negotiating any issues relating to intellectual property. These will follow the relevant PHS policies in force at the time the research is conducted.

Conflict resolution

If a conflict develops, the PIs shall meet and attempt to resolve the dispute. If they fail to resolve the dispute, the disagreement shall be referred to an arbitration committee consisting of one

senior executive chosen by each PI and a third impartial senior executive mutually agreed on by both PI's. No members of the arbitration committee will be directly involved in the research grant or disagreement.

Change in PI location

If a PI moves to a new institution, every attempt will be made to transfer the relevant portion of the grant to the new institution. In the event that a PI cannot carry out his/her duties, a new PI will be recruited as a replacement from Indiana University.

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LETTERS OF SUPPORT

Please see the Overall Component for Letters of Support

RESOURCE AND DATA SHARING PLAN

Please see the Overall Component for the Resource and Data Sharing Plan

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Validation of antibodies:

We have more than thirty-five years-experience in applying antibodies against components of the endocannabinoid and other neurotransmitter systems. Many of the antibodies widely used in the cannabinoid field were generated and validated in the Mackie lab (the PI for administrative core). Antibodies against protein epitopes are generally validated in the following way in our laboratories:

1. If KO is available, then this is our preferred test, using the same application (e.g., if the antibody is to be used for Western blotting, then it is tested for Western blotting using KO tissue, if for ICC, then it is tested in ICC using the same conditions (e.g., fixation and detection)) that will be used. Occasionally we will use shRNA knockdown of protein expression in cultures as an alternative approach.
2. If a KO is not available, then we will use two antibodies directed against different epitopes. A similar staining or detection pattern is taken as strong evidence that the antibodies recognizing the same protein.
3. All antibodies from Mackie Lab will be tested by detection of epitope-tagged protein expressed in HEK or similar cells (overlap of epitope tag and antibody being tested is expected) and block by immunizing protein.
4. When using GFP antibodies, native GFP fluorescence is compared to signal (e.g., fluorescence) from GFP antibody staining of adjacent sections, to ensure qualitative similarity.
5. We check to make sure commercially purchased antibodies have undergone rigorous screening and have an RRID.

Validation of fluorescent small molecules for PharmacoSTORM imaging:

Fluorescent small molecules, the pharmacoprobes will be performed in two independent manners:

1. Using brain slices from knockout mice of the target protein. These slices will be prepared, incubated, imaged and analyzed in parallel with brain slices obtained from wild-type animals.
2. Using ex vivo and in vivo displacement assays. Pretreatment of live mice or acute brain slices with excess concentration of the unlabeled pharmacoprobes readily displaces the subsequent binding of the fluorescent pharmacoprobe.

Validation of transgenic animals used in these experiments:

For tissue-specific Cre transgenic lines, we will cross them to reporter mouse lines such as the Ai9 (tdTomato) (JAX mice Stock No:007909) or mGFP lines (JAX mice Stock No:004077). The distribution of tdTomato or GFP positive cells in the progeny carrying one copy of Cre and one copy of reporter allele will be examined with multiple staining to confirm the brain regions and cell types in which recombination occurs. We have these mouse lines in the IUB vivarium.

Validation of recombinant adeno-associated viral vectors (rAAVs):

We have more than ten years of experience in injecting AAVs into live mice to label or manipulate specific neuronal populations. All of the rAAVs that will be used in this project have been documented with extensive literature and will be acquired from a reputable commercial source such as Addgene. The expression of target genes as well as viral transduction efficacy will be evaluated with immunostaining.

Here are a list of potential rAAVs that we will use in MSIC:

From Addgene (this company has internal quality control procedures including lot numbers and titer measurements): pAAV.Syn.GCaMP6f.WPRE.SV40 (#100837-AAV1), pAAV.hSyn.Cre.WPRE.hGH (#105553-AAV9), pAAV.CAG.Flex.NES-jRGECO1a.WPRE.SV40 (#100852-AAV1), pAAV.CAG.Flex.GCaMP6f.WPRE.SV40 (100835-AAV9), pAAV.Syn.NES-jRGECO1a.WPRE.SV40 (100854-AAV9), and pAAV.Syn.eCB2.1 (gift from Dr. Yulong Li, Peking University).

Validation of novel chemical compounds:

No novel compounds will be used in these studies. All chemicals used will be obtained from commercial companies and accompanied by a certificate of analysis.

**APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)**

5. APPLICANT INFORMATION**UEI***: YH86RTW2YVJ4

Legal Name*: TRUSTEES OF INDIANA UNIVERSITY
 Department:
 Division:
 Street1*: 509 E 3RD ST
 Street2:
 City*: BLOOMINGTON
 County: MONROE
 State*: IN: Indiana
 Province:
 Country*: USA: UNITED STATES
 ZIP / Postal Code*: 474013654

Person to be contacted on matters involving this application

Prefix: First Name*: Middle Name*: Last Name*: Suffix:
 Mr. STEVEN ALLEN MARTIN

Position/Title: ASSOCIATE VP FOR RESEARCH ADMINISTRATION

Street1*: 509 E 3RD ST

Street2:

City*: BLOOMINGTON

County: MONROE

State*: IN: Indiana

Province:

Country*: USA: UNITED STATES

ZIP / Postal Code*: 474013654

Phone Number*: 317-278-3473

Fax Number:

Email: IUAWARD@IU.EDU

7. TYPE OF APPLICANT*

H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type Women Owned Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*

Bioactive Lipid Mediators Core (BLMC)

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2023	06/30/2028

Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: TRUSTEES OF INDIANA UNIVERSITY

UEI: YH86RTW2YVJ4

Street1*: 702 North Walnut Grove Ave

Street2:

City*: BLOOMINGTON

County: MONROE

State*: IN: Indiana

Province:

Country*: USA: UNITED STATES

Zip / Postal Code*: 474052204

Project/Performance Site Congressional District*: IN-009

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* Yes No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes No

If YES, check appropriate exemption number: — 1 — 2 — 3 — 4 — 5 — 6 — 7 — 8

If NO, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* Yes No**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an Yes No environmental assessment (EA) or environmental impact statement (EIS) been performed?

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* Yes No

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* 220922_BLMC_abstract.pdf**8. Project Narrative*****9. Bibliography & References Cited** 2022_BLMC_references_20220927.pdf**10. Facilities & Other Resources** Facilities_OtherResources.pdf**11. Equipment** Equipment.pdf

The *Bioactive Lipid Mediators Core* (BLMC) is designed to support the ongoing work of affiliated scientists aiming to understand the systemic and neurophysiological outcomes of chronic drug use on lipid signaling. The focus on lipid biomarkers targets an emerging field that has unique promise to add a novel framework for understanding how lipid signaling in a wide range of tissues (e.g., plasma, CNS, liver, breast milk) is related to homeostatic dysregulation associated with the development and maintenance of drug use disorders. The Bradshaw lab has developed lipid extraction and analytical techniques aimed specifically at small molecule lipid metabolites like the endocannabinoids, lipoamines, prostaglandins, leukotrienes, and resolvins. These classes of lipids have been shown to change in response to exposure to drugs of abuse and understanding this regulation has the potential to provide unique insight into how both acute and chronic drug use drives both systemic and central nervous system changes. This understanding would then drive the discovery of novel therapies to treat drug addiction. We will accomplish this by 1) Evaluation of plasma lipidomics patterns in multiple drug abuse models. Using animal models of drug use (THC, opioids, alcohol) we will use our optimized analytical techniques for analysis of bioactive lipid metabolite signaling molecules in plasma to determine how acute and chronic drug exposure causes systemic changes in these lipid classes. This analysis technique will then be used to determine how endogenous cannabinoid and related lipid signaling molecules changes in different disease states (PTSD, concussion, drug addiction) in human plasma. 2) Analyze sex difference in bioactive lipids in targeted areas of the CNS with chronic drug use as a function of genetic sex. This will provide a novel framework to evaluate unique characteristics across and between drug models and sex. 3) Identification of changes in bioactive lipids in breast milk with exposure to drugs of abuse (THC, opioids). Lipidomics analysis of breastmilk in rodent models of drug use (THC, opioids) will provide a clearer understanding on how the presence of these drugs changes the lipid profile of breastmilk in a way that may have long term health consequences for offspring. 4) Develop data science analytical techniques of bioactive lipid metabolites to drive novel hypotheses on how drugs of abuse cause changes in systemic and CNS lipid signaling. Coupling the power of increasingly powerful analytical techniques with novel data mining and cluster analyses developed in-house will provide the field with new tools for interpreting lipid metabolomic information. 5) Increase the pipeline for underrepresented minority (URM) students for careers in STEM with a focus on lipidomic mass spectrometric techniques. In partnership with minority serving programs at IU, URM students will be provided unique opportunities for training and research that are tailored to the field of lipidomics and mass spectrometry as well as multiple programmatic opportunities for professional development.

FACILITIES AND OTHER RESOURCES

Please see the Overall Component for Facilities and Other Resources

EQUIPMENT

Please see the Overall Component for Equipment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Heather	Middle Name Bryte	Last Name*: Bradshaw	Suffix: Ph.D
Position/Title*:	Professor			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	1101 East 10th Street			
Street2:				
City*:	Bloomington			
County:	MONROE			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	812-856-1559		Fax Number:	
E-Mail*:	hbbradsh@indiana.edu			
Credential, e.g., agency login:	HBBRADSH			
Project Role*:	Other (Specify)		Other Project Role Category: Core Lead	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Clare	Middle Name Therese	Last Name*: Johnson	Suffix:
Position/Title*:	Research Associate			
Organization Name*:	Trustees of Indiana University			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	515 W 6th St. Apt 3			
Street2:				
City*:	Bloomington			
County:				
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474040000			
Phone Number*:	8282428128		Fax Number:	
E-Mail*:	clthjohn@iu.edu			
Credential, e.g., agency login: claretheresejohnson				
Project Role*:	Other (Specify)		Other Project Role Category: Core Technical Staff	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Mehmet	Middle Name	Last Name*: Dalkilic	Suffix: Ph.D
Position/Title*:	Professor			
Organization Name*:	Trustees of Indiana University			
Department:				
Division:				
Street1*:	700 N Woodlawn Ave			
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City*:	Bloomington			
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E-Mail*:	dalkilic@indiana.edu			
Credential, e.g., agency login: dalkilic				
Project Role*:	Other (Specify)		Other Project Role Category: Core Technical Staff	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Heather		Bradshaw	Ph.D	PD/PI	119,710.00		2.0	1.0	39,175.00	14,070.00	53,245.00
2.	Mehmet		Dalkilic	Ph.D	Data Management	140,406.00		1.0		0.00	0.00	0.00
3.	Clare		Johnson		Research Associate	50,000.00	12.0			50,000.00	19,970.00	69,970.00

Total Funds Requested for all Senior Key Persons in the attached fileAdditional Senior Key Persons: File Name: Total Senior/Key Person 123,215.00**B. Other Personnel**

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students		16.9		40,500.00	2,819.00	43,319.00
	Secretarial/Clerical						
1	Research Data Scientist	5.6			18,000.00	1,253.00	19,253.00
4	Total Number Other Personnel					Total Other Personnel	62,572.00
					Total Salary, Wages and Fringe Benefits (A+B)		185,787.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

	Funds Requested (\$)*
1. SCIEX TRIPLE QUAD 7500 SYSTEM	177,801.00
2. Nitrogen Generator	1,855.00
3. Zero Air Generator	2,038.00

Total funds requested for all equipment listed in the attached file**Total Equipment** **181,694.00**

Additional Equipment: File Name:

D. Travel**Funds Requested (\$)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	

Total Travel Cost **0.00****E. Participant/Trainee Support Costs****Funds Requested (\$)***

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs** **0.00**

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	40,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	1,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
	Total Other Direct Costs
	41,000.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	408,481.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	226,787.00	132,670.00
	Total Indirect Costs			
	132,670.00			
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	541,151.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	541,151.00

L. Budget Justification*	File Name:
	MS_Core_budget_justification_20220906.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2024**End Date*:** 06-30-2025**Budget Period:** 2**A. Senior/Key Person**

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Heather		Bradshaw	Ph.D	PD/PI	122,104.00		2.0	1.0	39,959.00	14,351.00	54,310.00
2.	Mehmet		Dalkilic	Ph.D	Data Management	143,214.00		1.0		0.00	0.00	0.00
3.	Clare		Johnson		Research Associate	51,000.00	12.0			51,000.00	20,369.00	71,369.00

Total Funds Requested for all Senior Key Persons in the attached fileAdditional Senior Key Persons: File Name: **Total Senior/Key Person** **125,679.00****B. Other Personnel**

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students	16.9			41,310.00	2,875.00	44,185.00
	Secretarial/Clerical						
1	Research Data Scientist	5.6			18,360.00	1,278.00	19,638.00
4	Total Number Other Personnel					Total Other Personnel	63,823.00
					Total Salary, Wages and Fringe Benefits (A+B)		189,502.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	40,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	1,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
	Total Other Direct Costs
	41,000.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	230,502.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	230,502.00	134,844.00
	Total Indirect Costs			
	134,844.00			
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	365,346.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	365,346.00

L. Budget Justification*	File Name:
	MS_Core_budget_justification_20220906.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2025**End Date*:** 06-30-2026**Budget Period:** 3**A. Senior/Key Person**

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Heather		Bradshaw	Ph.D	PD/PI	124,546.00		2.0	1.0	40,758.00	14,639.00	55,397.00
2.	Mehmet		Dalkilic	Ph.D	Data Management	146,078.00		1.0		0.00	0.00	0.00
3.	Clare		Johnson		Research Associate	52,020.00	12.0			52,020.00	20,777.00	72,797.00

Total Funds Requested for all Senior Key Persons in the attached fileAdditional Senior Key Persons: File Name: **Total Senior/Key Person** **128,194.00****B. Other Personnel**

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students		16.9		42,136.00	2,933.00	45,069.00
	Secretarial/Clerical						
1	Research Data Scientist	5.6			18,727.00	1,303.00	20,030.00
4	Total Number Other Personnel					Total Other Personnel	65,099.00
					Total Salary, Wages and Fringe Benefits (A+B)		193,293.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	40,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	1,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
	Total Other Direct Costs
	41,000.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	234,293.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1. MTDC		58.5	234,293.00	137,061.00
	Total Indirect Costs			
	137,061.00			
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)
	371,354.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	371,354.00

L. Budget Justification*	File Name:
	MS_Core_budget_justification_20220906.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2026**End Date*:** 06-30-2027**Budget Period:** 4

A. Senior/Key Person												
	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Heather		Bradshaw	Ph.D	PD/PI	127,037.00		2.0	1.0	41,573.00	14,931.00	56,504.00
2.	Mehmet		Dalkilic	Ph.D	Data Management	149,000.00		1.0		0.00	0.00	0.00
3.	Clare		Johnson		Research Associate		12.0			53,060.00	21,192.00	74,252.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons: File Name:										Total Senior/Key Person		130,756.00

B. Other Personnel									
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*		
	Post Doctoral Associates								
	Graduate Students								
3	Undergraduate Students		16.9		42,979.00	2,991.00	45,970.00		
	Secretarial/Clerical								
1	Research Data Scientist		5.6		19,102.00	1,329.00	20,431.00		
4	Total Number Other Personnel					Total Other Personnel	66,401.00		
					Total Salary, Wages and Fringe Benefits (A+B)		197,157.00		

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 4

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 4

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	40,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	1,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
	Total Other Direct Costs
	41,000.00

G. Direct Costs	Funds Requested (\$)*
	Total Direct Costs (A thru F)
	238,157.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	238,157.00	139,322.00
	Total Indirect Costs			
	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
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)
	377,479.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	377,479.00

L. Budget Justification*	File Name:
	MS_Core_budget_justification_20220906.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY**Start Date*:** 07-01-2027**End Date*:** 06-30-2028**Budget Period:** 5

A. Senior/Key Person												
	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Heather		Bradshaw	Ph.D	PD/PI	129,578.00		2.0	1.0	42,404.00	15,230.00	57,634.00
2.	Mehmet		Dalkilic	Ph.D	Data Management	151,980.00		1.0		0.00	0.00	0.00
3.	Clare		Johnson		Research Associate	54,122.00	12.0			54,122.00	21,616.00	75,738.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	133,372.00
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B. Other Personnel						
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits* Funds Requested (\$)*
	Post Doctoral Associates					
	Graduate Students					
3	Undergraduate Students		16.9		43,839.00	3,051.00 46,890.00
	Secretarial/Clerical					
1	Research Data Scientist	5.6			19,484.00	1,356.00 20,840.00
4	Total Number Other Personnel					Total Other Personnel 67,730.00
						Total Salary, Wages and Fringe Benefits (A+B) 201,102.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	40,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	1,000.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	41,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	242,102.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1. MTDC		58.5	242,102.00	141,630.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	383,732.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	383,732.00

L. Budget Justification*	File Name:
	MS_Core_budget_justification_20220906.pdf

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

BUDGET JUSTIFICATION

A. Senior/Key Personnel:

Dr. Heather Bradshaw (effort: 2 Academic Months and 1 Summer Month): Dr. Heather Bradshaw will be responsible for the development and oversight of the BLM core, personnel, training, fiscal management, and research compliance. She will work with C3A research affiliates and PIs of approved pilot grants to optimize experimental design, data collection, data analysis, and summaries of experimental outcomes. She will be a member of the IUB C3A internal advisory board. She will develop and implement an 8-week summer training workshop in lipidomic mass spectrometric techniques targeted for up to 8 students who are underrepresented minorities in STEM associated with the long-running Summer Stem Scholars program through IU. Dr. Bradshaw will be the C3A liaison to the SSI program, which is campus program that began in 2007 and is focused on providing summer research experiences for underrepresented minorities at IU and those from Minority Serving Institutions (see details in Admin core and letters of support).

Dr. Mehmet Dalkilic (effort: 1 Academic Month): Dr. Dalkilic will be responsible for the development, design, and oversight of the data science tools for large-scale lipidomics analyses. He will be responsible for the training and oversight of the Research Data Scientist (see hourly position below) who will implement the network analyses of these large lipidomic data sets. Dr. Dalkilic will take part in monthly data discussions with the C3A team and advise as appropriate on data science aspects for each of the C3A cores. Dr. Dalkilic's effort will be paid by institutional funds, and thus, no salary support is requested.

B. Other Personnel:

Ms. Clare Johnson, Research Associate (12 Calendar Months). Ms. Johnson is a senior graduate student in the Bradshaw lab who plans to continue in a non-tenure track research role after she graduates in December 2022. She has 5 years of training and implementation of lipid extraction and mass spectrometric techniques and data analysis. She will be responsible for purchasing, coordination of mass spectrometric running schedules with affiliated scientists and performing lipidomics analysis.

Undergraduate research assistants, TBD (12 Calendar, hourly). TBD (12 Calendar, hourly). As part of the training component with the summer course in mass spectrometry aimed at underrepresented minorities in STEM (see Admin Core description), 3-4 students per year will be employed to perform tasks associated with lipid extractions and mass spectrometric analysis from this group of students.

Research Data Scientist, TBD (12 Calendar, hourly). This individual will be recruited from the Data Science undergraduate or graduate program and will support the development and design of novel data tools for the analysis of large scale lipidomics data sets. They will be directly supervised by Dr. Dalkilic; however, they will also report to Dr. Bradshaw and present to the C3A research team during monthly data meetings.

Fringe Benefits:

The fringe benefit rates are set by Indiana University and approved by the Board of Trustees. For the PI, the academic year fringe rate is 39.94% of the requested salary. For the Research Associate, the fringe rate is 39.04% of the requested salary. For the hourly students, the maximum fringe rate is 6.96% of the requested salary.

C. Equipment

1) SCIEX Triple Quad 7500 System – QTRAP and UHPLC and 5-year service contracts: (\$177,801 [25% of \$711,205]). This instrumentation is essential for the implementation of the goals of the BLM core. The mass spectrometric analysis system currently in use by Dr. Bradshaw (API 3000) is at maximum capacity with current and projected projects and the advanced technology of the API 7500 will allow for both an increase in output capabilities but also an evolution in analytic capabilities. See attached quote for additional details. In brief, this is an enhanced high performance hybrid triple quadrupole LC-MS/MS mass spectrometer with linear

ion trap enabled. QTRAP License is installed. This system offers a mass range of m/z 5 to 2000 in triple quadrupole mode and 50-2000 in LIT mode. Includes Optiflow Pro Ion Source, Optiflow Pro ESI tower with analytical probe and E-Lens optimized for flow rates 200-1000 μ L/min. Addition options available for alternate flow rates and ionization modes. System includes integrated syringe pump and a 6 port, 2 position switching valve. Two wet pumps (roughing pumps) included. System ships with Data Acquisition Computer running Windows 10 64 bit and standard monitor. SCIEX OS Software is required for operation and must be purchased separately. Instrument will not operate without the purchase of the SCIEX OS software. Limited Warranty: Standard parts and labor warranty for one year starting from the completion of instrument commissioning {as provided in SCIEX's written limited warranty statement and accompanying terms in the user manual or other product documentation}. Includes our StatusScope Remote Monitoring Service and one no-charge Preventative Maintenance (PM) during the one-year warranty period. In addition, the price includes PROTECTPLUS 1PM|QT7500 4 Year, which extends the total coverage to 5 years. CONFIGURED EXIONLC AD SYSTEM Component based high performance UHPLC system. Includes the following ExionLC components: ExionLC Controller, ExionLC Degasser, ExionLC Tray, 2 x ExionLC AD Pumps, ExionLC AD Autosampler, ExionLC AD Column Oven. The package includes 5 solvent bottles, 5 solid caps and solvent lines, Auxilliary I/O synch cable and a 20 μ L binary micro mixer module. Plus, 5 years of ExionLC AD service contract.

2) Nitrogen generator (\$1,855 [25% of \$7,418.]); Liberty Gas Systems. This is a requirement for the gas needs for the API 7500.

3) Zero air generator (\$2,038 [25% of \$8,150.00]); Fisher Scientific. This is a requirement for the gas needs for the API 7500.

D. Other Direct Costs

1. Materials and Supplies (\$40,000 annually for 5 years)

Consumable reagents for lipid extraction and partial purification (e.g. solid phase extraction columns, analytical columns, HPLC-grade solvents, autosampler vials, Preppy apparatus, filters for gas generation instruments). Blood and tissue extraction supplies (e.g. vacuum tubes, needles, scalpels), liquid nitrogen for dissections and storage.

4. ADP / Computer Services

GraphPad and SPSS for data analysis (\$1,000 per year for all years).

5. Subawards/Consortium/Contractual Costs

None

6. Other Expenses

None

INDIRECT COSTS

The indirect cost rate for Indiana University is set by DHHS and was negotiated on 5/22/2019 at 58.5% for research, excluding capital equipment, subawards greater than \$25,000, and graduate student fee remissions.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	641,216.00
Section B, Other Personnel	325,625.00
Total Number Other Personnel	20
Total Salary, Wages and Fringe Benefits (A+B)	966,841.00
Section C, Equipment	181,694.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	205,000.00
1. Materials and Supplies	200,000.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	5,000.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other 1	0.00
9. Other 2	0.00
10. Other 3	0.00
11. Other 4	0.00
12. Other 5	0.00
13. Other 6	0.00
14. Other 7	0.00
15. Other 8	0.00
16. Other 9	0.00
17. Other 10	0.00
Section G, Direct Costs (A thru F)	1,353,535.00
Section H, Indirect Costs	685,527.00

Section I, Total Direct and Indirect Costs (G + H)	2,039,062.00
Section J, Fee	0.00
Section K, Total Direct and Fee (I + J)	2,039,062.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 09/30/2024

1. Vertebrate Animals Section

Are vertebrate animals euthanized? Yes No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

Yes No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

Yes No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount (\$) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? Yes No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? Yes No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: Yes No

If the answer is "Yes" then please answer the following:

*Previously Reported: Yes No

6. Change of Investigator/Change of Institution Section

Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	2022_BLMC_intro_20220927.pdf
Research Plan Section	
2. Specific Aims	20220923_BLMC_SA.pdf
3. Research Strategy*	2022_BLMC_research_strategy_20220927.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	VERTEBRATE_ANIMALS_BLMC-final.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	LettersOfSupport.pdf
10. Resource Sharing Plan(s)	Resource_DataSharing.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication.pdf
Appendix	
12. Appendix	

Introduction – Core 2 (Bioactive Lipid Mediators Core)

We thank the reviewers for their enthusiasm for this aspect of the P30 proposal and for the thoughtful comments aimed at specifically increasing the rigor and feasibility of running this type of core facility as well as leveraging the findings from projects in this core to drive hypotheses that can be addressed in the imaging core and beyond. Key concerns are addressed here.

1. How to effectively manage multiple, perhaps competing demands on core time.

We appreciate this concern and have worked with the data analysts on the project (see *Administrative Core*) to develop a system for project management and workflow that includes predicted timelines and instrument usage. By using an “Electronic Project Notebook” (EPN), each project will be tracked from its inception with Affiliates to the endpoint of data analysis (see *updated “Programmatic Design” section for detailed outlines*).

2. Collaborative projects are too ambitious with 100's of animals proposed.

We agree that the proposal was ambitious. Core availability for processing specific tissues will be under continual review for feasibility as discussed above and in the revised “Programmatic Design” section. We have reduced the number of initial Affiliates; however, it is still necessary to continually monitor Affiliate needs with realistic data output timelines. It is important to highlight that the animal models being proposed, and the tissue collection are being done in Affiliate labs and not in the core; therefore, the central focus by the BLMC is on the processing and analysis of these tissues. Using the EPN workflow will be a key aspect of regulating when tissues can be shipped and analyzed for each of these projects to manage expectations and project output. Currently, the Bradshaw lab processes ~1000-1500 samples per year, including preliminary projects to assess feasibility. Therefore, with the additional MS instrument, staff, and increased effort percentage by the PI proposed here, we do not foresee any bottle necks in processing and analyzing up to twice that number.

3. Integration of projects between BLMC and Core 1 (Multiscale Imaging Core)

Several proposed BLMC studies exploit the natural synergy between the cores. There is now a clearer description of how these projects integrate. In addition, we have implemented a process to discuss potential new integrative studies in our monthly data meetings so specific findings (e.g., a novel finding of dynamic changes in endocannabinoids in a specific CNS area from an Affiliate’s animal model investigating drugs of abuse) would be referred to the imaging core to work with the Affiliate to design an imaging study to determine possible underlying circuit-specific enzymatic mechanisms driving these dynamic changes.

4. Measurement of tissue levels of THC and metabolites is not proposed.

This type of analysis is routinely done in the Bradshaw lab, and we expect to continue to incorporate these analyses in many projects [1-5]. An example of detection of THC, CBD, and their metabolites is shown in the proposal (Figure 3 of the BLM Core section) and several more listed in the planned experiments.

5. More structure for summer lipids course.

The course structure by week is outlined in the Administrative Core, **Aim 3**, Section A. The structure of this course is augmented by the weekly structure of the Summer Stem Initiative program that has been run through IU campus administration each summer since 2007 (see *letter of support*).

6. Not enough senior scientist (PI) oversight for core 2.

The PI is extremely appreciative that this was pointed out. Dr. Bradshaw’s percent effort and budget have been increased to allow for a teaching release giving her more time and effort to oversee the core projects.

7. Are lipid measurements exploratory or hypothesis-driven?

Most, if not all, of the studies proposed here are both hypothesis-driven and exploratory. Only about 30% of the lipid species (*both endogenous and phytocannabinoids*) in a typical screen using our current equipment have known targets. This means that we can hypothesize how changes in their regulation would change signaling and treatment outcomes; however, evaluating how the additional ~70% of lipids without known targets would change in these screens is exploratory. With the greater sensitivity of the new HPLC/MS/MS instrument, we will be able to add more lipid species with known targets that are lower in abundance and traditionally harder to evaluate with current instrumentation (e.g., oxylipins, epoxides, bile acids, HETEs, HPETEs) to inspire hypothesis-driven questions as well as add to the exploratory nature of these types of screens with additional low-abundance or as yet unknown lipid species.

Bioactive Lipid Mediators Core Specific Aims

The bioactive lipid mediators core (BLMC) is designed to support the ongoing work of associated scientists (referred to herein as Affiliates) aiming to understand the systemic and neurophysiological outcomes of chronic drug use on lipid signaling. The focus on lipid biomarkers targets an emerging field that has unique promise to add a novel framework for understanding how lipid signaling in a wide range of tissues (e.g., plasma, CNS, liver, breast milk) is related to homeostatic dysregulation often associated with the development and maintenance of drug use disorders. The Bradshaw lab has developed lipid extraction and analytical techniques aimed specifically at small molecule lipid metabolites like the endocannabinoids, lipoamines, prostaglandins, leukotrienes, and resolvins. These classes of lipids have been shown to change in response to exposure to drugs of abuse and understanding their regulation has the potential to provide unique insights into how both acute and chronic drug use drives changes in systemic and CNS physiology. These insights would then help guide the discovery of novel therapies to treat drug addiction.

Aim 1: Determine plasma lipidomics patterns in preclinical drug abuse models and human samples. The use of plasma biomarkers is an important component of understanding and predicting disease, formulating therapies, and predicting therapeutic responses. Here, first using animal models of drug use (THC, opioids, alcohol) we will apply our optimized analytical techniques to analyze bioactive lipid metabolite signaling molecules in plasma to determine how acute and chronic drug exposure causes systemic changes in these lipids. This analysis technique will be extended to determine how endogenous cannabinoids and related lipid signaling molecules change in disease states (e.g., PTSD, concussion, drug addiction) in human plasma. Comparisons of plasma lipid biomarkers in these animal model and human disease systems will drive novel hypotheses on using plasma lipids biomarkers as predictors of disease progression or therapeutic response.

Aim 2: Characterize sex differences in bioactive lipids in the CNS in models of chronic drug use. Emerging data from multiple levels of analysis demonstrate sex differences in neurophysiological and behavioral aspects of drug use. Evaluation of bioactive lipid mediators in targeted areas of the CNS in multiple models of drug use (THC, opioids, alcohol) as a function of genetic sex will provide a novel framework to evaluate unique characteristics across and between drug models and sex. Identification of sex differences in lipid biomarkers associated with models of drug use will provide novel hypotheses towards understanding these unique neurophysiological differences that can be exploited for novel therapies.

Aim 3: Characterize changes in bioactive lipids in breast milk with exposure to drugs of abuse. Recent data have shown that the lipidome of breastmilk has a broader complexity than previously theorized. Endocannabinoids and oxylipins play a significant role in infant health and human and rodent data show that drugs of abuse like THC readily concentrate in breastmilk. Lipidomics analysis of breastmilk in rodent models of drug use will (THC, opioids) will provide a clearer understanding on how the presence of these drugs changes the lipid profile of breastmilk in a way that may have long term health consequences for offspring.

Aim 4: Expand our current bioactive lipid library and apply data science analytical techniques for bioactive lipid metabolites to drive novel hypotheses on how drugs of abuse cause changes in systemic and CNS lipid signaling. The enhanced analytical technology in the API 7500 will allow for an expansion of the number of small molecule bioactive lipids that can be analyzed from each sample, which will simultaneously reduce overall run times and increase the power of the lipidomics analyses. Coupling the increased power of analytical techniques with novel data mining and cluster analyses developed in-house will provide the field with new tools for interpreting lipid metabolomic information. Models will be developed using lipidomics data from cell-based assays that allow for rapid and economical responses to drug exposure on specific cell types (C6 glioma, BV2 microglia, N18 neuronal). These analysis models will then be applied to analyze animal and human studies to generate novel hypotheses on the intersections of lipids, genetic sex, drug use, and disease.

Aim 5: Increasing the pipeline of underrepresented minority (URM) students prepared for careers in STEM, with a focus on lipidomic mass spectrometric techniques. In partnership with minority serving programs at IUB, URM students will be provided unique opportunities for structured summer training and research that are tailored to the field of lipidomics and mass spectrometry. Then, during the academic year students will supplement their summer learning with funded, hands-on research experiences. For the summer component, these students will be immersed in a fully developed STEM Summer Scholars Institute program that began in 2008 at IU and partners with Minority Serving Institutions to provide research opportunities and professional development programming.

Research Strategy

Bioactive Lipid Mediators Core (BLMC) Programmatic Design

Since 2008, research in the Bradshaw lab has focused on small molecule lipidomics and operated as a model of collaborative science. To date, we have published with 30 unique laboratories [1,5-33], have 2 additional laboratory contributions in preprint [34,35], and 5 additional collaborative projects ongoing. My laboratory has essentially been functioning as a lipidomics mass spectrometric core for the entirety of my career at Indiana University, which uniquely prepares me for developing and implementing the BLMC. It's been my philosophy that to understand how lipid signaling functions, we need to investigate this functioning in a variety of different model systems. As the BLMC, we will focus lipidomics analyses on models of drug abuse from a wide range of collaborators (C3A Affiliates), which will provide an important opportunity to combine data across multiple model systems that will both test and drive novel hypotheses. The BLMC will also provide a lipidomics resource to those who are studying the underlying endogenous systems that are utilized by drugs of abuse (e.g., endogenous cannabinoids, endogenous opioids) to enhance their research efforts. Together, each of the research models described here will provide novel lipidomics information that can be used for understanding how lipid signaling plays an important role in addiction.

Working within the premise that lipidomics data will provide novel information to advance research, we have developed a set of protocols for hypothesis driven experimental and exploratory study designs with collaborators using the following key procedural outcomes.

- 1) What are the key questions that lipidomics can answer in their model system?
- 2) What type of tissue would provide key information about the experimental question (e.g., plasma, brain, spinal cord, liver, isolated cells)?
- 3) How much material can be harvested in their model system?
- 4) What is the most feasible means of harvesting that tissue that will maintain the integrity of the lipidome?
- 5) What are the procedures for tissue harvesting to ensure consistency?
- 6) What is the process for labeling tissue that maintains blindness while processing and a backup of the labeled sample codes?
- 7) What is the expected timeline for completion of analysis?
- 8) What are the authorship expectations?

This process is accompanied by phone or Zoom meetings to work out design specific details and finalized with a written agreement using the “electronic project notebook” (EPN) system established for each project through our IU data science partners (see *Administrative core*). In addition to transparent project management for Affiliates and core labs, the EPN will manage blinding procedures (e.g., generating a randomized notation for samples), timelines, and overall data management. Affiliates will load information (e.g., subjects, treatment timelines, tissue types, and tissue harvesting times) and the BLMC will load lipidomics data, both raw and analyzed, into the EPN. Project workflow using the EPN will allow us to know when samples will be harvested from our affiliates and to better schedule mass spectrometric run times so that all samples in large batches are analyzed in succession. Adding an additional instrument will decrease the overall run time for each large batch; however, it remains an important parameter that all the same type of analytes be analyzed on the same instrument within the same time period. The EPN will contain an updated MS analysis scheduling system that tracks when a project’s samples were processed for lipid extraction through endpoint statistical analysis, which will allow each member of the research team to stay updated on research progress. This will also allow for realistic expectations for when samples can be analyzed for Affiliates, BLMC members, and our IAB.

Understanding what can and cannot be learned from lipidomic data is also a key aspect of experimental design and part of the front-end discussions with collaborators. If the procedural steps listed above are followed, our established analytical processes allow us to determine when a specific lipid species in our lipidomics library is increased or decreased in a specific tissue type compared to a baseline control and/or another treatment. Being consistent with expectations from each side of the collaboration is important as is maintaining good communication, including manuscript preparation, and authorship. In addition to the expectation that both sides of the collaboration will embargo external discussion of data until publication unless given permission, there will be the understanding that all data gathered through the BLMC will be used to create a larger lipidomics database aimed at determining patterns of lipid signaling molecule regulation with acute and chronic drug use across multiple modalities and as a function of genetic sex and drug dose. Any publication from these secondary analyses will also include authorship from collaborators; however, it will be understood that these analyses are separate from the primary analyses that are linked to the specific research model of the researcher.

A. Significance: Bioactive lipid metabolites as markers for and effectors of drug use disorders

Lipidomic techniques that allowed the scientific community to isolate the endogenous cannabinoid (eCB) ligands Anandamide (AEA; [36]) and 2-arachidonoyl glycerol (2-AG;[37,38]) encompasses multiple processes by which lipid species in biological systems are identified and characterized. These techniques have allowed the scientific community to discover a vast new world of molecules that were heretofore unknown and still represents a wide-open frontier. Metabolomics is the umbrella term under which lipidomics often resides and recognizes that bioactive lipids are not formed directly from the genome like proteins but are formed as the products of enzymes. Therefore, the number of lipid species in an organism in specific contexts is arguably exponential to those of the many proteins, in that a single enzyme can potentially interact with 10s or even 100s of different lipids. We recently outlined how this process is working for selected enzymes specifically associated with the biosynthesis and metabolism of AEA and 2-AG (e.g., NAPE-PLD, FAAH, MAGL; Fig. 1) and highlighted the problems that emerge if a field views these individual enzymes as being only involved in metabolizing endogenous cannabinoids [39]. In that review we summarized the data showing that there are significantly more bioactive lipids (theoretically hundreds) whose levels are modulated by those enzymes in addition to the two eCB ligands that are the desired “targets” of pharmacological enzyme modulation [4,40-42]. Therefore, the focus on one or two bioactive lipids as a representative for an entire class is not informative or accurate when the goal is to understand how a drug is affecting the entire organism.

A primary focus of the Bradshaw laboratory is research into a class of lipids that are metabolites of phospholipids, of which the eCBs, AEA and 2-AG, are the most studied of the species. The largest subgroup of these eCB congeners are structural analogs of AEA of which there are over 80 identified species. These congeners are very biologically active and promiscuous: Many activate diverse GPCRs and TRP channels, though some remain orphan ligands [21,43,44]. Many of the AEA congeners are metabolites of *N*-acyl ethanolamines (NAEs) with FAAH being a rate-limiting enzyme in their synthesis ([41,45,46]; Fig. 1). In addition, our lab has also shown that THC, CBD, and synthetic CBs significantly regulate levels of this class of lipids across the CNS [2-4].

We arguably have the largest working library of these lipid species, as most are still not commercially available and we have published extensively characterizing these lipids in a variety of model systems [6-11,32,47,48]. While no other labs are focused primarily on broad scale lipidomics of this class of lipids, numerous additional labs are studying members of this class of lipids adding to our understanding of their importance as ubiquitous signaling molecules. With this ubiquity, it is not surprising that this field has the potential to be confusing. In the latest count, researchers have assigned at least 13 different names to describe this specific class of lipids across the scientific literature: acyl amide [49], alkylamide [50], amides of amino acids [51], endocannabinoid-like [52,53], fatty acid amide [19,54-69], fatty acyl amide [70], lipid amide [71], lipid-related compound [72], lipoamino acid [73-83], *N*-acyl amide [84-90], *N*-acyl amino acid [91-95], and *N*-fatty acyl amino acid [96]. The Bradshaw lab has used an umbrella term of “lipoamines” in an attempt to consolidate these terms [6,8,48,97,98]; however, recognize that a nomenclature consensus has not been met. Regardless of the lack of an official nomenclature, what remains consistent is that *this class of lipid metabolites is ubiquitous, understudied, and are regulated by disease and drugs of abuse*.

We also investigate oxylipins, another major class of bioactive lipid metabolites that have a more agreed upon umbrella term. These are a broad category of oxygenated metabolites of fatty acids with the major subtypes being prostaglandins, leukotrienes, bile acids, and resolvins produced via the major enzyme families: COX, LOX, and cytochrome P450s (CYPs) and are implicated in a wide range of disease models [98-101]. Each of these subcategories has, at minimum, 10s of species and likely have many undiscovered species given that reports show that lipoamines and 2-acyl glycerol species are, likewise, metabolized by COX and CYPs [102,103]. Methodological limitations have constrained the broad scale study of these lipid metabolites, wherein specialist researchers largely focus on just one subclass and not all of them. *Here, we will combine the analysis of each of these lipid classes to drive novel hypotheses.*

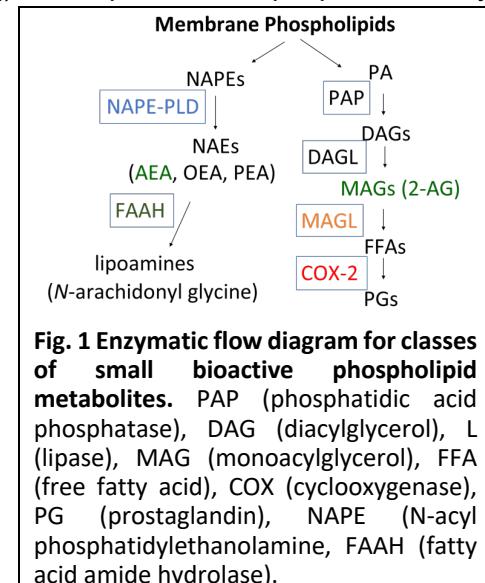


Fig. 1 Enzymatic flow diagram for classes of small bioactive phospholipid metabolites. PAP (phosphatidic acid phosphatase), DAG (diacylglycerol), L (lipase), MAG (monoacylglycerol), FFA (free fatty acid), COX (cyclooxygenase), PG (prostaglandin), NAPE (*N*-acyl phosphatidylethanolamine), FAAH (fatty acid amide hydrolase).

Innovation

The increased analytical power of the API 7500 will completely change the paradigm we have been operating under, allowing for the analysis of hundreds of small molecule signaling lipids from a single sample and in a fraction of the time of previous techniques. **Fig. 2A** are data provided by Applied Biosystems Sciex analytical team upon request that illustrates an example of the leap in sensitivity of the API 7500, which has only been in production within the past year, compared to the API 6000, which has been in production for less than 5 years. The Bradshaw lab has extensive experience with Sciex instruments and has long working experience with the API 3000, API 4000, and the QSTAR TOF. Technical notes provided on the API 7500 mirror the data in **Fig. 2A** and state that the increase in sensitivity translates into 30% more lipid metabolites being detected in the same plasma sample.

It is also important to note that the samples in the demonstration in **Fig. 2A** underwent no partial purification via solid phase extraction columns, illustrating the instrument's level of increased sensitivity. However, even with the overall increase in sensitivity, the signal to noise for lower abundance lipid metabolites would be challenging to measure in such a complex matrix without partial purification. We recently summarized the importance of this type of sample preparation for the measurement of small molecule lipid metabolites and explained the importance of sample preparation targeted to these specific lipid classes [104]. **Fig 2B** illustrates the current sensitivity of a 20 μ l injection into an API300 from 25 μ l of plasma using our optimized extraction and partial purification techniques in the Bradshaw lab. A logical question would be why not simply increase the injection volume to 50 μ l and/or dry down and reconstitute the purified sample to get a better signal. However, this is where the limitations of the original API electrospray ionization cause a significant drop in sensitivity due to the global effect of "signal suppression". In the original design, the more your matrix is concentrated, the higher the noise and the less likely you are to measure the signal. The significantly modified filtering system of the API 7500 allows for matrix to be more complex and to more effectively filter out the mass range of molecules to analyze, which is why so many analytes can be detected without the need for purification and matrix dilution. Therefore, used together, coupling the increases in sensitivity gained by the API 7500 with our advances in sample preparation, we can use these tools to maximize the identification and measurement of multiple classes of lipids that are particularly difficult to measure and to measure more lipids in smaller samples, such as specialized brain regions like the nucleus accumbens core and shell. Bringing together a team of at least 30 different labs with a focus on understanding how drugs of abuse change molecular, cellular, and behavioral responses will allow us to generate substantial and useful databases of how lipid signaling molecules are regulated within and between each of these different model systems.

C. Approach

Aim 1: Determine plasma lipidomics patterns in preclinical drug abuse models and human samples. The need for identification and characterization of plasma biomarkers of disease has a long history in biomedical research. Much of the focus in plasma lipidomics analysis has been on key proteins and classes of lipids such as phospholipids, cholesterol, ceramides, and triglycerides [89,105-107]. However, analysis of lipid metabolites, like endocannabinoids and oxylipins, as markers of disease [98-101] highlight the value in targeting these signaling molecules as specific predictive biomarkers for disease. An important translational mechanism in identifying these biomarkers is the evaluation of systemic changes in lipid signaling molecules in plasma. The Bradshaw lab recently showed that use of plasma lipid biomarkers (including the eCBs, especially 2-AG and its analogs, and free fatty acids) in patients with chronic gastroparesis motility disorder identified those subjects with more severe symptoms and in some cases these changes were sex-dependent [6].

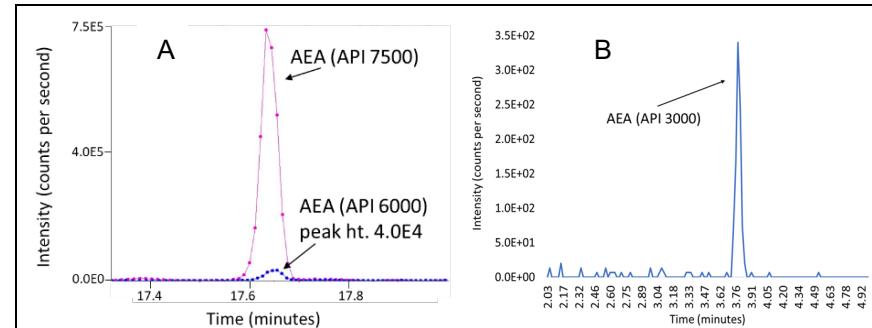
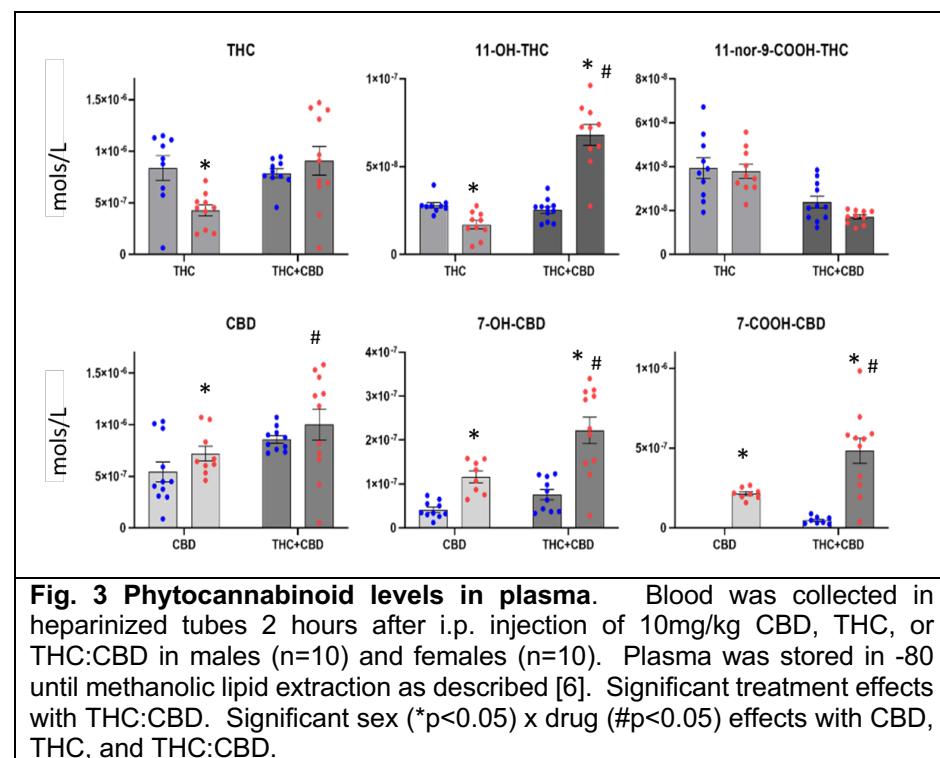


Fig. 2 Comparisons of AEA method used on plasma between Sciex API 7500, Sciex API 6000, and API 3000. **A)** Data are from equivalent amounts of NIST human plasma (100 μ l) dissolved in methanol, supernatant dried, and reconstituted in 50 μ l in 50:50 water: methanol and 50 μ l injected on a C18 Phenomenex analytical column. The pink line represents the output from the API 7500 and the blue line is the API 6000. **B)** Data are from a 20 μ l injection on a Zorbex C18 column from 25 μ l of plasma partially purified on C18 solid phase extraction column and eluted in 1.5ml 100% methanol. The identical parent and fragment pairs [H+]348/62 in positive ion mode were used for analysis in each instrument.

How drugs of abuse drive changes in these plasma biomarkers will provide important information on both acute and long-term changes occurring in these systemically active signaling molecules. **Fig 3** provides a novel insight into one way in which the CBs, THC and CBD, function differently between males and females. Both THC and CBD are metabolized by CYP enzymes and here we show that females have significantly more THC and CBD metabolites than males in plasma at 2 hours post CB treatment. We hypothesize that a key component of the mechanism of action of THC and CBD is its broad effect on the lipidome, though previous work was done primarily in tissue samples and the relationship to plasma levels was not established [4]. Here, in **Fig. 4** we show that plasma lipids are also modified 2 hours after 10 mg/kg THC or THC+CBD injection IP and that some of these changes are sex dependent. Through a series of novel studies in collaboration with C3A core and Affiliate labs, plasma lipidomics will determine changes in bioactive signaling lipids as a function of different drug abuse modalities (opioids, cannabinoids, alcohol) and disease (mTBI, PTSD, chronic pain). In studies using mouse models, blood will be collected via tail snip, which has been shown to be the least invasive and reliable blood collection procedure in mice [108] and a final time via core blood at sacrifice. In studies using rat models, blood will be collected via tail vein and a final time via core blood at sacrifice [109,110]. All blood collection materials and shipping costs will be provided by C3A. Blood is collected in heparinized tubes and rapidly separated via centrifugation, with plasma collected and stored in -80°C until lipid extraction. In studies using human plasma, samples will be collected using standardized phlebotomy and will be anonymized, lack personal identifiers, and stored at -80°C until lipid extraction. All plasma will undergo partial purification through methanolic extraction and solid phase extraction as previously described [6]. The overall experimental design will allow us to monitor ~100-200 bioactive lipids in plasma per sample at different time points using different treatment modalities. Standard analytical techniques will be used as previously described [42]. In addition, data will be analyzed using novel data science techniques



determine changes in bioactive signaling lipids as a function of different drug abuse modalities (opioids, cannabinoids, alcohol) and disease (mTBI, PTSD, chronic pain). In studies using mouse models, blood will be collected via tail snip, which has been shown to be the least invasive and reliable blood collection procedure in mice [108] and a final time via core blood at sacrifice. In studies using rat models, blood will be collected via tail vein and a final time via core blood at sacrifice [109,110]. All blood collection materials and shipping costs will be provided by C3A. Blood is collected in heparinized tubes and rapidly separated via centrifugation, with plasma collected and stored in -80°C until lipid extraction. In studies using human plasma, samples will be collected using standardized phlebotomy and will be anonymized, lack personal identifiers, and stored at -80°C until lipid extraction. All plasma will undergo partial purification through methanolic extraction and solid phase extraction as previously described [6]. The overall experimental design will allow us to monitor ~100-200 bioactive lipids in plasma per sample at different time points using different treatment modalities. Standard analytical techniques will be used as previously described [42]. In addition, data will be analyzed using novel data science techniques

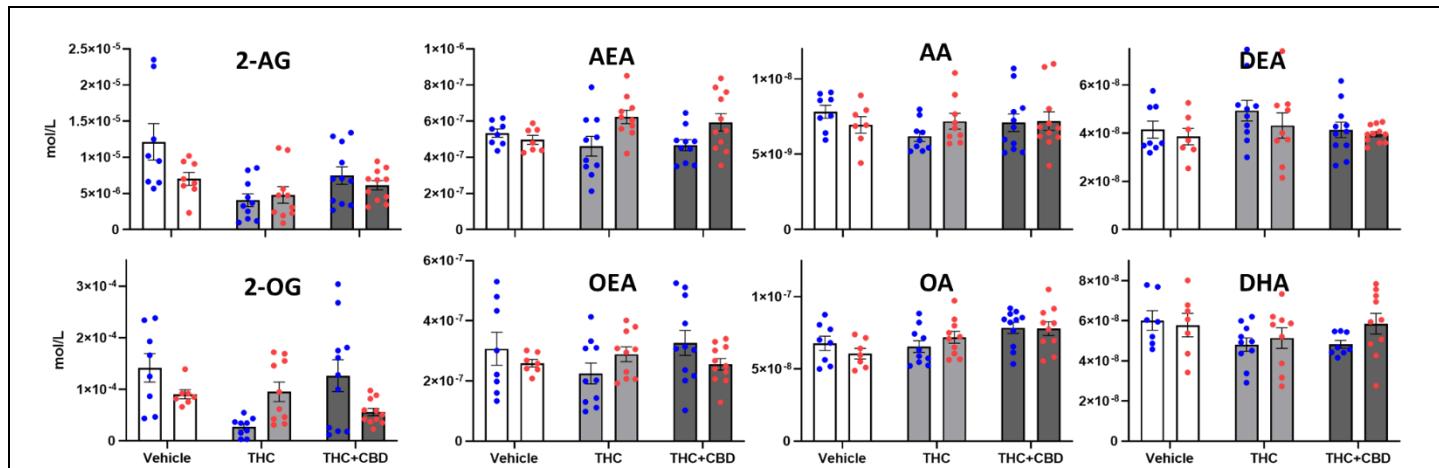


Fig 4. Plasma lipidomics after treatment with THC or THC+CBD. Blood was collected in heparinized tubes 2 hours after i.p. injection of 10mg/kg THC or THC:CBD in males (n=10) and females (n=10). Plasma was stored in -80 until methanolic lipid extraction as described [6]. Significant overall drug effects were measured in 2-AG, 2-OG, and OEA; whereas, sex x drug effects were measured in 2-AG, AEA, AA, 2-OG, OEA, and DHA. In addition, sex effects at baseline (vehicle) were detected for 2-AG and 2-OG.

to uncover novel interactions and networks (see *data science analytics in Aim 4*). Following are several projects proposed for this aim that are tightly integrated with currently funded or pilot projects.

Study 1.1: Evaluation of plasma lipids after morphine and CB2 agonist exposure. C3A core grant “CB2 Cannabinoid Mechanisms for Suppressing Opioid Tolerance and Dependence” (DA047858), showed that a CB2 agonist blocked the development of opioid tolerance in a mouse model of chemotherapy-induced peripheral neuropathy (CIPN). In male mice with established neuropathy, the CB2 agonist LY2828360 (LY) Phase 1 (LY injection on days 1-12; *phase I*) blocked the development of tolerance to morphine administered in Phase 2 (morphine injection on days 16-27; *phase II*) [111,112]. However, LY given during phase I was ineffective in blocking morphine tolerance *in females*. Here, we will use this same testing strategy (CIPN; Phase I LY; Phase II morphine) to examine the lipidome. Plasma samples will be collected on days 0, 6, 12, 16, 21, 27. On day 27 animals will be sacrificed and CNS, liver, and core blood collected for studies outlined in Aim 2. This study requires two therapeutic treatment groups [LY, veh] x 2 pain states [paclitaxel-treated; veh-treated] x 2 opioid challenge conditions [morphine; veh] x 2 sexes [M; F] x 10 mice per group (**N = 160; in vivo treatments, blood and tissue collection performed in Hohmann lab**).

Study 1.2: Evaluation of plasma lipids after oral oxycodone self-administration and post-surgical pain. C3A affiliate Andrea Hohmann recently developed an oral oxycodone self-administration model in mice as part of a funded proposal through the “Indiana Addiction Grand Challenges” initiative. They have used this approach to model opioid-self-medication in the presence and absence of post-surgical pain as well as examining the role of microglial CB2 receptors in this context. Strikingly, mice lacking CB2 receptors in microglia showed lower levels of oral oxycodone self-administration compared to their wildtype CB2f/f counterparts (*unpublished data*). Here, we will examine the plasma lipidome in mice during the initiation and maintenance of oxycodone self-administration and the impact of subsequent incisional injury. Blood will be collected every week beginning before self-administration. CNS, liver, and core blood will be collected on final sacrifice. This study requires 2 oral self-administration groups [oxycodone, water] x 2 pain states [injury; sham] x 2 sexes [M; F] x 10 mice per group (**N = 80; in vivo treatments, blood and tissue collection performed in Hohmann lab**).

Study 1.3: Evaluation of plasma lipids after chronic adolescent THC exposure. C3A core grant “Mechanisms and treatment of adolescent phytocannabinoid impairment of prefrontal cortex function” (DA053746) is focused on the long-term effects of THC exposure on CNS development. As outlined in the proposal and supported by previous findings [113], THC exposure during PND 28-49 (adolescence) drives behavioral deficits in working memory and increases in anxiety-like behaviors in the adult (testing days ~PND90-120), whereas THC exposure during PND 69-89 (young adult) do not drive these behavioral changes. Here, we will examine changes in the plasma lipidome during THC exposure and during adulthood behavioral testing to determine if there are key changes in circulating THC and its metabolites as well as the endogenous lipidome that are associated with these differences in exposure. Blood samples will be collected on days PND 28, 38, 49, 69, 79, and 89. Animals will be sacrificed on PND 100, and CNS and core blood collected for studies outlined in aim 2. This study requires an adolescent THC treatment group [THC-PND28-49, veh] x young adult THC treatment [THC-PND69-89; veh-treated] x 2 sexes [M; F] x 10 mice per group (**N = 80; blood and tissue collection performed in Lu lab**).

Study 1.4: Evaluation of plasma lipids after prenatal opioid exposure and adult alcohol consumption. C3A core affiliate, Brady Atwood (IUPUI), has utilized the drinking-in-the-dark (DID) model of binge drinking in both adult or adolescent male and female mice ([114] AA027214). The Attwood labs’ prior work demonstrates that alcohol and opioid exposure disrupts opioid and cannabinoid plasticity in the dorsal striatum [115-118]. Another key aspect of their work shows that developmental opioid exposure (prenatal methadone; PME) has profound effects on both behavior and neural circuitry involved in alcohol consumption and that this effect is sex-dependent [119,120]. Here, we will examine the plasma lipidome from animals using the DID model that also have PME. Blood samples will be collected on days at the beginning of the 4-week DID paradigm on either PD 40, 47, 54, 60 (adolescent) or PD 80, 87, 94, and 100 (adult). Animals will be sacrificed on PND 100, and CNS and core blood collected for studies outlined in Aim 2. This study requires 2 prenatal treatment group [PME, veh] x 2 DID [DID; veh-treated] x 2 age groups [Adolescent, Adult] x 2 sexes [M; F] x 10 mice per group (**N = 160; blood and tissue collection performed in Atwood lab**).

Study 1.5: Evaluation of plasma lipids after chronic adolescent THC exposure and adult mild traumatic brain injury (mTBI). C3A affiliate, Gabriel Nah (IUB) has developed a model of mTBI in rats that elicits behavioral cognitive deficits and activates hippocampal microglial cells (*unpublished data and pilot study 1*). Previously, the Bradshaw lab showed that mTBI causes significant changes in the CNS lipidome. Here, this

study aims to understand the effects of adolescent exposure of THC on adult mTBI lipidome. Animals will be injected with 3mg/kg THC from PND 28-49. At age PND 60, animals will undergo behavioral training on cognitive tasks (see *Pilot study 1 for more details on behavioral analyses*). Blood samples will be collected on days at the beginning of the two-week training paradigm, one day after the mTBI treatment, and on 3 additional days during the 3 weeks of post-mTBI behavioral analyses. Animals will be sacrificed after the last testing day, and CNS and core blood collected for studies outlined in Aim 2. This study requires 2 adolescent treatment groups [THC, veh] x 2 mTBI [mTBI; sham-treated] x 2 sexes [M; F] x 10 rats per group (**N = 80; blood and tissue collection performed in Nah/Crystal lab**).

Study 1.6 Evaluation of plasma lipids after long-term alcohol exposure. C3A core affiliate and Assistant Prof Adam Kimbrough (Purdue) has developed a research protocol that investigates changes in CNS network functions in differing drug abuse patterns using whole-brain imaging of immediate early genes to identify brain regions that are recruited during drug withdrawal and protracted abstinence (AA027301 and AA029985). This study will use the testing strategy his lab has developed for long-term alcohol dependence in adult rodents including weekly blood analysis [121,122]. Animals are started in the paradigm at PND 60 and finish ~PND 180. Blood samples will be collected and plasma lipidomics analysis determined for two time points during acquisition, 3 times points during dependence, and a final point at sacrifice. This study requires 3 alcohol dependence development groups [alcohol vapor, alcohol injection, veh] x 2 sexes [M; F] x 10 mice per group (**N = 60; blood and tissue collection performed in Kimbrough lab**).

Study 1.7: Evaluation of plasma lipids after adolescent Cannabis use in humans. C3A core affiliate Cece Hillard (Medical College of Wisconsin) recently showed that levels of 2-AG are dysregulated in individuals with PTSD [123,124]. Currently, her team is investigating circulating endocannabinoids (AEA and 2-AG) levels of neurocognitive, psychopathology, and early Cannabis use outcomes in a subset of 2000 youth aged 11- 14 who are already enrolled in the longitudinal Adolescent Brain Cognitive Development (ABCD) Study (DA049109). Samples from this study will be analyzed using these broader endocannabinoid lipidomics techniques to determine associations well beyond the two endogenous cannabinoids previously proposed. The number of samples will be determined by those that are available to Dr. Hillard through the ABCD consortium.

Study 1.8: Evaluation of plasma lipids in adolescence with mTBI in humans. C3A core affiliate Keisuke Kawata (IUB) is an Associate Professor who has investigated multiple protein blood biomarkers to understand post-concussion syndrome [125-128]. He is currently investigating "Subconcussive neurodegenerative progression in adolescent athletes" (NS113950) through the investigation of blood biomarkers and cognitive behavioral and imaging analysis. Here, we will evaluate endocannabinoids and related lipids in subjects who are enrolled in these long-term studies to determine lipid blood biomarkers that may be used to predict those individuals who will develop long lasting post-concussion syndrome. The number of samples will be determined by those enrolled in the study and number of samples available per subject.

Study 1.9 Evaluation of plasma lipids in human cohorts with developmental cognitive dysfunction. C3A core affiliate Debra Karhson is a new Assistant Professor at a minority-serving institution (The University of New Orleans) who studies the relationship between endocannabinoid signaling and the development of autism spectrum disorders [129,130]. Through her previous position at University of Texas Permian Basin, she has access to the University of Texas System Health Biobank, which allows her access to a wide range of human plasma samples. Given that her previous work showed strong relationships between plasma endocannabinoids and autism, she has identified two studies in the Biobank on development and cognitive dysfunction, and she plans to evaluate for lipid biomarkers of disease such as endogenous cannabinoids. The number of samples will depend on the number available in these ongoing studies.

Study 1.10 Evaluation of plasma lipids in individuals with cannabis use disorder undergoing cognitive behavioral therapy. C3A core affiliate and Assistant Professor, Shanna L Babalonis (Univ. Kentucky) investigates therapeutic and abuse liability of opioid and cannabinoid combinations in the human laboratory [131-135]. In a recently funded proposal (Cannabis Modulation of Outcomes Related to Opioid Use Disorder: Opioid Withdrawal, Opioid Abuse Potential and Opioid Safety, R01DA054347) they aim to examine the effects of inhaled cannabis on outcomes related to opioid use disorder in a sample of participants who are physically dependent on opioids. As outlined in the pilot grants here, they plan to enroll these participants into their inpatient hospital research unit and examine acute (Study 1) and repeated (Study 2) doses of cannabis during periods of opioid withdrawal to assess how cannabis modulates opioid withdrawal severity. They will also examine how cannabis modulates acute doses of intranasal opioids to examine abuse potential (i.e., does cannabis increase or

decrease opioid drug liking outcomes) and opioid-related safety (i.e., does cannabis exert protective or deleterious effects on opioid-induced respiratory depression). Patients in each of these studies have blood analyses throughout and would reserve plasma for lipid analysis at each time point. The number of subjects in each study would be variable between 20-40 and the number of samples per patient between 5-10. This study represents an important translational intersection between preclinical and clinical populations within the BMLC core proposal and is outlined in more detail in *Pilot study 2*.

Aim 2: Characterize sex differences in bioactive lipids in the CNS in models of chronic drug use.

Substantial data at multiple levels of analysis demonstrate sex differences in neurophysiological and behavioral aspects of drug use [136-142]. As outlined in **Aim 1** and illustrated in **Fig. 5** there are inherent sex differences in the response to drug exposure. Here, we use animal models to evaluate bioactive lipid mediators in targeted CNS areas in multiple models of drug use (THC, opioids, alcohol) and analyzed these data as a function of genetic sex to provide a novel framework to evaluate unique characteristics across and between drug models and sex. **Fig 5** illustrates how these types of data can drive novel hypotheses. Lipidomics evaluation of cerebellar lipids show that AEA and related N-acyl ethanolamines are significantly decreased in the NAPE-PLD KO mouse [42]. Acute treatment with CBD causes an increase in NAEs and other lipoamines but a decrease in prostaglandins [4]. However, acute treatment with CBD in the NAPE-PLD KO mouse does not increase NAEs and lipoamines, however PGs are still reduced [4]. These types of data provide critical insights to form novel hypothesis on how cannabinoids function in the body. Finally, novel data (**Fig. 5**) show that there are baseline differences in these classes of lipids between males and females, which could drive different types of signaling systems in the context of different drug exposures. Here, all studies are designed to first be analyzed as previously described [2,4] and as illustrated in **Fig.5**. In each of these studies analysis will focus on genetic sex as variable as well as drug effects. In addition, Power analyses will be generated to determine if the initial N of 10/group that our previous work was sufficient to show potential sex effects is sufficient with a probabilistic cut off threshold of $N \leq 20$. If so, specific studies may be run with additional subjects to evaluate these potential differences. Finally, all data will undergo novel network analyses that are being generated in-house (See *Aim 5*).

	NAPE-PLD KO vs WT#	WT+CBD vs VEHT	NAPE-PLD KO + CBD vs VEHT	Female WT vs Male WT*
N-acyl ethanolamine				
N-palmitoyl ethanolamine	↓↓		↓	↓↓
N-stearoyl ethanolamine	↓↓↓			↓
N-oleoyl ethanolamine	↓			
N-linoleoyl ethanolamine	↓↓↓↓	↑		
N-arachidonoyl ethanolamine	↓↓↓	↑		
N-docosahexaenoyl ethanolamine	↓↓↓↓	↑		
N-acyl glycine				
N-palmitoyl glycine				↓
N-stearoyl glycine				↓
N-oleoyl glycine				
N-linoleoyl glycine		↑	↓	
N-arachidonoyl glycine	↑		↑	
N-docosahexaenoyl glycine				
N-acyl serine				
N-palmitoyl serine		↑		↓↓
N-stearoyl serine				
N-oleoyl serine		↑		↓
N-linoleoyl serine				
N-arachidonoyl serine	↓	↑	↓	
N-docosahexaenoyl serine		↑		
2-acyl-sn-glycerol				
2-palmitoyl-sn-glycerol				
2-oleoyl-sn-glycerol				
2-linoleoyl-sn-glycerol	↓			
2-arachidonoyl-sn-glycerol				
Free Fatty Acids				
Oleic acid		↑		↓
Linoleic acid			↓	↓↓
Arachidonic acid				↓
Prostaglandins				
PGE2			↓	↓↓
PGF2α		↓	↓	↓↓
6-ketoPGF1α		↓	↓	

Fig. 5. Changes in lipid levels in cerebellum. Adapted from Leishman et al. that used 3mg/kg CBD in female mice [4,42]. *unpublished male versus female. Orange cells =sig. decreases, green cells=sig. increases. Arrows show effect size.

Study 2.1: Evaluation of CNS lipids with opioid and CB2 agonist exposure. The treatment protocol is outlined in Study 1.1. Here, lipidomics will be performed on spinal cord, midbrain, and thalamus, which are areas that are being evaluated for changes in microglial activation with the chronic opioid and CB2 agonist treatment protocols outlined in DA047858. Liver will also be evaluated. These tissues will be collected with Study 1.1.

Study 2.2: Evaluation of CNS lipids with oxycodone self-administration and post-surgical pain. The treatment protocol is outlined in Study 1.2. Here, lipidomics will be performed on spinal cord, midbrain, and thalamus, which are areas that are being evaluated for changes in microglial activation due to changes in CB2 activity in this testing strategy. Liver will also be evaluated. These tissues will be collected with Study 1.2.

Study 2.3: Evaluation of CNS lipids after chronic adolescent THC exposure. The treatment protocol is outlined in Study 1.3 for the study with C3A core grant DA053746. Here, lipidomics will be performed on thalamus, PFC, and striatum, which are areas that are being evaluated for changes in functional activity in the DA053746 proposal. These tissues will be collected with Study 1.3.

Study 2.4: Evaluation of CNS lipids after prenatal opioid exposure and adult alcohol consumption. The treatment protocol is outlined in Study 1.4 for the study with C3A core affiliate Brady Atwood and Pilot Project 2. Here, lipidomics will be performed on dorsal striatum, midbrain, and PFC. These tissues will be collected with Study 1.4. The dorsal striatum is a specific area of focus in the Atwood lab as multiple studies have shown this area of the CNS is particularly vulnerable after opioid and alcohol exposure and for which proteomics data have been published [114,116,118,143,144]. Specific attention will be made to determine the minimum amount of tissue needed for lipidomics analyses. Some samples may need to be pooled, which could modify the original N of 10/group as outlined in Study 1.4.

Study 2.5: Evaluation of CNS lipids after chronic adolescent THC exposure and adult mTBI. The treatment protocol is outlined in Study 1.5 for the study with C3A core affiliate Gabriel Nah and Pilot Project 1. Here, lipidomics will be performed on hippocampus, PFC, and thalamus. Initially, half of the subjects (see Pilot project 1) will be used for lipidomics and half for imaging. Power analysis after initial studies will be performed to determine if additional animals are needed for lipidomics. These tissues will be collected with Study 1.5.

Study 2.6 Evaluation of CNS lipids after long-term alcohol exposure. The treatment protocol is outlined in Study 1.6 for the study with C3A core affiliate Adam Kimbrough (Purdue). Here, lipidomics will be performed on nucleus accumbens, prefrontal cortex, and midbrain. The nucleus accumbens is a special area of focus for this research model and methodological attempts will be made to evaluate lipids in the accumbens core and shell. This may require pooling of samples and adjustment of the number of animals outlined in Study 1.6. Tissues will be collected under the protocol of Study 1.6 in the Kimbrough lab.

Study 2.7. Evaluation of CNS lipids in opioid withdrawal and anxiety with cannabidiol treatment. C3A Core Affiliate and Ward-Coleman Chair of Translational Neuroscience and the Director of the Addiction Institute at Mount Sinai, Yasmin Hurd, is investigating the efficacy of CBD treatment for opioid withdrawal symptoms such as craving associated with chronic opioid use in humans and in animal models [145-150]. A key aspect of withdrawal craving in humans is anxiety. Using a model of anxiety-like behaviors in rodent (foot-shock), their data show that CBD (3mg/kg daily) is efficacious in reducing these behaviors (*data not shown*). Previous data using therapeutic CBD for opioid withdrawal and in the anxiety-model both indicate a CNS target, which may be the nucleus accumbens. Data here will compare the two testing strategies to determine if the lipid signaling regulation in the CNS by CBD is the same in both conditions. Tissues collected at sacrifice: core blood, liver, CNS tissue (accumbens core, nucleus accumbens shell, and medial prefrontal cortex). This study requires two independent treatment paradigms. Part 1 opioid treatment [heroin, veh] x 2 drug treatments [CBD, veh] x 2 sexes [M; F] x 10 mice per group; Part 2 anxiety-like behavior [shock, sham] x 2 drug treatments [CBD, veh] x 2 sexes [M; F] x 10 mice per group. (**N = 160; CNS tissue collection performed in the Hurd lab.**)

Study 2.8. Evaluation of CNS lipids in adult with adolescence THC exposure. C3A Pilot Core PI and new Gill Chair Professor Norbert Hájos (IUB) studies innate behaviors associated with motivation and investigates network signaling between the basal lateral amygdala and the mPFC. For this project, adolescent CD1 mice (P28) will be chronically treated with THC (3 mg/g) for 14 days to mimic long-term adolescent cannabis usage. At P42-45, AAV5-CamKII-ChR2-EYFP vector will be injected into the BLA region of the animals to allow for optogenetic stimulation of BLA to mPFC projections as previously described [151-154]. Following a 30-day recovery period, a second surgery is performed to insert a 64-channel silicone probe combined with an optical fiber into the PFC to stimulate BLA > mPFC projections while monitoring mPFC neural activity. Treatment parameters will mimic those for evaluation of innate behaviors. After the electrophysiological experiments, mPFC, amygdala, and VTA will be harvested for lipidomics analysis. This study requires 2 drug treatments [ThC, veh] x 2 viral conditions [vector; control] x 2 sexes [M; F] x 10 mice per group (**N = 80; CNS tissue collection performed in the Hájos lab.**)

Aim 3: Characterize changes in bioactive lipids in breast milk with exposure to drugs of abuse. Nursing moms who report postpartum cannabis use cite physical health and better parenting as reasons for consumption; however, they also report receiving little or conflicting guidance on its perinatal safety [155]. Highlighting a need for better counseling, drugs that act on the cannabinoid (CB) signaling system can have significant effects on infant growth and development. Early THC exposure in mice can influence behavior later in life, as evidenced by significant changes in locomotion at 2 months after a single injection of THC (10mg/kg) on PND10 [156]. The CB1 antagonist SR141716A administered to mice on postnatal days (PND) 2-8 results in starvation via loss of

suckling behavior, while THC administration results in significant weight gain [157]. Members of the C3A core labs (Lu, Mackie, and Bradshaw) recently showed that perinatal cannabinoid exposure leads to long-term behavioral changes on repetitive tasks and alters the effect of fluoxetine on immobility time during a forced swim task [5]. Additional studies by the Mackie lab show that THC exposure during lactation impairs developmental changes in GABA neural circuitry [158]. Because of the effects of early-life CB exposure and the detection of THC, THC metabolites, and CBD in human breast milk [159-162], it is important to evaluate how CBs given to lactating females transfer into milk and how CBs affect the lipid composition of milk, including eCBs and related signaling lipids. In a preliminary study, nursing mouse dams were treated with either THC (3mg/kg), CBD (3mg/kg), or a combination of THC:CBD (3mg:3mg/kg) all given subcutaneously from P0 to P10. Pups were allowed free access to nurse throughout the period and were sacrificed 2 hours after the last injection maternal injection on P10 and milk extracted from pup stomachs. **Fig. 6** shows that, like in humans, both THC and CBD are concentrated in breast milk. Importantly, levels of CBD are significantly higher than THC at this time point and there is a further increase when the compounds are combined. We also report that the CBD metabolite, 7-OH is present in breastmilk and, like the THC metabolite, 11-OH THC, its levels are significantly higher when the drugs are combined. **Fig. 7** shows that both eCBs and related lipids are modified differentially depending on drug treatment. This is a subset of the 80 lipids analyzed in breastmilk and further illustrates the complexity of the regulation of small molecule bioactive lipids in response to drug exposure. In this aim, we will partner with the Lu laboratory to continue these studies with different THC/CBD doses and with the Atwood lab to determine the effects of maternal opioid exposure on breast milk lipids.

Study 3.1: Examination of breastmilk lipidome with cannabinoid exposure: C3A core labs (Lu, Mackie, and Bradshaw) showed that perinatal cannabinoid exposure leads to long-term behavioral changes on repetitive tasks and alters the effect of fluoxetine on immobility time during a forced swim task [5]. Preliminary data in **Figs. 6 and 7** illustrate that exposure of 3mg/kg of cannabinoids THC, CBD, and THC:CBD from P0-P10 drive changes in the breast milk lipidome. Here, we will determine a dose response of these phenomenon by evaluating the breastmilk lipidome in with 1, 3, and 10mg/kg of each drug combination. We will collect breastmilk in three cohorts at P1, P5, and P10. With the assumption of an average litter of 8 pups, 2 dams per treatment group there will be 2 dams per treatment group. This study requires 4 treatment groups [THC, CBD, THC:CBD, veh] x 3 doses [1, 3, 10mg/kg] x 2 dams per group (**N = 24 dams; ~192 pups; breastmilk collection performed in Lu lab**). We will also examine a cohort that has cannabinoid exposure from P0-P3, P0-P7 and collect breastmilk at P10. This evaluation strategy will provide important information when these changes in breastmilk lipids occur and if the changes persist after cannabinoid exposure is halted. This study requires 4 treatment groups [THC, CBD, THC:CBD, veh] x 1 doses [3mg/kg] x 3 treatment timelines, 2 dams per group (**N = 24 dams; ~192 pups; breastmilk collection performed in the Lu lab**).

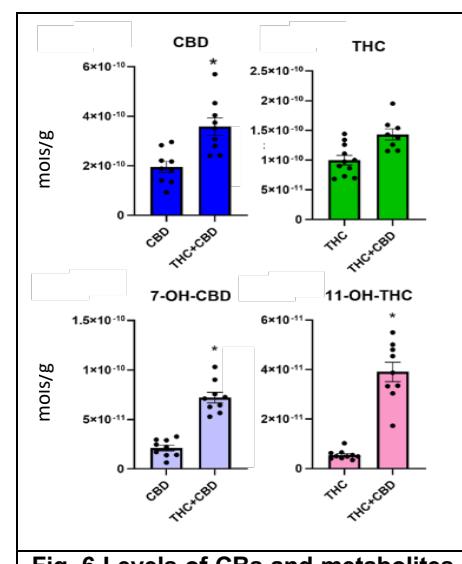


Fig. 6 Levels of CBs and metabolites in breastmilk. Milk extracted from pup stomachs 2 hours after final injection to dams on P10 and analyzed as previously described for plasma analysis [6]. *p<0.05

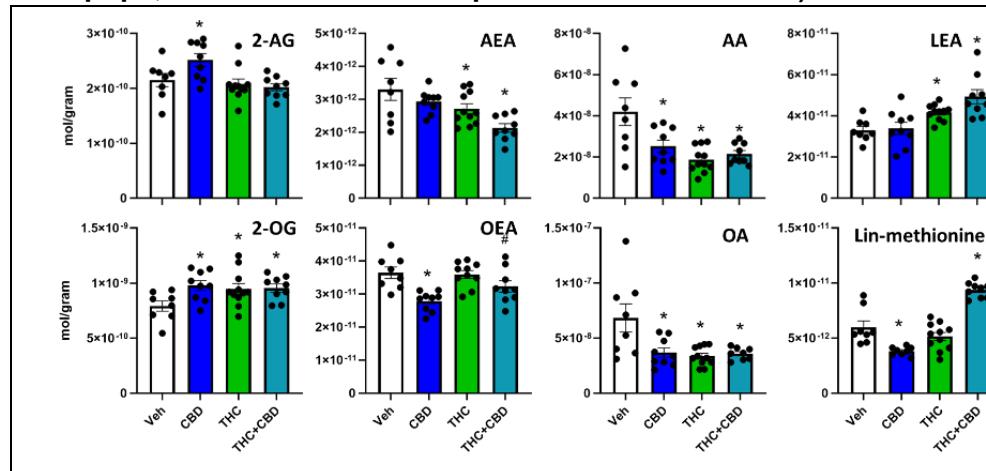


Fig. 7 Bioactive lipid levels in breastmilk change after cannabinoid exposure. Levels of 2-AG, AEA, arachidonic acid (AA), linoleoylethanolamine (LEA), 2-oleoyl glycerol (2-OG), oleoylethanolamine (OEA), oleic acid (OA), and linoleoyl methionine are all significantly modified by drug treatment (see text for methods). *p<0.05 compared to veh. Milk was analyzed as previously described for plasma [6].

Study 3.2: Examination of breastmilk lipidome in post oxycodone use methadone treatment. C3A affiliate Brady Atwood (IUPUI) has shown that prenatal exposure to methadone has a profound effect on drug seeking behaviors as adults [163,164]. In this paradigm dams are first given chronic oxycodone prior to pregnancy, then transitioned to methadone prior to and during pregnancy and lactation as previously described (i.e., similar to women being on methadone maintenance therapy during pregnancy) [164]. Here, we will test the hypothesis that methadone exposure to dams drives changes in the breastmilk lipidome, including eCBs and related lipids. This study requires 2 oxycodone groups [oxy, veh] x 2 methadone groups [meth, veh] x 4 dams per group (**N = 16 dams; ~126 pups; breastmilk collection performed in Atwood lab**).

Aim 4: Expand our current bioactive lipid library and data science analytical techniques for bioactive lipid metabolites to drive novel hypotheses on how drugs of abuse cause changes in systemic and CNS lipid signaling.

Recent studies have shown that levels of abundant bioactive lipids are influenced by sex, age, and disease condition [72,89,105-107,165,166]. In most studies the key lipids species analyzed are from the major classifications of lipids (e.g., a collection of phospholipids, sphingomyelins, ceramides). Understanding the changes in these larger, ubiquitous lipids are important in understanding health and disease; however, it is the metabolites of these lipids that will likely provide more insight into how changes in these lipids drive cellular signaling. Here, we will use cell-based lipidomics analyses to optimize analytical tools to study bioactive lipid metabolites (e.g., endocannabinoids, lipoamines, oxylipins) in response to treatment with drugs of abuse. In previous work, we showed that treatment with THC, CBD, or THC:CBD has differential effects on each of three different cell lines (C6 glioma, BV2 microglia, N18 neuronal; **Fig. 8**). Preliminary data in **Fig. 8** also shows that

Lipid Species	BV2 Microglia				HepG2	
	THCT	CBD†	THC+CBD†	Morphine	CDCA	
<i>N</i> -palmitoyl ethanolamine		↑	↑	↓	↑	
<i>N</i> -stearoyl ethanolamine			↑	↓	↑↑	
<i>N</i> -oleoyl ethanolamine	↑		↑↑		↑↑	
<i>N</i> -linoleoyl ethanolamine	↑		↑↑		↑↑	
<i>N</i> -arachidonoyl ethanolamine	↑		↑↑		↑↑	
<i>N</i> -docosahexaenoyl ethanolamine	↑	↑			↑	
<i>N</i> -arachidonoyl GABA	BAL	BAL	BAL	BAL	BAL	
<i>N</i> -arachidonoyl glycine	↑↑	↑				
<i>N</i> -arachidonoyl phenylalanine	↑		↑			
<i>N</i> -arachidonoyl serine	↑		BAL			
<i>N</i> -arachidonoyl taurine	↑	↑	↑↑			
<i>N</i> -arachidonoyl tyrosine						
2-arachidonoyl glycerol				↓	↑	
Arachidonic acid	↑	↓	↓		↑↑	
PGE ₂	↑↑	↓			BAL	
PGF _{2α}	↑↑	↓↓	↑	↓	BAL	
6-ketoPGF _{1α}	↑				BAL	

Fig. 8. Changes in the lipidome in cell-based assays. 2-hour incubation with 1μM THC, CBD, or THC+CBD in BV2 microglial cells † as previously described [4]. 30-minute incubation with 1μM morphine in BV2 cells. 2-hour incubation with 50μM bile acid chenodeoxycholic acid (CDCA) in HepG2 cells. Green cells denote significant increases, orange cells denote significant decreases, and arrows denote effect sizes.

morphine drives changes in the lipidome of BV2 cells and that bile acids drive changes in eCBs and related lipids in the human hepatocyte cell line, HepG2. Cell-based assays are a more economical means for generating a high volume of uniform, enriched samples and have the benefit of modeling lipidomics data on specific cell types. These data will provide a framework to drive hypotheses for *tissue-based* lipidomics to target specific cell types (i.e., microglia versus astrocytes) and provide data sets for building network databases described below that can be used to develop hypotheses to be tested in animals.

Study 4.1: Evaluation of lipidome in neuronal cell types of drugs of abuse. The Bradshaw lab has shown that lipidomic profiles change with acute (1μM) exposure to cannabinoids (Fig. 8 [4]). Here, broad scale lipidomics profiles of small molecule lipid metabolites will be compared in C6 glioma, BV2 microglia, and N18 neuronal cells after treatment with 1) acute(2hr)/chronic(24hr) THC (0, 100nM, 1μM, 10μM [4]); 2) acute/chronic morphine (0, 100nM, 1μM [167]), 3) acute/chronic nicotine (0, 10, 50 μM [168]), 4) acute/chronic alcohol (0, 50, 100mM; [169]). A minimum of 10 replicates per treatment and cell type. Data will be analyzed as previously described [4] and by novel network analyses outlined below.

Study 4.2: Evaluation of lipidome in TRPV1-4, TRPM8, and TRPA1-transfected HEK cells treated with minor cannabinoids. The Bradshaw lab has shown that over 20 of the endocannabinoids and related lipids in the current lipid library modulate TRP channels in novel ways [90]. We also recently showed that activation of

TRPV1 by capsaicin drives changes in endocannabinoids and related lipids [47], providing another model system to investigate novel lipid signaling. Here, we will use so-call “minor” cannabinoids in the molarities that drive changes in TRP channel activity [170,171] to determine changes in the lipidome in TRPV1-4, TRPM8, and TRPA1-transfected HEK cells. Using the same treatment and analysis protocols previously described [47] these experiments will be carried out in the Bradshaw lab and data will also be used in the network analyses.

Study 4.3. Lipidome comparisons of bile acids and cannabinoids in hepatic cells. C3A affiliate Associate Professor Sayee Anakk (Univ Illinois-Urbana Champaign) specializes in the study of bile acid signaling in hepatocytes and their relation to liver diseases like alcoholic hepatitis [172-174]. In a collaboration with the Bradshaw lab, preliminary data (Fig. 8) suggests that hepatocyte treatment with bile acids causes a similar lipidome pattern to general CBD treatment in multiple cell types with an increase in NAEs. Given that THC was shown to decrease NAEs in the CNS [2], that NAPE-PLD regulates NAE production, and that bile acids regulate NAPE-PLD [175-177], these data suggest a link between bile acid signaling in the liver and cannabinoid pharmacology. This link has particular relevance to recent studies in humans where hepatocellular toxicity with CBD is a frequent finding [178]. Here, bile acid treatment in hepatocytes will be compared to cannabinoid treatment to identify similar signaling pathways and determine the areas of cross over that may drive changes in liver function with chronic drug use. Experiments will be conducted in the Anakk laboratory and cell pellets shipped to the BLMC.

Study 4.4. Integration of lipidomics data using machine learning algorithms

The well-known physicist Richard Feynman observed more than 50 years ago that over time, the ratio of compute time to the size of data would approach zero. The computing community has begun addressing this in various ways *i.e.*, data science (DS), big data (BD), and data-centric AI (DCAI). What is shared among these approaches is to focus as much on the data—its management, maintenance, provenance, cost—as much as on the models. A small cast of excellent techniques have taken center stage, *e.g.*, the Recurrent Neural Network (RNN) for time series analysis and Extreme Gradient Boosting. Our approach with these lipidomics data will be drawn from our successful work in material science [179-181]. We will build a hybrid machine learning framework (HMLF) drawn specifically from Generalized Linear Model; Generalized Linear Model with Stepwise Feature Selection; Multivariate Adaptive Regression Splines; Projection Pursuit Regression; Random Forest; Extreme Gradient Boosting; Monotone Multi-Layer Perceptron Neural Network; Support Vector Machines with Linear and Radial Kernel Functions; K-Nearest Neighbor; Decision Tree (CART); and Gaussian Process Regression

Our HMLF employs various approaches to the general ML task: given an experiment of inputs (levels of lipids at baseline) and either drug exposure (drugs, genetic sex, age) $x \in X$ and observable outputs (levels of lipid with condition) $y \in Y$ construct a function $F: X \rightarrow Y$ that describes the relationship. We describe this more formally in **Fig. 9**. We have an initial set of lipid levels (1) then levels affected by drug exposure clustered on various factors like genetic sex or age. We generate data that pairs these two sets of lipid levels and look for patterns. Our current work leverages ensembles of models that we can apply to this problem. Central to our HMLF is training and examining the performance of each model individually. We then prune the models that are either highly correlated or perform poorly. **Fig. 10** is a heat map indicating the correlation between the various individual ML models we constructed to understand TiO₂ nanoparticles (*under review*). This research is very similar to our proposed research here since we are investigating outcomes based on related chemical properties

$$\begin{aligned} \mathcal{L} &= \ell_0, \ell_1, \dots, \ell_n & (1) \\ \mathcal{L}_i &= \ell_0^i, \ell_1^i, \dots, \ell_n^i & (2) \\ \mathcal{I} &= \text{experimental factor} & (3) \\ &\quad (\text{e.g., drug exposure, genetic sex, age}) \\ D &= \{(x, y) | x \in \mathcal{L}, y \in \mathcal{L}_i, i \in \mathcal{I}\} & (4) \end{aligned}$$

Fig. 9. The Experimental Data and relationship we are investigating. (1) are the existing lipid levels. (2) are the lipid levels with experimental factors with drug exposure. (3) There are two sets of patterns ultimately described by the data sets. (4) The ML task is to build functions at describe the relationship in D .

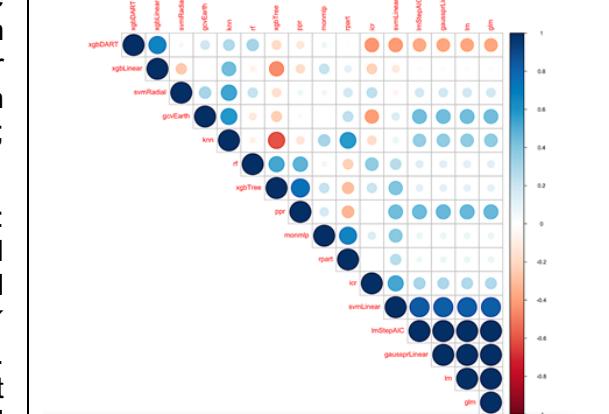


Fig. 10 Visualization of correlation of disparate models in HMLF approach to analyzing experimental data. This is the upper half of a matrix of model \times model in terms of measurement of efficacy like root means square error. The darker the color the more alike the models behave with the data.

that are perturbed based on different environments. Quantum properties are affected by the environment (e.g., temperature and pressure) which in turn affect quantum properties. What is clear is that the different models perform differently and in different ways. Our technique is to build a meta-ensemble model that leverages the best performing models together. We continue with the standard assessment of performance by doing a V-fold cross validation to predict error. Although our previous and current work includes time and 3D Euclidean space, our proposal here presents some new challenges. At its most rudimentary, we are interested in determining **patterns of lipid regulation → drug exposure → effects on lipid regulation, drug effects, genetic sex, behavior changes**. There are two broad approaches in our analytics: static analysis (output from a single experiment) and temporal analysis (changes over time). For static analysis we will utilize our successful HMLF model modifying it for this project. For the time series data, we will leverage RNN. We also propose to look at changes with respect to both dual combination of drugs and dual treatments. The aim of this is to begin a solid foundation for ultimately combinations of drugs and treatments.

Deliverables (Analysis, Novel Algorithms, Software, and Data)

The deliverables from this work will be a thorough understanding of the relationship between lipid regulation and drug exposure through novel extensions of our data-centric approach HMLF. We will be extending RNN for time-series lipid level patterns through encoding the output as a mapping from the space of drugs to a bit vector that encodes level and presence/absence of lipid and drugs, respectively. The degree of discretization will be determined experimentally. With these algorithms we will provide a repository of well-annotated raw and cleaned and transformed data sets for scientists to study, models available via Python or R (or some combination) to both verify results and conduct new analysis, ability to automatically do V-fold validation (means of determining the quality of the model), and various help guides to make use of data and software.

Aim 5: Increasing the pipeline for underrepresented minority (URM) students for careers in STEM with a focus on lipidomic mass spectrometric techniques.

The Bradshaw lab has many years of experience in training undergraduate researchers in lipidomics and mass spectrometry techniques. These undergraduate researchers have co-authored at least 25 manuscripts since 2008 [4,7,14,19-23,27,28,32,46-48,90,113,166,182-189]. Many of these students took part in the STEM Summer Scholars Institute (SSI; see *letter of support from SSI Directors*). The Bradshaw lab has hosted students in this program during every summer since 2008. SSI is a long-running summer research program that brings in students from Minority Serving Institutions (MSIs) throughout the US as well as underrepresented minorities from IU and provides them with well-developed research and professional development programming. We have also recruited and mentored students from the two programs at IU with a focus on professional development opportunities for URMs, Groups scholars (see *letter of support from Groups Director*) and Hudson and Holland (HHSP) Scholarship program (see *letter of support from HHSP Director*). In this aim we are working with each of these programs to develop a year-long opportunity for training in lipidomics and mass spectrometric techniques to supplement our summer training program. Working with the directors at Groups, HHSP, and SSI, we will identify students who want to apply for this year-long opportunity and support them throughout the process. The SSI-associated 10-week summer training course plan and objectives are outlined in the Administrative Core documents.

Logistically, training on lipidomics and mass spectrometric techniques is straightforward in the Bradshaw lab. For example, all equipment is housed in the laboratory; therefore, the typical issue of using shared equipment present in many training programs is not a detriment in terms of timing and cost. The Bradshaw lab has a 14-year history of training undergraduate researchers in lipidomic research and on this instrumentation. As evidenced by the preliminary data presented throughout this proposal, the API 3000 is still highly capable for the analysis of many small molecule lipids and can be used as a key instrument in both student training and data collection. Students would undergo training primarily on the existing API 3000 instrument prior to working on the API 7500 that would be purchased as part of this P30 Center. Each student will also be engaged in an independent research project chosen to match the student's interest with the goals of the project and will have weekly individual meetings with Dr. Bradshaw to discuss progress. As members of the Bradshaw lab, they will also attend weekly laboratory meetings and data meetings with C3A core participants as needed to discuss individual projects. They will also benefit from the many neuroscience opportunities (e.g., seminar series) provided by labs adjacent to Dr. Bradshaw's in MSBII. As projects finish up, trainees will be encouraged to take advantage of the opportunities to present at local and national conferences, which is a wonderful and transformational opportunity for undergraduates considering a career in Neuroscience. (Typically, IUB funding is available for these opportunities that involve undergraduate travel to meetings such as the SfN meeting.)

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

Yes No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

[Explanation-PrivateInfoBiospecimens.pdf](#)

Are Human Subjects Involved

Yes No

Is the Project Exempt from Federal regulations?

Yes No

Exemption Number

1 2 3 4 5 6 7 8

Other Requested Information

Research Involving Private Information or Biospecimens

Some work in the Bradshaw lab involves specimens obtained from humans. Specimens analyzed in this way are subject to the following:

1. Specimens will be obtained from a provider that is prohibited from releasing identifiers by established regulations or policies

Or

2. Specimens won't be collected solely for analysis in the BLMC AND BLMC personnel will not be able to link the specimens to private information identifying the donor AND the provider is solely providing (vs collaborating) AND the specimens will not be provided to the BLMC with a code linking them to private information of living individuals.

If the above criteria are not met in future experiments, then the IUB Institutional Review Board will be consulted on whether or not the research is considered human subjects research and the appropriate institutional approvals will be sought.

VERTEBRATE ANIMALS

All vertebrate animals associated with the studies in this proposal will be housed, maintained, and manipulated in the laboratories of the C3A core affiliates performing the study. Those labs will be responsible for ensuring that the appropriate IACUC protocols are in place for their studies. The Bradshaw lab will only receive tissue from those animals and no live vertebrate work will be conducted in her lab. See *Letters of Support* and Administrative Core for full details of the university affiliations for C3A Affiliates. All studies are associated with ongoing studies in these affiliate laboratories with their institutional compliance.

Aim 1 studies using animal tissues:

Study 1.1: Evaluation of plasma lipids after morphine and CB2 agonist exposure. Hohmann laboratory, Indiana University.

Study 1.2: Evaluation of plasma lipids after oral oxycodone self-administration and post-surgical pain. Hohmann laboratory, Indiana University.

Study 1.3: Evaluation of plasma lipids after chronic adolescent THC exposure. Lu laboratory, Indiana University.

Study 1.4: Evaluation of plasma lipids after prenatal opioid exposure and adult alcohol consumption. Atwood laboratory, IUPUI.

Study 1.5: Evaluation of plasma lipids after chronic adolescent THC exposure and adult mild traumatic brain injury (mTBI). Nah/Crystal laboratory, Indiana University.

Study 1.6 Evaluation of plasma lipids after long-term alcohol exposure. Kimbrough lab, Purdue.

Aim 2 studies using animal tissues:

Study 2.1 Evaluation of CNS lipids with opioid and CB2 agonist exposure. Hohmann laboratory, Indiana University.

Study 2.2: Evaluation of CNS lipids with oxycodone self-administration and post-surgical pain. Hohmann laboratory, Indiana University.

Study 2.3: Evaluation of CNS lipids after chronic adolescent THC exposure. Lu laboratory, Indiana University.

Study 2.4: Evaluation of CNS lipids after prenatal opioid exposure and adult alcohol consumption. Atwood laboratory, IUPUI.

Study 2.5 Evaluation of CNS lipids after chronic adolescent THC exposure and adult mTBI. Nah/Crystal laboratory, Indiana University.

Study 2.6 Evaluation of plasma lipids after long-term alcohol exposure. Kimbrough laboratory, Purdue.

Study 2.7. Evaluation of CNS lipids in opioid withdrawal and anxiety with cannabidiol treatment. Hurd laboratory, Mount Sinai

Study 2.8. Evaluation of CNS lipids in adult with adolescence THC exposure. Hájos laboratory, Indiana University.

Aim 3 studies using animal tissues:

Study 3.1: Examination of breastmilk lipidome with cannabinoid exposure: Lu Laboratory, Indiana University

Study 3.2: Examination of breastmilk lipidome in post oxycodone use methadone treatment. Atwood laboratory, IUPUI

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LETTERS OF SUPPORT

Please see the Overall Component for Letters of Support

RESOURCE AND DATA SHARING PLAN

Please see the Overall Component for the Resource and Data Sharing Plan

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES PLAN

Please see the Overall Component for the Authentication of Key Biological and/or Chemical Resources Plan

**APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)**

5. APPLICANT INFORMATION**UEI***: YH86RTW2YVJ4

Legal Name*: TRUSTEES OF INDIANA UNIVERSITY
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 Division:
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 City*: BLOOMINGTON
 County: MONROE
 State*: IN: Indiana
 Province:
 Country*: USA: UNITED STATES
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Person to be contacted on matters involving this application

Prefix: First Name*: Middle Name*: Last Name*: Suffix:
 Mr. STEVEN ALLEN MARTIN

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ZIP / Postal Code*: 474013654

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7. TYPE OF APPLICANT*

H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type Women Owned Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*

IUB C3A Pilot Project Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2023	06/30/2028

Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: TRUSTEES OF INDIANA UNIVERSITY

UEI: YH86RTW2YVJ4

Street1*: 702 North Walnut Grove Ave

Street2:

City*: BLOOMINGTON

County: MONROE

State*: IN: Indiana

Province:

Country*: USA: UNITED STATES

Zip / Postal Code*: 474052204

Project/Performance Site Congressional District*: IN-009

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* Yes No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes No

If YES, check appropriate exemption number: — 1 — 2 — 3 — 4 — 5 — 6 — 7 — 8

If NO, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* Yes No**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed?

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* Yes No

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* Pilot_project_abstract_20220930.pdf**8. Project Narrative*****9. Bibliography & References Cited** 2022_PPC_references.pdf**10. Facilities & Other Resources** Facilities_OtherResources.pdf**11. Equipment** Equipment.pdf

Pilot Project Core Abstract

The IUB Center for Cannabis, Cannabinoids and Addiction (C3A) Pilot Project Core will establish a pilot project program designed to support 2 to 4 new projects/year. The Pilot Project Core PI is Professor Norbert Hájos, who holds a Gill Chair of Neuroscience. Its goal is to support and encourage innovative and exploratory projects from trainees and establish investigators exploring new lines of research related to substance abuse. Pilot projects will be solicited annually from C3A Affiliates and their trainees. We anticipate that some of these pilot project proposals will come from trainees supported by our NIDA-T32, DA024628. Each proposal will be reviewed by 2 members of the External Advisory Board and 2 members of the Internal Advisory Board. Proposals will be ranked by the following priorities: 1. Support of young investigators/investigators new to the addiction field. 2. Support of groups historically underrepresented in drug abuse research. 3. A project that will maximally benefit from access to either the BLM or MSPM cores. 4. Obtain preliminary data for NIDA grant application. 5. Includes a component of high risk/high payout. Low priority will be given to proposals that merely supplement ongoing funded research. All applicants will receive feedback on their proposals and will be offered the opportunity to be mentored by a C3A PI or Affiliate in subsequent applications to the C3A or external agencies. Successful applicants will be assigned a Core staff member to optimize experimental design with the technical capabilities of the relevant core(s) utilized and matched with a IUB C3A Center Affiliate to provide guidance in the substance abuse field. Awardees will present their work at least three times to the monthly C3A research meetings and once to the external advisory board. A summary report will also be prepared when the project is completed.

Based on these criteria, we have chosen two projects for the initial submission of this P30. Pilot Project 1 has been proposed by Gabriel Nah, a graduate student working with Dr. Jonathan Crystal, a Provost Professor at IU Bloomington. Mr. Nah's project uses a rat model to examine the impact of adolescent THC exposure on the lipidomic, anatomical, molecular, and cognitive changes following mild traumatic brain injury and will use both the lipidomics and imaging cores. Pilot Project 2 has been proposed by Center Affiliate, Dr. Shanna Babalonis, as assistant professor at the University of Kentucky studying multi-drug use in human populations. Dr. Babalonis' project will use the lipidomics core to examine the plasma lipidome and phytocannabinoid levels in opioid users in a controlled setting with and without inhaled cannabis exposure. These two pilot projects meet the goals of the Pilot Project Core in the following ways: Project 1 supports an African American graduate student working on a significant question addressing the impact of prior drug use on the sequelae of traumatic brain injury. Project 2 supports an early-stage investigator examining the interactions between cannabinoids, lipids, and opioids in a clinical population.

FACILITIES AND OTHER RESOURCES

Please see the Overall Component for Facilities and Other Resources

EQUIPMENT

Please see the Overall Component for Equipment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Norbert	Middle Name	Last Name*: Hajos	Suffix:
Position/Title*:				
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	702. N. Walnut Grove Ave.			
Street2:				
City*:	Bloomington			
County:				
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*: 8128552012		Fax Number:		
E-Mail*: nhajos@iu.edu				
Credential, e.g., agency login: NHAJOS				
Project Role*: Other (Specify)		Other Project Role Category: Core Lead		
Degree Type:		Degree Year:		
Attach Biographical Sketch*: File Name:				
Attach Current & Pending Support: File Name:				

PROFILE - Senior/Key Person				
Prefix:	First Name*: Heather	Middle Name Bryte	Last Name*: Bradshaw	Suffix: Ph.D
Position/Title*:	Professor			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:	PSYCHOLOGICAL & BRAIN SCIENCES			
Division:	COLLEGE OF ARTS + SCIENCES			
Street1*:	1101 East 10th Street			
Street2:				
City*:	Bloomington			
County:	MONROE			
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	474052204			
Phone Number*:	812-856-1559		Fax Number:	
E-Mail*:	hbbradsh@indiana.edu			
Credential, e.g., agency login:	HBBRADSH			
Project Role*: Other (Specify)	Other Project Role Category: Project Lead			
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Brady	Middle Name	Last Name*: Atwood	Suffix: Ph.D
Position/Title*:	Assistant Professor			
Organization Name*:	TRUSTEES OF INDIANA UNIVERSITY			
Department:				
Division:				
Street1*:	302 W 15th St			
Street2:	NB-400C			
City*:	Indianapolis			
County:				
State*:	IN: Indiana			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	462022266			
Phone Number*:	2062901649		Fax Number:	
E-Mail*:	bkatwood@iu.edu			
Credential, e.g., agency login:	atwoodb			
Project Role*: Other (Specify)	Other Project Role Category: Project Lead			
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:			
Attach Current & Pending Support:	File Name:			

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Norbert		Hajos		Core Lead	186,594.00		0.1		0.00	0.00	0.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	0.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel				Total Other Personnel		0.00
					Total Salary, Wages and Fringe Benefits (A+B)		0.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2023

End Date*: 06-30-2024

Budget Period: 1

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Pilot Project #1	36,900.00
9. Pilot Project #2	36,900.00
10. Pilot Project #3	36,900.00
Total Other Direct Costs	110,700.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	110,700.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	110,700.00	64,760.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	175,460.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	175,460.00

L. Budget Justification*	File Name:
	2022_PPC_budget_justification_20220930.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Norbert		Hajos		M.D. PD/PI	186,594.00		0.1		0.00	0.00	0.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	0.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel				Total Other Personnel		0.00
					Total Salary, Wages and Fringe Benefits (A+B)		0.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2024

End Date*: 06-30-2025

Budget Period: 2

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Pilot Project #1	30,000.00
9. Pilot Project #2	30,000.00
10. Pilot Project #3	30,000.00
Total Other Direct Costs	90,000.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	90,000.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1. MTDC		58.5	90,000.00	52,650.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				
Total Indirect Costs	52,650.00			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	142,650.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	142,650.00

L. Budget Justification*	File Name:
	2022_PPC_budget_justification_20220930.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

A. Senior/Key Person												
	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Norbert	Hajos		M.D.	PD/PI	186,594.00		0.1	0.0	0.00	0.00	0.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons: File Name:										Total Senior/Key Person		0.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar	Months	Academic	Months	Summer	Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates									
	Graduate Students									
	Undergraduate Students									
	Secretarial/Clerical									
0	Total Number Other Personnel							Total Other Personnel		0.00
								Total Salary, Wages and Fringe Benefits (A+B)		0.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2025

End Date*: 06-30-2026

Budget Period: 3

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Pilot Project #1	30,450.00
9. Pilot Project #2	30,450.00
10. Pilot Project #3	30,450.00
Total Other Direct Costs	91,350.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	91,350.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1. MTDC		58.5	91,350.00	53,440.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	144,790.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	144,790.00

L. Budget Justification*	File Name:
	2022_PPC_budget_justification_20220930.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 4

A. Senior/Key Person												
	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Norbert		Hajos		M.D. PD/PI	186,594.00		0.1	0.0	0.00	0.00	0.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons: File Name:										Total Senior/Key Person		0.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar	Months	Academic	Months	Summer	Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates									
	Graduate Students									
	Undergraduate Students									
	Secretarial/Clerical									
0	Total Number Other Personnel							Total Other Personnel		0.00
								Total Salary, Wages and Fringe Benefits (A+B)		0.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 4

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2026

End Date*: 06-30-2027

Budget Period: 4

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Pilot Project #1	30,900.00
9. Pilot Project #2	30,900.00
10. Pilot Project #3	30,900.00
Total Other Direct Costs	92,700.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	92,700.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	92,700.00	54,230.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	146,930.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	146,930.00

L. Budget Justification*	File Name:
	2022_PPC_budget_justification_20220930.pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium**Enter name of Organization:** TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Norbert		Hajos		M.D. PD/PI	186,594.00		0.1	0.0	0.00	0.00	0.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	0.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel				Total Other Personnel		0.00
					Total Salary, Wages and Fringe Benefits (A+B)		0.00

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

UEI*: YH86RTW2YVJ4

Budget Type*: Project Subaward/Consortium

Enter name of Organization: TRUSTEES OF INDIANA UNIVERSITY

Start Date*: 07-01-2027

End Date*: 06-30-2028

Budget Period: 5

F. Other Direct Costs	Funds Requested (\$)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Pilot Project #1	31,300.00
9. Pilot Project #2	31,300.00
10. Pilot Project #3	31,300.00
Total Other Direct Costs	93,900.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	93,900.00

H. Indirect Costs	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
	1. MTDC	58.5	93,900.00	54,932.00
Cognizant Federal Agency	Department of Health and Human Services (D.H.H.S.) POC Name and Phone Number: Arif Karim, Director 214-767-3261			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	148,832.00

J. Fee	Funds Requested (\$)*

K. Total Costs and Fee	Funds Requested (\$)*
	148,832.00

L. Budget Justification*	File Name:
	2022_PPC_budget_justification_20220930.pdf

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

Budget Justification: Pilot Research Project Core

Personnel

Norbert Hájos, PhD. Gill Chair and Professor, Psychological and Brain Sciences Indiana University, Bloomington. As PI of the pilot project core, Dr. Hájos will oversee requests for pilot proposals and the pilot review process, and he will also coordinate assignment of trainers and mentors for pilot awardees. He will devote 1.2 academic months to the Pilot Core. Professor Hájos has extensive mentoring experience, has an expansive and open view of science, and is thus ideally suited for this task. He has a substantial experience in organizing review panels as he oversaw the development of the review criteria for the Bolyai Scholarship of the Hungarian Academy of Science and for the Neuroscience panel of the Hungarian Scientific Research Fund, which he chaired for 6 years. This experience positions him well to make mentor assignments for pilot applicants and awardees who are at an early stage of their career or entering the drug abuse field. Dr. Hájos will devote 1.2 academic months to Pilot Core activities. No salary is requested from the Pilot Project Core as his salary is supported via the Administrative Core.

Other expenses: Pilot Research Project Grants

We will award 2-4 pilot project grants per year. Preference will be given to applicants submitting rigorous proposals but are at an early stage in their career, to more established investigators moving into drug abuse research, or applicants who will increase the diversity of the drug abuse community at IUB.

The amount of support per pilot project will naturally vary year-by-year depending on the nature of the proposed work. Project expenses will include salaries, living expenses for scientists needing to travel to IUB to complete their projects, project-specific research expenses, etc. Following are the budget justifications for the two pilot projects proposed for the first year of the C3A.

Pilot Project 1:

Scope of work: The primary expenses for this pilot project involves animal purchase and housing, technician time for training the rats in the behavioral tasks, summer salary for Gabriel Nah, drug costs, supplies for injections, blood sampling, and tissue sampling. Imaging and lipidomics expenses are covered by the respective cores.

A. Personnel

Gabriel Nah, Fourth year graduate student in the Department of Psychological and Brain Sciences, Indiana University. Mr. Nah's supervisor is Jon Crystal, Provost Professor of Psychological and Brain Sciences and a respective experiment in rodent cognition. Mr. Nah acquired the preliminary data for this proposal and will supervise the technician who will perform the behavioral testing. Guided by Drs. Johnson (BLMC) and Dr. Barna (MSIC), Mr. Nah will perform the plasma sampling, tissue extraction and processing necessary for lipidomics analysis as well as the tissue preparation, imaging, and data analysis necessary for the imaging component of the studies, respectively. Guidance for the molecular studies will be provided by the Hohmann or Mackie labs, whichever is more convenient in terms of timing. Both labs have worked with and extensively published with the Crystal Lab. Summer salary and benefits for Mr. Nah are requested (\$7,000).

B. Other Personnel

TBD, Hourly research technician. An hourly technician will be recruited to perform the behavioral training. Training rats for these tasks is time intensive, requiring approximate 20 hrs/week x 16.05 \$/hr x 30 weeks = \$9630

D. Travel. The opportunity for Mr. Nah to present his results to the scientific community and to network with other trainees and established PIs is an important component of his training experience supported by this pilot project proposal. Potential meetings would include the GRC on Cannabinoids, ICRS, or SfN. \$2,500 is requested to cover travel, registration, lodging, and per diem.

F. Other direct costs

1. Materials and supplies

Consumable supplies for drug injections and blood collection. This project involves multiple injections during adolescence and subsequent tail blood sampling during adolescence and adulthood. This cost category includes syringes, needles, prep/cleaning supplies, tubes, vials, etc. It is estimated at \$2,800.

Drugs. This category includes the cost of purchasing THC, isoflurane, Cremophor, sterile saline, etc., for these experiments. This is estimated to be \$1,200.

qPCR reagents. This category includes the cost of purchasing rat Taqman immunology arrays (#4414081). These arrays are a cost-effective way to screen several dozen immune-related genes simultaneously. The largest or most provocative (in terms of pathways) results will be validated by qPCR with specific Taqman probes. This cost category also includes reagents necessary for isolated mRNA from frozen rat brain and time on the Quantstudio 7 for running the arrays and individual reactions. The expenses in this category are estimated at \$15,052.

General disposables.

This cost category includes PPE, weigh boats, pipette tips, etc. and is estimated to be \$1,000

2. Publication costs

We expect one major publication from these studies and request \$1,500 to partially cover open access charges.

3. Animal purchase charges and per diems

Working with adolescent mice, it's most cost effective and ensures reproducible rearing conditions to purchase time pregnant dams. We anticipate using approximately 16 dams (to account for vagaries in sex, different size of litters, pregnancy loss, etc. At an estimated cost of \$180/dam, this comes to \$2,880. Rat per diems at IUB are ~\$1.05/cage/day. Estimated per diem costs (adolescents will be group housed (~4 rats/cage) until adult injury after which they will be single house) will be $(16 \times 10 \times \$1.05 = \$168) + (16 \times 21 \times \$1.05 = \$353) + (9 \times 7 \times 25 \times 1.05 = \$1,654) + (4 \times 7 \times 100 \times 1.05 = \$2,940) = \$5,115$. A buffer of 20% to per diems is added in case increased training is needed post injury. Thus, the combined estimated purchase and per diem costs are estimated to be \$9,018

4. Imaging and lipidomic costs

These expenses will be covered by the respective cores and are not requested here.

Pilot Project 2:

Scope of Work: The primary workload for this project involves obtaining, processing, and shipping the plasma samples collected during screening and inpatient enrollment. We anticipate collecting 6 samples per participant enrolled in the inpatient study and 1 sample for each participant completing screening for various other controlled drug administration studies at our facility.

Sample estimates: We anticipate enrolling 15 completers in the inpatient study (total of 90 samples). In addition, we estimate screening 75 -100 participants in our other studies. Overall, we estimate collecting, processing, and shipping a total of approximately 190 samples to the Bioactive Lipid Mediators Core.

B. Other personnel

Research Nurse (effort = 6 months calendar) will be ACLS-certified and will be responsible for all phlebotomy for the pilot project. The RN will obtain, process, aliquot, label, and freeze the plasma samples. This individual will also obtain consents for the study, explain procedures to subjects and family members, and prepare and keep all necessary documentation (e.g., sample log) for all collected samples. **Total = \$50,000 (salary and benefits)**

F. Other Direct Costs

1. Materials and Supplies

Phlebotomy and Sample Storage Supplies: This project will require phlebotomy kits (alcohol wipe, tourniquet, butterfly needle, vacutainer adapter), blood collection tubes, pipettes, labels, cryovials, and cryoboxes. **Total = \$3,000**

Sample Shipment Supplies: Samples are shipped in padded sample shipment boxes (DOT-approved cooler boxes). For these shipments, we will also need sufficient dry ice, and sample bags for approximately 8 shipments. **Total = \$1,000**

Shipment Costs: Based on current FedEx rates, we estimate each priority overnight shipment to cost approx. \$125. We anticipate shipping approximately 8 shipments. **Total = \$1,000**

F. Participant Costs

Patient compensation for blood draws. 16 blood draws that are not part of the original study will be required. Participants will receive \$25/draw x 16 draws = \$400/participant. With 15 participants, **Total = \$6,000**

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	0.00
Section B, Other Personnel	0.00
Total Number Other Personnel	0
Total Salary, Wages and Fringe Benefits (A+B)	0.00
Section C, Equipment	0.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	478,650.00
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. Other 1	159,550.00
9. Other 2	159,550.00
10. Other 3	159,550.00
11. Other 4	0.00
12. Other 5	0.00
13. Other 6	0.00
14. Other 7	0.00
15. Other 8	0.00
16. Other 9	0.00
17. Other 10	0.00
Section G, Direct Costs (A thru F)	478,650.00
Section H, Indirect Costs	280,012.00

Section I, Total Direct and Indirect Costs (G + H)	758,662.00
Section J, Fee	0.00
Section K, Total Direct and Fee (I + J)	758,662.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 09/30/2024

1. Vertebrate Animals Section

Are vertebrate animals euthanized? Yes No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

Yes No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

Yes No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount (\$) *Source(s)

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3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? Yes No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? Yes No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: Yes No

If the answer is "Yes" then please answer the following:

*Previously Reported: Yes No

6. Change of Investigator/Change of Institution Section

Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	20220928_Introduction_Pilot_Project_Core.pdf
Research Plan Section	
2. Specific Aims	20220930_Pilot_Project_Core_SA.pdf
3. Research Strategy*	20220930_Combined_PPC_strategy_and_proposals.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	VAS_pilots_20220927.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	Resource_DataSharing.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication.pdf
Appendix	
12. Appendix	

Introduction – Pilot Project Core

We thank the reviewers for their careful reading of our original proposal, thoughtful comments, and enthusiasm for the proposal. Of the cores, the Pilot Project Core (PPC) had the most significant concerns, so we have completely revised this core for the resubmission. Following are the most significant changes.

- 1. ...There is a limited amount of detail on the processes established in the Core, the format of the request for funding and the evaluation and expected outcomes or a discussion of how mentorship of the project/individual be facilitated and monitored by the Core.** These are now better explained. Briefly, request for funding will be in a “short” (4 page) R21 format, outcomes tracked include completion of project aims, grant submission, publication of results, and applicant’s career advancement, mentees will be matched by the PPC director to an appropriate mentor (based on mentee’s interests, goals, experience, etc.). The PPC director will monitor the mentoring relationship directly through regular conversations with the mentor and mentee and indirectly by project progress.
- 2. Eligibility of graduate students for PPC funds.** We anticipate that graduate students would be eligible and benefit from PPC funds in the later stage of their career (e.g. after passing their qualifying exam).
- 3. Details of the application process and variability of pilot projects proposed in the first submission.** The application process will be standardized as described in the PPC Research Strategy along the lines of an R21 proposal, including rationale, methods, expected outcomes, pitfalls, alternative approaches, etc. How they will benefit from the C3A is more thoroughly explained.
- 4. Concern that PPC awardees will only be at an early career stage.** The PPC competition will be open to all career stages. Priorities for choosing awardees is articulated in the PPC research strategy. The mentoring offered to applicants receiving PPC funds will be tailored to career stage.
- 5. Logistical challenges associated with a PPC awardee working between their institution and the PPC and tracking awardee progress.** Approaches to addressing logistics are more thoroughly discussed in the resubmission. Approach will necessarily vary by project. Some, especially those involving the BLMC, may mostly involve supporting experiments done at the awardee’s home institution using a model they have developed and now want to measure bioactive lipids, which will be done at the C3A. Others, such as *in vivo* 2P projects will involve extensive time at IUB. Progress is tracked via the PPC and relevant service core PI’s at least quarterly.
- 6. Outreach by PPC awardees to their communities.** This was an excellent point raised in the review. Many of our Affiliates work in regions of the US particularly hard hit by SUDs and already engage in this type of outreach. We will leverage the experiences of these outreach-engaged Affiliates broadly across our mentees to incorporate community outreach as a component of our mentoring structure.
- 7. Ensuring rigor and reproducibility.** This is now emphasized throughout the application, including in the PPC.
- 8. Additional details of pilot grant applicant mentoring.** While advice, primarily technical, will be provided to applicants as they are preparing their proposals, most mentoring will occur after proposal submission. This is both for practical and educational reasons. More details on mentoring of successful and unsuccessful applicants are provided in the PPC research strategy.
- 9. Increase support of pilot projects to \$40-50K annually.** The P30 RFA restricts the pilot core budget to 10% of the proposal’s annual direct costs. To maximize funding to support pilot projects, the salary for the pilot project core PI is in the administrative core, since their task is primarily administrative. Our goal in the PPC is to maximize support of the most meritorious pilot projects. Given the nature of the C3A service cores, we anticipate the cost of the pilot projects will vary widely. For example, a project to measure bioactive lipids from a small number of samples will cost much less than a multi-month 2 photon microscopy experiment. Our IAB and EAB will be a resource to help us to balance scientific merit and availability of funds in making these decisions.

Pilot Project Core Specific Aims

Fresh ideas and new scientists must continuously be introduced to drug addiction research if the field is to remain vigorous, relevant, and have a positive impact on society. There are two primary strategies we will use to ensure a continuous influx of new ideas and investigators into drug addiction research. The first is to encourage early career investigators to apply their skills, enthusiasm, perspectives, and knowledge to problems of drug addiction. The second is to convince established investigators from other fields to apply their wisdom, techniques, and abilities from their current fields to unanswered questions in drug addiction research. By pursuing both paths, a strategy that likely yields the greatest success, the IUB Center for Cannabis, Cannabinoids and Addiction (C3A) Pilot Project Core (PPC) will establish a pilot project program designed to support 2 to 3 new projects/year that is aimed both at early career investigators as well as established investigators from other fields with substance abuse-related projects that could be approached using the resources of the C3A.

The goal of the pilot projects are to (1) provide resources and mentoring for early career drug addiction researchers to obtain preliminary data for preparing competitive grant applications, (2) provide resources and appropriate for career-stage mentoring to recruit more individuals from groups traditionally underrepresented in drug addiction research into the field, and (3) provide the necessary resources to allow talented neuroscientists outside of the drug addiction field to obtain preliminary data for grant applications that will provide novel insights into the study of drug addiction. Funding from the C3A Pilot Project Core is not appropriate for proposals that are only incremental in nature or are intended to provide "bridge" funding to established investigators.

Pilot projects will be solicited annually from C3A Affiliates, their trainees, and their colleagues.

Pilot project proposals will undergo reviews for scientific merit and program fit (*i.e.*, the three criteria listed above) and all applicants will receive written feedback on their proposals. Projects selected for possible funding will be shared with and reviewed by NIDA Program, before a final funding decision is made. The PPC PI will provide applicants (successful or not) career stage-appropriate mentoring. The mentor/applicant match will be done by the PPC PI (Dr. Hájos) and will be designed to best fit the applicant's needs. Outcomes of the pilot project process will be assessed at least annually and the pilot project process adjusted if indicated by the feedback we obtain from applicants, grantees, and others involved in the process.

To implement the above pilot project program the PPC has four Specific Aims:

- 1. Establish a process for solicitation of Pilot Projects:** Develop and implement an inclusive Pilot Project program to recruit a range (topic and level of training) of pilot projects from C3A Affiliates and their trainees.
- 2. Establish an equitable and helpful system to review the pilot projects:** We anticipate that many Pilot Projects will be submitted by trainees (late-stage graduate students and post-docs) and others who will benefit from a review process with a strong and supportive educational component. The C3A PI's, Core technical staff, and the External and Internal Advisory Boards, supplemented by additional content area experts as needed (for example, provided by Affiliates), will be assigned by the PPC core PI to perform the reviews.
- 3. Provide technical and field-specific mentoring to ensure pilot project and career success.** Since pilot project awardees are likely to either be early in their career or newly entering the realm of drug addiction research (or a new area of drug addiction research) it will be important that they receive supportive mentoring in addition to the funds to finance their project. This mentoring will come in two forms. The first is technical, so the awardee has a deep understanding of the methodologies used for their experiments. The second is conceptual, designed to provide mentoring that will identify, focus, and formulate strategies to address the most relevant scientific questions in drug addiction research consistent with the trainees' interests. This will be provided by one of the Core PIs or IUB C3A Affiliates, depending on the awardee's background, location, and career interests.
- 4. Establish criteria and mechanisms for evaluating the success of the PPC and for continuous process improvement.** Criteria for evaluating the success of the PPC include publications, grants submitted by PPC awardees, and if PPC funding enhances a trainee's career progression.

In the Research Strategy we will first describe the PPC. This will be followed by a detailed explanation of the pilot project application, how applications will be ranked and funded, and how outcomes of the pilot project awardees will be determined. Finally, we will describe the two pilot projects that we have chosen (based on the criteria listed below) for the first year of the P30, should it be funded.

Pilot Project Core Research Strategy

A. Significance

Fresh ideas and new scientists must continuously be introduced to drug addiction research if the field is to remain vigorous and have a positive impact on society. To fulfill this goal, the C3A will establish a Pilot Project Core (PPC) to support early career investigators or investigators entering the substance abuse field for the first time and wishing to use the technologies present in the C3A, give them financial and technical support to perform mass spectrometric or multiscale imaging experiments relevant to understanding the neurobiology of addiction (especially, to provide preliminary data for grant applications to NIDA), and/or develop novel mass spectrometric or light microscopic imaging technologies. PPC applications will be solicited annually from C3A Affiliates, their trainees, and their colleagues. Applications will be reviewed by a C3A steering committee member as well as by members of the internal and/or external advisory board for scientific merit, feasibility, relevance to drug addiction, etc. One of the intended benefits of the pilot grant program is to assist trainees in making the potentially difficult transition from post-doc/research scientist to tenure track junior faculty by allowing them to acquire significant preliminary data for successful grant applications as well as to provide mentoring in addition to the mentoring they receive from their "home" lab.

The funding provided to PPC awardees will help cover the cost of conducting experiments and/or preparing samples for analysis the BLM or MSI Cores. Since there is no charge to the awardees for using Core resources, PPC awardees will be able to heavily leverage modest PPC funding to gather a significant amount of data. We anticipate PPC funds paying costs such as animal purchase and per diems, tissue culture supplies, graduate student summer stipends, technician salaries, or the other expenses that are incurred to conduct experiments and to prepare materials for analysis by either Core. Depending on the nature of the experiments, they may be partially carried out at the awardees home institution or at IUB. In either case, special attention will be paid to the logistics associated with executing the projects, including compliance-related tasks like DEA scheduling, IACUC approvals, etc. PPC awards will be for one year, corresponding to the fiscal year of the C3A P30. Awardees who successfully complete their projects will have the opportunity to become Center Affiliates. This is one way that the C3A will identify and recruit talented new investigators into the Center.

B. Innovation

Innovation will be an important consideration in evaluating PPC applications. Thus, we will encourage applicants to think creatively and propose high risk-high benefit projects that have promise to generate important knowledge. Applications like these will be evaluated by considering the potential payoff to the potential risk (*i.e.*, negative result or technical failure). In this way PPC awards can be used to establish feasibility and obtain preliminary data, which will then be used to seek funding to continue the work.

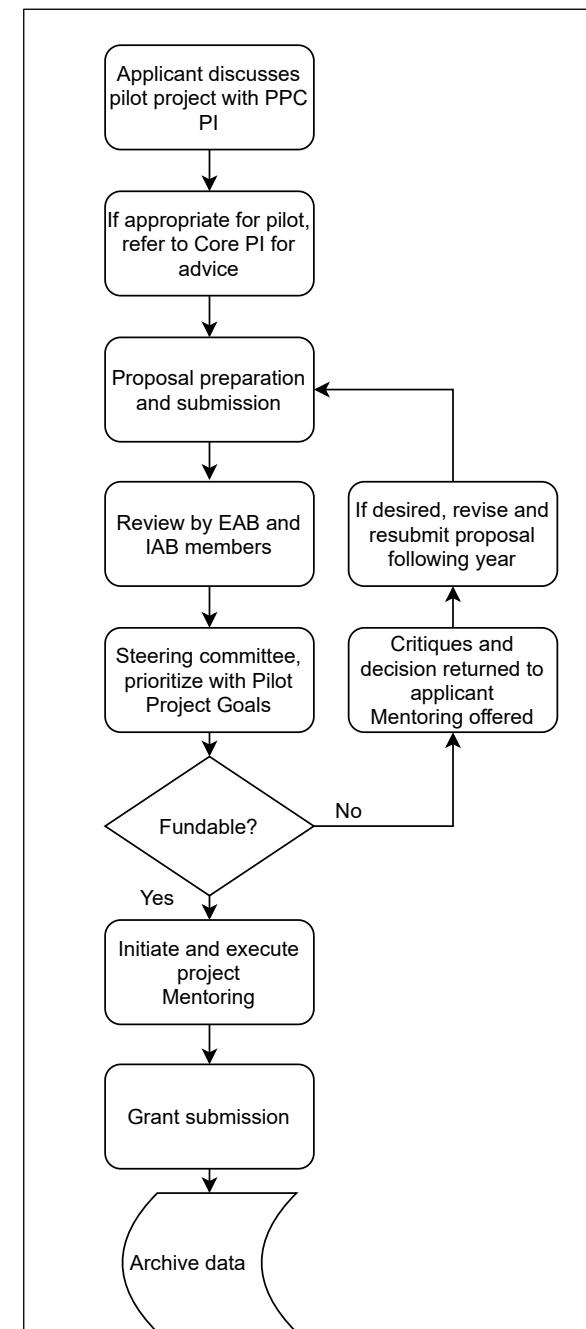


Fig. 1. Process for Pilot Project submission. See text for details. Briefly, applicants discuss potential projects with the PPC PI, who refers feasible projects to the appropriate core's PI. This PI then works with the applicant to craft a competitive application, which is reviewed by members of the EAB and IAB. Successful applicants are mentored through their project and unsuccessful applicants are offered constructive feedback and mentoring if desired to increase the competitiveness of their subsequent applications.

C. Approach

The Pilot Project Core (PPC) oversees and helps develop pilot projects that bring new (either chronologically or outside of drug abuse research) investigators into the Center, encourages young investigators in our Center's and Affiliate's laboratories to embark on careers in substance abuse research, and disseminates the Center's core technologies to researchers investigating the neurobiology of addiction whose research would benefit from these techniques. An important component of the PPC process is to provide useful feedback to all PPC applicants, including those that are unsuccessful. Our approach and philosophy model the approach used by the highly successfully Yale Neuroproteomics of Addiction P30 as outlined below (Dr. Mackie is a member of this P30 and a reviewer of their pilot projects).

Pilot Project application and initial review process: Fig. 1 highlights the key steps in the Pilot Project application process. Applications for Pilot Projects will be solicited annually. The solicitation will be broad, as we want to attract a wide range of applicants in addition to C3A Affiliates. For example, one of the Pilot Project Proposals here is from Gabriel Nah, an African American Neuroscience graduate student working with Dr. Jon Crystal (not a C3A Affiliate), Dr. Heather Bradshaw (a C3A Affiliate and co-PI), and Dr. István Katona (a C3A Affiliate and co-PI). The other Pilot Project proposal is from Dr. Shanna Babalonis, a C3A Affiliate and translational neuroscientist working with Dr. Heather Bradshaw. Before submitting a proposal, potential applicants are encouraged to communicate with the Core PIs to fully understand the Core's capabilities and to give the Core Director an opportunity to discuss technical issues and rigorous experimental design relevant to the core (and substance abuse research, if appropriate) and answer any questions from the applicant. Submitted proposals will be reviewed by 4-5 reviewers, 2-3 from IUB (not involved in the project) and 2 external reviewers, either by unconflicted Center Affiliates or EAB members. These reviewers will be selected to ensure the expertise needed to give the proposal the best review is utilized.

Scoring will use the current 5 category, 9-point NIH scale and applicants will receive written feedback on the strengths and weaknesses of their applications for each category. Our goal in giving such detailed feedback is to strengthen unfunded applications so they well be competitive in the next round as well as to provide general mentoring for the applicants who are at an earlier career stage. In addition, specific *criteria* beyond the usual 5-point NIH scale will also be used in evaluating the Pilot Applications are shown in **Table 1**. These criteria will be used to ensure pilot project awards align with PPC goals. Trainees and researchers new to drug abuse research will be prioritized in the review process and all applicants will be aware of this prioritization prior to submission.

Second stage of review: For the next stage, the ranked proposals will be reviewed by the Steering Committee (Comprised of the C3A PIs and Core technical staff) to ensure the top scoring applications are compatible with Core capabilities and capacity. The funds requested by each application will be reviewed with the goal of maximizing the amount of impactful work that will be accomplished, the number of applications that can be funded, and the career stage of the applicants. The final funding decision is made by the five C3A PIs (Bradshaw, Hájos, Katona, Lu, and Mackie).

NIDA Program Officer approval for all Pilot Projects will be obtained before they are awarded. Information provided to the Program Officer will include a table outlining the Project summaries from all Pilot Projects submitted, a summary of the review process and scores, the proposed pilots selected for funding, and the justification for the pilot projects chosen. In addition, assurance will be provided by the Pilot Investigator before funding that all applicable Federal regulations, policies, and guidelines for research involving vertebrates and protection of human subjects, including the evaluation of risks and protections and appropriate ethical oversight will be followed. Such assurances will be in place (C3A administrative staff will assist in this process as necessary, especially for specialized approvals such as those involved in using Schedule I compounds). In addition, awardees will need to agree to follow the C3A's Resource Sharing Plan.

Pilot project review criteria	
1.	Young investigator or investigator new to addiction field
2.	Support groups historically underrepresented in drug abuse research
3.	Project cannot be done without accessing BLM or MSI Cores
4.	Generate preliminary data for NIDA application
5.	Component of high risk/high reward

Table 1. Evaluation Criteria for pilot projects

Award mentoring and expectations: Each successful applicant will be assigned a Core technical staff member (e.g., Barna, Huang, or Johnson) who will provide background and training in the technology used for the relevant Core. Awardees entering the drug abuse field or already working in the drug addiction field, but at an early career stage, will be matched with a Center PI or C3A Affiliate experienced in drug addiction research (e.g., Hohmann, Katona, Mackie, etc.), based on the awardee's interests, geographic location (e.g., if an awardee is from an Affiliate's institution it will make sense to match that awardee with the Affiliate if scientifically appropriate) and needs for guidance in choosing and evaluating appropriate models of substance abuse and experimental design.

Importantly, trainees supported by the PPC will present their work before initiation and during the early, middle, and late stages (either in person or Zoom, depending on the nature of the project and the location of the awardee). The venues for these presentations include the monthly steering committee meetings (this will be the most frequent venue for presentation) and at least one external advisory board meeting (typically when the project is completed). The goal of these presentations is to encourage feedback and discussion on the awardee's project (such a discussion is likely to be productive at all stages of a project, but are especially important during the early stages), the most profitable future directions the research could go, how best to prepare for grant submission, and to enable the awardee to network with a broad range of accomplished drug abuse researchers. All grantees will be required to submit a standardized final report at the end of the project. This report will include research accomplishments, challenges that arose and how they were addressed, presentations/manuscript submissions arising from the funded work, and grant applications that were supported by PPC funding. As much as is practical and appropriate, we will encourage past awardees to mentor current awardees, as past awardees are keenly aware of the challenges facing scientists at an early career stage (or recent transition to a new field) and can offer perspectives complementary to those of the established investigators (*i.e.*, Center PIs and Affiliates). We aim to support 2-4 pilot projects annually, depending on the funds available and the budgets of the pilot projects funded.

Criteria for evaluating Pilot Projects and the Pilot Project Core: Criteria that will be used to evaluate the success of the Pilot Project Program includes:

- (1) publications and poster presentations arising from studies support by the PPC,
- (2) the frequency of pilot projects transitioning from pilot projects into full projects, and
- (3) the number of grant submissions (and evaluating the role of Pilot Project funding in the success of the proposal).

For the latter, the contribution of the pilot project funding towards the grant submission will be an important criterion for evaluation. For trainees receiving Pilot Project funding, funding that contributed to their transition from trainee to independent investigator will be considered as a strong marker of success. This information will be conveyed annually to the NIH (via the C3A's RPPR) and to the External Advisory Board as a component of the C3A's annual report.

PILOT PROJECT 1: ROLE OF ADOLESCENT EXPOSURE TO THC ON THE DEVELOPMENT OF POST-CONCUSSION SYNDROME AS A RESULT OF MILD TRAUMATIC BRAIN INJURY

A. BACKGROUND AND SIGNIFICANCE

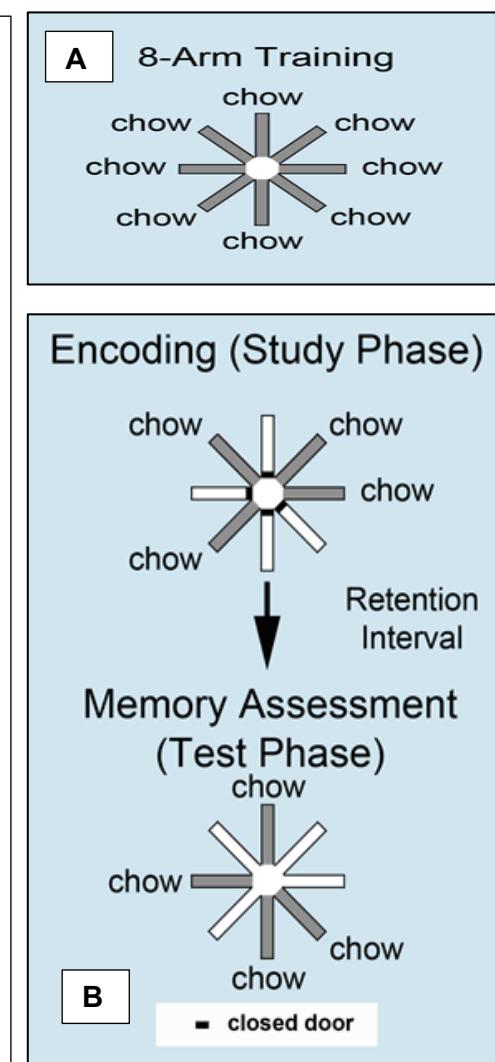
Between 10 and 25 percent of mild traumatic brain injury (mTBI) patients suffer from a cluster of long-term neurological problems. Known as post-concussion syndrome (PCS), symptoms are predominately headache, a range of cognitive deficits, and depression [1]. Currently, there are no established treatments for PCS and no clear predictive biometrics to determine which patients are at increased risk [2]. Hampering these goals is that the fact that, while there are preclinical models of mTBI, there is no preclinical model of PCS. One potential cause of PCS is preexposure of neural insult through prior drug use [3,4] Adolescence is a particularly vulnerable time in brain development and exposure to drugs of abuse, like THC, can have detrimental effects on development and adult behavior and CNS function [5-7] Therefore, there may be an even greater impact on neuronal function with a combination of adolescent THC exposure and later mTBI.

The Crystal lab has developed and utilized sophisticated behavioral assays for determining changes in rodent cognition, including spatial working memory (**Fig. 1**) [8-10]. In my graduate work in the Crystal lab, I (Gabriel Nah) recently developed an mTBI model that mimics the biomechanics of sport-related mTBI and showed that this head injury produces long-term changes in spatial memory (**Fig. 2**) and is accompanied by changes in hippocampal microglial phenotype and morphology (**Figs. 3, 4**).

Researchers have endeavored to understand the biological and environmental risk factors that increase PCS incidence; however, there is currently no unifying set of criteria to predict who will ultimately develop this often-debilitating condition. The Bradshaw lab recently showed that the CNS endocannabinoid-related lipidome is dramatically altered in a rodent model of mTBI [11].

Adolescent THC exposure produces long-lasting cognitive deficits and microglial activation [5,12]. Given that a risk factor for PCS may be preexposure to a neural insult from earlier drug use, and that adolescence is a particularly vulnerable time in CNS development when exposure to drugs of abuse, like THC, may have detrimental effects on development, adult behavior, and CNS function, **we hypothesize that adolescent THC exposure will worsen cognitive deficits and microglial activation after mTBI as an adult.**

Figure 1 Behavioral assessment of spatial working memory: Food is available at each open runway that radiates from a central hub. An initial visit to a baited runway is considered a correct choice; a revisit to an already depleted runway is considered an error. Accuracy is measured as a proportion of visits to baited (correct) versus depleted runways. **A.** In *8-arm training*, all runways are accessible, each runway provides food, and the daily session ends when all food has been depleted. **B.** In *2-phase testing*, a daily session consists of a study phase, a brief retention interval delay, and a test phase. In the study phase, 4 randomly selected runways are accessible, and the rats are permitted to eat food in each accessible runway; the remaining 4 runways are blocked by closed guillotine doors, thereby preventing access to these locations. In the test phase, all 8 runways are accessible, and food is available only in the runways previously blocked by closed doors in the study phase. The session ends when the rat finds the food in the 4 baited locations in the test phase.



This proposal utilizes both the C3A BLMC and MSIC to characterize a novel model of adolescent THC-exacerbated PCS by testing the hypothesis **that adolescent THC exposure will worsen cognitive deficits and microglial activation after adult mTBI**. We will evaluate this hypothesis using a rodent model of adolescent THC exposure followed by adult mTBI and analyze behaviors, inflammatory gene expression, changes in CNS and plasma lipid biomarkers (BLMC), and microglia/astrocyte activation (STORM analysis in MSIC).

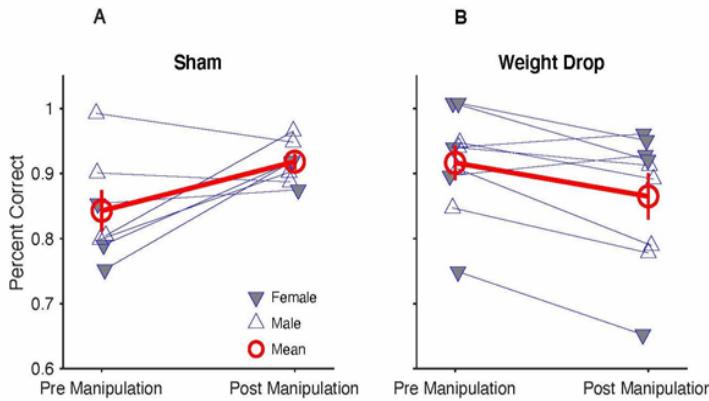


Figure 2. mTBI impairs spatial working memory in rats. Spatial working memory was assessed pre- and post-manipulation in sham and weight drop (head injury) groups. Accuracy in the sham group (**A**) improves with additional behavioral training (pre vs. post manipulation) whereas accuracy declines in the weight drop group (**B**) after being subjected to the weight drop. Data were subjected to an analysis of covariance: significant effects of injury, behavioral training status, and injury X training status interaction ($p < 0.01$); sex and other interactions were not significantly different ($p > 0.05$), though the low number of subjects lacks statistical power to make a confident determination. Accuracy is measured by the proportion of visits to baited locations in the first 4 choices in test phases. Error bars are ± 1 SEM. (Source: Nah et al. in preparation)

B. INNOVATION

Investigating the relationship between adolescent THC exposure, mTBI, and CNS lipid signaling dysregulation as a risk factor for PCS is novel. While studies have linked Cannabis use to poorer mTBI outcomes [3,4], no previous studies have examined the effect of CNS lipid signaling, adolescent THC exposure and mTBI in an animal model in relation to multiple, comprehensive outcome indicators: behavioral assays, brain morphology, gene expression, and morphology, and changes in the plasma and CNS lipidome. Should we find that adolescent THC exposure worsens PCS promotes neuroinflammation, this would suggest a possible strategy that should be examined for Cannabis users suffering from a mTPI. Similarly, there are currently no published reports on the use of the eCB-related lipids as plasma biomarkers for mTBI or PCS. Should these prove sensitive, this could lead to a breakthrough in the field.

C. APPROACH

Our approach toward developing an animal model of mTBI leverages our work on animal models of memory in the Crystal lab with lipidomics and imaging techniques provided by the BMLC and MSIC cores, respectively. The translational potential of this work stems from utilizing behavioral assays in animals that mirror (as closely as possible) the types of cognitive deficits seen in humans following mTBI. For example, the Crystal lab has developed several rodent models to quantify spatial working memory (the ability to briefly maintain information in mind) and episodic memory (memory for unique, personally experienced events from the past). These aspects of memory in people are quite fragile and are frequently impaired following mTBI and diseases of memory, such as Alzheimer's disease. Thus, our preclinical work has high translational potential as we are examining a form of memory that are specifically impaired in PCS. We propose to use this pre-clinical rodent model for mTBI to *test the hypothesis that THC exposure during adolescence exacerbates memory, cellular, and biochemical outcomes following mTBI in adulthood.*

The overall experimental approach (Fig. 5) involves administering THC or vehicle administration during adolescence, training in memory tasks during early adulthood (after THC has been metabolized and excreted), induction of mTBI (or sham), and then further training of memory tasks. Blood will be collected for lipid analysis before and after the weight drop treatments and brains will be collected at the end of the experiment (see Fig. 5 for time points for each intervention).

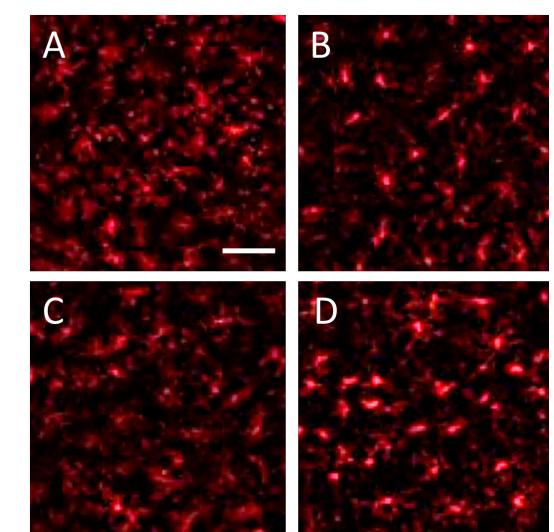


Fig. 3. Representative images of hippocampal CA1 (A-B) and DG (C-D) 24 hours after sham (A,C) and mTBI (B,D). A-B: The soma of CA1 microglia (red) appears to be “swollen” after mTBI (B) compared to the sham (A) indicating a change in their morphology consistent with activation. Similar changes occurred dentate gyrus microglia (C-D). In addition, fine processes are decreased after mTBI. Together, these suggest that microglia are activated at 24 hours post injury. mTBI = mild traumatic brain injury; CA1 = hippocampal cornu ammonis; DG = dentate gyrus. (Nah et al. in preparation)

Plasma and brain lipids will be identified using the large and expanding lipid screening library outlined in the BLMC core proposal, ~150-300 lipids will be analyzed from each sample: (see **Fig. 4** in the BLMC Research Strategy for preliminary data of plasma analysis 2-hours post THC treatment for an example of a subset of lipids). At the end of behavioral testing, all animals will be sacrificed, and brain tissue will be analyzed for changes in lipids by HPLC/MS/MS. Associated alterations in the expression levels of key molecular players of the endocannabinoid system will be determined at the mRNA level by qPCR [13]. The lipid and mRNA measurements will orient subsequent correlated confocal and STORM super-resolution imaging experiments to establish brain region- and cell-type-specific quantitative changes in the nanoscale distribution of the endocannabinoid metabolic enzymes or the CB₁ receptors associated with mTBI and adolescent THC exposure.

In line with our preliminary data that show microglia activation and morphological changes after mTBI (see **Figs. 3, 4**), other studies demonstrate prolonged microglia activation after adolescent THC [12]. We hypothesize that adolescent THC, by eliciting a persistent neuroinflammatory state, impairs the P2Y12-mediated protective effects normally occurring after brain injury [13,14] and this will worsen behavioral outcomes after mTBI. This will be examined by measuring the anatomical density and molecular composition of the recently discovered somatic purinergic junctions that are contact sites between microglial processes and neuronal cell bodies. The co-PI of MSIC was involved in the original description of these structures that are identified by STORM super-resolution imaging of P2Y12-containing junctions. Therefore, we will perform STORM imaging in CA1 and DG in the different treatment groups using techniques outlined in the MSIC core proposal [13].

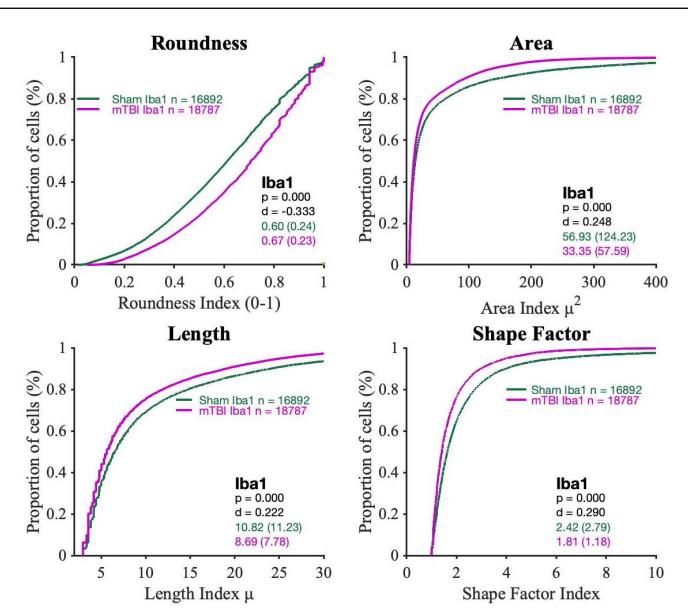


Fig. 4. Microglial morphological changes observed by Iba1 staining 24hrs after mTBI. Brains were removed and stained for Iba1 to identify microglia 24 hours after sham or weight drop. Four parameters were measured: roundness, area, length, and shape factor and cumulative frequency histograms plotted. Green and purple represent microglia from sham, and weight drop groups, respectively. There were significant differences between the sham group and the mTBI group for all parameters ($p < 0.001$). (Nah et al. in preparation)

In line with our preliminary data that show microglia activation and morphological changes after mTBI (see **Figs. 3, 4**), other studies demonstrate prolonged microglia activation after adolescent THC [12]. We hypothesize that adolescent THC, by eliciting a persistent neuroinflammatory state, impairs the P2Y12-mediated protective effects normally occurring after brain injury [13,14] and this will worsen behavioral outcomes after mTBI. This will be examined by measuring the anatomical density and molecular composition of the recently discovered somatic purinergic junctions that are contact sites between microglial processes and neuronal cell bodies. The co-PI of MSIC was involved in the original description of these structures that are identified by STORM super-resolution imaging of P2Y12-containing junctions. Therefore, we will perform STORM imaging in CA1 and DG in the different treatment groups using techniques outlined in the MSIC core proposal [13].

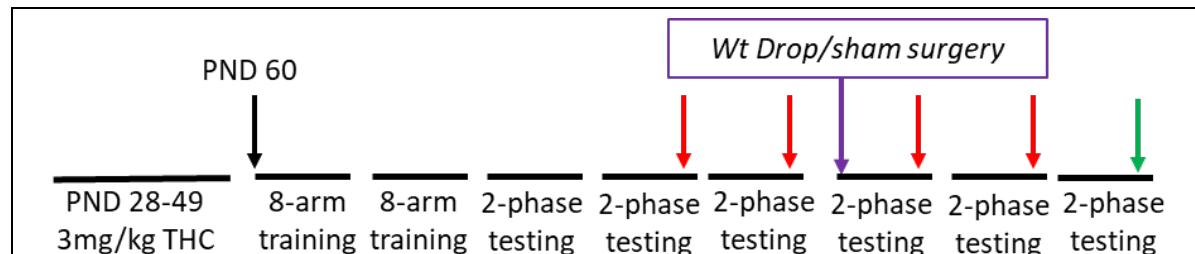


Fig. 5. Schematic of overall experimental design. Black bars after PND 60 denote a week. The red arrows are time points for tail blood collection. The purple arrow denotes the time of the weight drop procedure. The green arrow is the final tissue collection of both blood and brain. Animals will be injected with 3mg/kg THC or vehicle from PND 28-49. At age PND 60, animals will start behavioral training on cognitive tasks. Blood samples will be collected on days at the beginning of the two-week training paradigm (red arrows), one day after the mTBI treatment, and on 3 additional days during the 3 weeks of post-mTBI behavioral analyses. Animals will be sacrificed after the last testing day, and CNS and core blood collected. This study requires 2 adolescent treatment groups [THC, veh] x 2 mTBI [mTBI; sham-treated] x 2 sexes [M; F] x 10 rats per group (N = 80)

Key experimental steps:

- Part 1: Analyze behaviors pre and post weight drop-induced mTBI after adolescent THC or vehicle exposure.
- Part 2: Collect and analyze plasma lipid biomarkers at key timepoints pre-post mTBI (see BLMC studies).
- Part 3: Collect/analyze CNS lipid biomarkers and inflammatory mRNAs after mTBI (see BLMC & MSIC studies).
- Part 4: Analyze changes in microglia and their markers in CNS post mTBI (approaches in MSIC).

Experimental Methods

All procedures have been approved by the IUB IACUC.

Adolescent THC exposure. 80 Male and female Sprague-Dawley rats will used. Half of each sex will receive 3mg/kg THC while the other half will receive vehicle i.p. from P28 to P49.

mTBI weight drop procedure. The Wayne State injury apparatus [15] consists of a 1-meter PVC tube that guides a 400 g weight aimed at the head of a lightly anesthetized (isoflurane) rat placed on perforated aluminum foil. When the weight contacts the head, the aluminum foil gives way, and the rat undergoes a 180° rotation, landing on its back on a foam pad below. A fishing line attached to the weight ensures a single impact. The sham procedure is as described above, except that the weight does not contact the head. Equal numbers (by sex and treatment group) receive either the weight drop or the sham treatment.

Behavioral procedures. To assess cognitive impairment after mTBI, PND60 rats are first trained in a behavioral assessment of spatial working memory (see **Figs. 1, 5**). After rats achieve a high level of accuracy of spatial working memory (as in our published work [9,10,16]), they undergo a weight drop or sham. Next, spatial working memory is re-assessed daily starting one day after mTBI. Multiple sessions are conducted over several weeks to improve accuracy of measurement and identify trends (i.e., speed of learning). The dependent variable is the proportion of correct arm visits in the first four choices of each session (**Fig. 1**, see preliminary data in **Fig. 2**).

Blood collection and analysis of lipids in plasma and CNS. Blood (see **Fig. 4** for sampling times) will be collected from the tail vein [17, 18], plasma prepared, and frozen at -80°C until brain region dissection (e.g., hippocampus, cerebellum, PFC), lipid extraction, or mRNA purification. Lipid extraction, partial purification, and HPLC/MS/MS or mRNA purification and qPCR are performed as previously described [17-19].

Immunohistochemical analysis. After the last day of post-injury behavioral testing, rats are anesthetized with ketamine/xylazine and transcardially perfused with 0.1% heparinized 0.1 M phosphate buffered saline (PBS) and perfusion-fixed using 4% paraformaldehyde (PFA) in PBS. Brains are extracted, post-fixed in 4% PFA for 2 days, and cryoprotected in 30% sucrose for 3 days. Brains are sectioned at 40 µm. Free floating slices are washed with 0.1M PBS and 0.3% TritonX100 PBS, blocked in 4% normal goat serum, and incubated overnight at 4°C with mouse anti-rat Iba-1 to label microglia. The next day, the sections are washed in 0.3% TritonX100 in PBS and incubated for 2 hours in AlexaFlour 488 goat anti-mouse antibody. Sections are washed and then incubated with DAPI, mounted on slides, and left to dry overnight. The next day, the slides are cover slipped using Prolong Diamond, sealed, and dried overnight. Images are captured using a Nikon A1 confocal microscope. For analysis, using ImageJ or Imaris, microglial morphological characteristics in CA1 and the dentate gyrus will quantified using standard morphological parameters [20]. STORM imaging to determine P2Y12 [13] and CB₁ nanoscale distribution and endocannabinoid-metabolizing enzyme distribution is described in MSIC Research Strategy.

Tissue collection for lipid and mRNA measurements. After the final testing session, the brains from half (i.e., 5) of the rats will be removed and stored frozen at -80°C and used for lipid (see *BLM Core for further details*) and mRNA (mRNA will be purified using Qiagen kits and the Taqman rat inflammation panel (catalog #: 414081) will be used to profile mRNAs associated with the onset and resolution of the inflammatory response) analysis.

Expected outcomes, pitfalls, and alternative approaches:

Based on our preliminary data, we anticipate that adolescent THC exposure will worsen cognitive deficits in rats after mTBI. Additionally, we anticipate mTBI exacerbates the mild cognitive deficits associated with adolescent THC. We also predict that inflammation (both morphological and molecular) will increase and that the degree of cognitive impairment will correlate with the level of inflammation. Such findings will motivate more studies to determine if anti-inflammatory approaches improve cognitive outcomes following mTBI after THC exposure.

While not proposed here, as time and resources allow, astrocytes will also be characterized as they are likely to be adversely affected by mTBI. As we have the technical expertise needed to complete the studies as evidenced by our preliminary data and previously published work, we do not anticipate any specific technical problems.

It is likely that adolescent THC exposure will produce a mild impairment of spatial working memory prior to mTBI or the sham procedures. We have two approaches to handle this important confound. First, we will use a statistical approach to quantify deviation scores pre- vs. post- mTBI induction. Second, several factors are known to influence levels of spatial working memory based on our own work and that of others [21,22]. Accordingly, we will vary proactive interference by preceding a target radial maze trial by 1, 2, or 3 preceding trials to match performance between adolescent THC and vehicle groups. Moreover, we will use a randomized block design to equate any remaining variation in spatial working memory prior to assigning rats to mTBI and sham conditions.

PILOT PROJECT 2: An “Add-on” Study to Examine Endocannabinoid and Lipidomic Profiles in Plasma as Biomarkers of Substance Use Disorder: Cannabis Administration in Individuals with Opioid Use Disorder - Controlled Inpatient Trial at the University of Kentucky

A. SIGNIFICANCE

Opioids and cannabinoids are the two most widely used/misused drug classes.[1,2] Despite high rates of licit and illicit use of these drug classes, there are no controlled data on the effects of inhaled cannabis in one of the most high-risk groups – individuals with opioid use disorder. Epidemiological studies provide conflicting data on how cannabis availability affects rates of opioid use and overdose rates.[3-5] One highly cited study reported that, in states with enacted medical cannabis laws, there were 25% fewer opioid overdose fatalities.[5] However, two recent studies[6,7] reported the opposite relationship – a 23% increase in opioid overdose mortality in states with medical cannabis. Despite inconclusive data, there has been strong public advocacy for using cannabis to mitigate the opioid crisis,[8] which has resulted in five US states enacting legislation that lists opioid use disorder as a qualifying condition for medical cannabis. Some of the unfortunate consequences of this legislation are that cannabis dispensaries are now: 1) promoting cannabis as an effective treatment for opioid use disorder and 2) suggesting that patients stop using their current FDA-approved medication for opioid use disorder (e.g., buprenorphine, methadone) and use cannabis instead.[9] These claims are potentially life-threatening for those with opioid use disorder and well-controlled data on this topic are critical. Additional research is needed to test the most commonly used forms of cannabinoid agonists, inhaled cannabis, to determine how route of administration and cannabinoid constituents (THC, CBD) may affect opioid withdrawal. It is also necessary to carefully assess how cannabis may alter the safety profile of opioids (e.g., abuse potential, respiratory depression) in individuals with opioid use disorder. The parent R01 (DA054347) critically examines these issues. This pilot project will examine changes in the plasma lipidome in response to the study interventions described over the following pages.

B. INNOVATION

This project is highly innovative because it will be the first controlled data on the effects of cannabis in individuals with opioid use disorder by providing 1) a careful evaluation of a full dose range of inhaled cannabis, 2) a risk/benefit profile of cannabis during opioid withdrawal and during active opioid use, and 3) *broad-scale lipidomics screens* to explore the biochemical impact of opioid use disorder and active opioid use and determine if cannabis administration modulates endocannabinoid/lipid dysregulation. The multi-dimensional assessments and experimental control in these carefully designed studies will also yield novel and innovative data on guiding dose selection for future clinical studies, generating novel lipid biomarkers in those with OUD, and will help determine if cannabis may be a useful therapeutic for mitigating symptoms of OUD. This project is also timely: U.S. Senators [10,11] have urged the research community to examine how cannabis use affects those with OUD and the opioid crisis at large; several states are voting to further expand cannabis access; Senate Majority Leader Chuck Schumer indicated that it is a party priority to fully legalize recreational cannabis nationwide;[12] and there is federal legislation under consideration that would remove cannabis from the DEA Controlled Substances Act.[13,14] Overall, this timely and innovative project will provide data to inform state policies, regulatory considerations, clinical practice, prescribing, and public safety.

C. APPROACH

Overview: We will collect plasma samples from participants enrolled in a newly awarded NIDA R01 at the University of Kentucky (UK). This inpatient trial examines the effects of acute cannabis administration on outcomes related to opioid use disorder – severity of opioid withdrawal, opioid reward, and opioid safety. The pilot project proposed here will allow us to carefully collect plasma samples in a highly controlled research environment across all experimental conditions (as outlined below) to assess the profile of endocannabinoids and lipids in individuals with opioid use disorder and determine if cannabis modulates those profiles. This pilot study presents a rich opportunity to collaborate with IU scientists and access state-of-the-art analyses from the BLMC to collect the first controlled data on plasmid lipids in humans in this research area. During the 12 months of funding, we will assess the endogenous plasma lipidome as well as evaluation of phytocannabinoids and metabolites in participants with opioid use disorder under the following conditions:

- 1) **Baseline:** Prior to enrollment, participants will present to the laboratory for screening - we will collect plasma to determine the lipidomic profile (*screening ~150-300 lipids in expanded lipid screen-see BLMC proposal*) during uncontrolled/naturalistic polydrug use (i.e., daily fentanyl; methamphetamine, benzodiazepine, cannabis, and other drug use). Drug use will be verified through urine sample immunoassay and/or LCMS.

2) **Opioid stabilization:** After participants are medically cleared for the study, they are admitted to the inpatient research unit at the hospital and will receive oral morphine (40 mg, 4 times/day for 7 days) to stabilize their opioid withdrawal symptoms. After the stabilization is complete, we will collect plasma to determine how consistent administration of morphine and wash-out from illicit opioids and other drugs affects the plasma lipidomic profile compared to samples collected during baseline.

3) **Opioid withdrawal:** During inpatient enrollment, participants undergo bouts of opioid withdrawal (spontaneous withdrawal produced by the double-blind omission of regularly scheduled morphine doses); plasma will be collected 5 hrs after session start (during mild-to-moderate opioid withdrawal). The samples collected during these sessions will reveal how opioid withdrawal impacts the plasma lipidome.

4) **Opioid withdrawal + active cannabis administration:** During 6 of the 7 opioid withdrawal bouts, active cannabis will be inhaled during opioid withdrawal sessions (samples collected 5 hrs after session start, equiv. to 4.5 hrs after cannabis administration). Six doses of cannabis will be tested: THC alone (5, 10, 20, 30 mg THC) and THC/CBD combinations (10 mg THC+10 mg CBD; 30 mg THC + 30 mg CBD). The data collected here will help inform if cannabis administration affects the analyte profiles relative to opioid withdrawal alone (e.g., placebo cannabis); we will also explore the role of CBD to assess if its addition to THC may alter the biochemical profiles relative to THC alone.

5) **Intranasal oxycodone administration:** During enrollment, participants will receive intranasal oxycodone (90 mg) during 7 separate sessions as a model of opioid reward (*i.e.*, administration of supratherapeutic doses of oxycodone models opioid misuse). Plasma samples will be obtained 4 hrs after intranasal oxycodone administration to explore how supratherapeutic acute doses of an opioid may alter the plasma lipidome.

6) **Intranasal oxycodone + cannabis administration:** During 6 of the 7 oxycodone administration sessions, cannabis will be administered immediately prior to oxycodone (designed to determine how cannabis affects opioid reward; samples collected 4 hrs after oxycodone). The plasma lipidome will be examined here to assess if cannabis modulates the analyte profile produced by oxycodone.

The data collected during this pilot project will provide the first controlled data in this population of humans and will provide 1) *a summary profile of the plasma lipidome in those with OUD*, 2) *how opioid use and/or THC +/- CBD may change these lipidomic profiles* (e.g., regulate, dysregulate), and 3) *how the potential therapeutic benefit/harms of cannabis effects the plasma lipidome over time*. Overall, this pilot project will collect high impact and innovative data in the context of a controlled inpatient study, which would not be feasible without access to the BLMC facility and support from the P30 grant.

Objectives: The primary objective of this study is to explore how plasma signaling lipids are modulated during the experimentally controlled conditions of 1) opioid withdrawal, 2) cannabis administration during opioid withdrawal, 3) opioid administration, 4) cannabis + opioid administration, and 5) opioid stabilization. We will also explore if plasma lipid concentrations are associated with opioid abuse potential (e.g., subjective ratings of drug effects) or opioid safety outcomes (e.g., O₂ saturation, EtCO₂ concentration, respiratory rate) - measures which are collected regularly throughout the primary study.

General Design: The parent study (R01DA54347) employs a within-subject, double-blind, randomized, placebo-controlled, crossover design. Participants (*n*=15 completers) will be enrolled as inpatients and will complete 1 safety session and 14 experimental sessions (7 pairs of sessions) across 5.5 weeks. Each session pair will be comprised of one opioid challenge session and one opioid withdrawal session, with the same cannabis dose tested within each session pair (*i.e.*, 7 pairs, 7 cannabis doses). The order of the sessions within each pair (challenge first, withdrawal second) will be counterbalanced across participants. Participants will be maintained on oral morphine throughout the study (40 mg/dose, 4 doses/day). Morphine doses will be withheld prior to opioid withdrawal sessions to produce spontaneous withdrawal (per protocol) and during the opioid challenge session for safety purposes (this procedure has been used successfully by our research group[15-17] and others.[18-20]).

The blood draws will occur at regularly scheduled times during the participant's stay. Blood draws will be conducted during each type of experimental condition; importantly, the timing of the blood draws will not compromise the double-blind experimental conditions as one blood draw will be collected during each session. All planned blood draws are indicated in the calendar and described below:

Detailed overview of blood draws:

- 1) **Baseline blood draw:** The baseline blood draw will occur prior to admission, as it will function to assess ongoing/naturalistic drug use (e.g., illicit formulations, polydrug use).

- 2) Post-stabilization blood draw: Blood draw 2 will be collected after the completion of opioid stabilization (approx. 6-7 days of morphine maintenance), approx. 4 hrs after oral morphine administration.
- 3) Acute opioid reward paired with cannabis/placebo pretreatment: Blood draws 3, 5, 7, 9, 11, 13 and 15 will be completed during a within-session dose-response administration of intranasal oxycodone (90 mg cumulative total). These opioid doses will be preceded by cannabis/placebo pre-treatment (7 different doses across the 7 sessions, one dose per session): THC (5, 10, 20, 30 mg), THC/CBD combination (10 mg THC:10 mg CBD; 30 mg THC:30 mg CBD), and placebo. Blood draws will occur approx. 4 hrs post-opioid dose. Plasma analytes will be assessed during opioid administration alone (no cannabis) and during cannabis pre-treatment across all active doses. Exploratory outcomes will examine if changes in the analyte profiles are associated with subjective ratings of drug effects (e.g., VAS reporting, standardized questionnaires of street value and drug liking outcomes) or safety outcomes (e.g., oxygen saturation, EtCO₂ concentration, respiratory rate).
- 4) Acute opioid withdrawal paired with cannabis/placebo administration: Blood draws 4, 6, 8, 10, 12, 14 and 16 will be completed during spontaneous opioid withdrawal sessions. During each of the opioid withdrawal sessions, one inhaled cannabis dose will be administered: THC (5, 10, 20, 30 mg), THC/CBD combination doses (10 mg THC:10 mg CBD; 30 mg THC:30 mg CBD), and placebo. Plasma lipids will be examined during opioid withdrawal alone (placebo cannabis) and when active cannabis is administered. Analyte profiles will also be explored to determine if changes are associated with subjective ratings of opioid withdrawal severity (e.g., VAS ratings of withdrawal severity, standardized assessments [Himmelsbach, COWS, SOWS]) subjective drug effects (e.g., VAS reporting, standardized questionnaires of street value and drug liking outcomes) or safety outcomes (e.g., oxygen saturation, EtCO₂ concentration, respiratory rate).

General Experimental Procedure: On the morning of experimental sessions, participants will receive a standardized breakfast 1.5 hrs prior to dose administration. The session will be conducted if urine samples test negative for drugs of abuse (outside of expected positives from experimental drug administration – a positive drug screen is not expected as participants are residing as inpatients at the hospital and the screen is conducted out of caution); pregnancy will be tested in those with child-bearing potential and breath samples must test negative (0.000 BAC) for alcohol. Participants will be permitted to smoke cigarettes/nicotine 30 min prior to and upon session completion. After data collection has been completed and participants have passed a sobriety test, they will be transported back to the CCTS Inpatient Unit and provided a full meal.

All participants will provide written informed consent and will be paid for their participation. This add-on pilot study will pay participants \$25 per blood draw (16 blood draws = additional \$400 payment per participant). Because we are requesting frequent blood samples and samples during opioid withdrawal sessions (during which participants are already quite uncomfortable), \$400 is well justified.

Research Setting: The proposed studies will be conducted by Shanna Babalonis, Ph.D. and her team at the Straus Behavioral Science Research Facility and at the Center for Clinical Translational Science (CCTS) Inpatient Research Unit at the University of Kentucky (UK) Chandler Hospital. We have also recently established the UK Cannabis Center (Dr. Babalonis is the Director). This team has a long history of enrolling participants with opioid use disorder and cannabinoid use history. The laboratory and CCTS staff have extensive experience working with and monitoring participants with opioid use disorder and are familiar with executing the protocols for clinical pharmacology research protocols.[21-27] The CCTS is a 12-bed unit supervised by nursing staff 24 hrs a day, 7 days a week. Participants are monitored by nursing staff who collect vital signs, weight, and query for adverse events and side effects.

Participants will be transported under supervision from the CCTS to the Straus Behavioral Science Research Facility (the location of the ventilated smoking laboratory) for cannabis administration and experimental sessions. The laboratory facility has on-site medical staff (Dr. Lofwall, Dr. Fanucchi, a team of clinical physicians, and RNs) and the offices for Dr. Babalonis, Co-I's, the lab supervisor, and research assistants and nurses. This facility is fully equipped for the proposed work (e.g., sample processing lab, fully-equipped crash cart, secure Schedule I drug storage facilities, fully equipped cannabis smoking laboratory, physiological monitoring equipment, experimental test areas, private exam rooms). After session completion, staff will transport participants back to the CCTS Inpatient Unit (<2 miles).

Sample Collection: An experienced and licensed medical professional (e.g., RN) will obtain the blood samples under sterile conditions following universal precautions. Samples will be processed on-site in the laboratory's sample processing area and stored in the facility's -80°C freezers. Samples will be shipped

overnight on dry ice to the core facility every 2 months (Dr. Babalonis has extensive experience shipping biological samples and has led sample collection, processing and shipment on four NIH grants). Back up samples will also be stored on-site in the event of sample loss during shipment.

Participants: Participants enrolled in the inpatient study will be adults, ages 18-50, with BMI of ≤ 30 . Participants will be non-treatment seeking and meet DSM 5 criteria for moderate-to-severe opioid use disorder and are physically-dependent on short-acting opioids (e.g., heroin, fentanyl, oxycodone), reporting daily/near-daily opioid use ($>21/30$ days in preceding month), as confirmed by observed, opioid-positive urine samples. Participants will be required to have a lifetime history of inhaled cannabis use (≥ 1 lifetime exposure) but have limited current use (≤ 5 times per month) to decrease the likelihood of enrolling those with high levels of cannabinoid tolerance/dependence. Participants will not be physically dependent on any substance that requires medical detoxification (i.e., benzodiazepines, alcohol) as determined by urinalysis, self-report, and observation (e.g., a negative urine sample/breath for alcohol in the absence of withdrawal signs). Potential participants enrolled in treatment programs and taking MOUD will be excluded.

Participants with appropriate drug use histories are screened through laboratory tests (i.e., blood chemistry with liver function tests, hematology; urinalysis and microscopic evaluation; 12-lead ECG), psychological assessments, and physical and psychiatric evaluation by a qualified study physician (Dr. Michelle Lofwall, board-certified in Addiction Medicine and Psychiatry; Dr. Laura Fanucchi, board-certified in Addiction Medicine and Internal Medicine; both are certified in Advanced Cardiac Life Support). Participants will be carefully screened to eliminate those presenting with seizure disorders, history of asthma/respiratory disorders, head injury, hypertension, or cardiovascular disease. Those with clinically significant abnormal laboratory or ECG results (e.g., liver function tests 3x upper limits of normal, arrhythmia) will be excluded (our cardiologist will evaluate all abnormal ECGs). Participants must not have a chronic illness nor require prescription medication (aside from oral contraceptives) on an ongoing basis (e.g., diabetes). Individuals with child-bearing potential are tested for pregnancy at screening, admission and prior to each session and are enrolled only if they are not pregnant or lactating and are using an effective method of contraception. Additional screening assessments will include: Addiction Severity Index,[28] SCL-90-R checklist,[29] Beck Depression Inventory,[30] NEO Personality Inventory,[31] Wide Range Achievement Test (literacy assessment),[32] Fagerstrom Test for Nicotine Dependency,[33] McGill Pain Inventory,[34] and a locally developed detailed time-line follow-back assessment of alcohol, opioid, cannabis and illicit drug use over the last 30 days capturing dose, route, frequency of use per day, and dollar amount paid. Together, these assessments are used largely for inclusion/exclusion (e.g., participants with current psychiatric symptomatology [e.g., bipolar disorder] will be excluded), assessment of competency (e.g., literacy) and characterization of the population.

Drugs: All doses will be prepared and blinded by the UK Investigational Pharmacy licensed pharmacists (Drs. Seth Larkin, Thomas Lyman). Oral morphine maintenance doses and intranasal oxycodone doses be obtained from Spectrum Chemical Corp. (Gardena, CA). Cannabis will be obtained through the NIDA drug supply. Each cannabis dose will weigh ~ 250 mg and will be vaporized using the Volcano Vaporizer using validated procedures. Research Triangle Institute (RTI) will provide reports of cannabinoid concentrations.

Data analysis: Lipidomic data will be analyzed as discussed in the BLMC.

Expected outcomes and pitfalls: The Bradshaw lab routinely measures human plasma lipids, so there are no expected problems with these being measured in the BLMC. Inter-subject variability in lipid profiles would likely drive the greatest amount of noise in these analyses; therefore, these samples will be analyzed independently per subject across time for change and then as changes from baseline in percentage to account for inter-subject variability. Since the studies from the parent grant are already underway with excellent subject enrollment, there is no expectation that there will be any impediments in recruiting enough subjects for these analyses during the year of the pilot study.

Regulatory: Dr. Babalonis currently holds an IND (#141,123) for opioids and cannabis and a DEA Schedule I drug license (DEA RB0545763).

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

Yes No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved

Yes No

Is the Project Exempt from Federal regulations?

Yes No

Exemption Number

1 2 3 4 5 6 7 8

Other Requested Information

VERTEBRATE ANIMALS

Rats will be housed and used in the Multiple Science Building-II (MSB-II) at Indiana University Bloomington. All experiments will be conducted in compliance with the NIH guide (as appropriate) and following institutional approval. The Animal Welfare Assurance number for IU Bloomington is D16-00587.

We estimate the animal numbers based on the nature of experimental procedures and time required for individual projects.

Here are the estimated experimental numbers for each Pilot Project:

Pilot project 1: 80 Sprague-Dawley rats (40 male, 40 female)

Pilot project 2: None (human study)

1. Description of Procedures

Species: Rat

Strains: Sprague-Dawley

Age: Adolescent through young adult (~P120 at time of sacrifice)

Sex: Male and Female

Number: 100 (to allow for deaths, unexpected illness, etc.) to yield 80 experimental rats

A total of 80 rats will be used over the course of this pilot experiment for all procedures.

Tissue harvesting.

Brains will be harvested after utilizing an AVMA-approved euthanasia technique.

Administration of substances:

Vehicle or THC will be given by subcutaneous (s.c.) or intraperitoneal (i.p.) injection in a volume of 5-10 μ l/g body weight as approved by our animal care and use committee.

mTBI injury:

The Wayne State injury apparatus consists of a one-meter PVC tube, which guides a 400 g weight aimed at the head of a lightly anesthetized (isoflurane) rat placed on a perforated aluminum foil. When the weight contacts the rat's head, the aluminum foil gives way, causing the rat to undergo a 180° rotation, landing on its back on a foam pad below. A fishing line attached to the weight ensures a single impact. The sham procedure is as described above, except that the weight does not contact the head.

Behavioral procedures (working memory assessment):

Food is available at each of 8 runways that radiate from a central hub. An initial visit to a baited runway is considered a correct choice; a revisit to an already depleted runway is considered an error. Accuracy is measured as a proportion of visits to baited (correct) versus depleted runways. A. In *8-arm training*, all runways are accessible, each runway provides food, and the daily session ends when all food has been depleted. B. In 2-phase testing, a daily session consists of a study phase, a brief retention interval delay, and a test phase. In the study phase, 4 randomly selected runways are accessible, and the rats are permitted to eat food in each accessible runway; the remaining 4 runways are blocked by closed guillotine doors, thereby preventing access to these locations. In the test phase, all 8 runways are accessible, and food is available only in the runways previously blocked by closed doors in the study phase. The session ends when the rat finds the food in the 4 baited locations in the test phase.

2. Justification for the Use of Animals

The use of animals (rats) is necessary for this project because the project seeks to elucidate the effects of mTBI +/-THC on memory, which is only measurable in an intact animal. No existing computer simulations are sophisticated enough to address the questions to be studied in this proposal.

As we are conducting these experiments, we will continuously monitor our results and adjust numbers of animals used as appropriate. We will also perform interim power analyses to verify sample sizes once experiments are underway and as guided by initial effect sizes and observed variance.

3. Procedures to Minimize Pain and Distress

All procedures are consistent with the NIH Guide for the Care and Use of Laboratory Animals and follow the guidelines of the International Association for the Study of Pain. In all cases, rats will receive ad lib access to food and water in their home cages. Moreover, rats in our study will not experience undue pain, injury, or discomfort. For tissue harvesting, prior to decapitation, the animal will be deeply anesthetized following IACUC-approved protocols. The rat will be visually monitored during this procedure and when unconscious, the depth of anesthesia will be assessed by the absence of a response to noxious stimulation (pinching of a paw).

If unexpected health findings are observed, we will consult with one of IUB's veterinarians.

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RESOURCE AND DATA SHARING PLAN

Please see the Overall Component for the Resource and Data Sharing Plan

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES PLAN

Please see the Overall Component for the Authentication of Key Biological and/or Chemical Resources Plan