CBC | CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

Mu-opioid receptor regulation by Golgi satellites in opioid use disorder

Name, degree, title, institution, and contact information including the email address of each PI

Christian J. Peters, Ph.D., Assistant Professor, University of Illinois at Chicago
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Do you have any current or pending grant applications that potentially overlap with this application?
Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

(ENTER THE OVERLAP HERE)

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects?	**Check ONE:**	**YES** ☒	**NO** ☐
Does the proposed research involve human subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve embryonic stem cells?	**Check ONE:**	**YES** ☐	**NO** ☒

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

One-paragraph lay-language summary of project. Opioid use disorder (OUD), often resulting from over-prescription of opiate-derived analgesics, is an escalating public health crisis in the USA. With addiction, drugs of abuse are thought to target and rewire the brain's reward circuits. Opioids interact with μ-opioid receptors (MORs) highly expressed in neurons in the brain's reward circuits, specifically ventral tegmental area (VTA) and nucleus accumbens (NAc) neurons. Significant questions remain about how chronic opioid exposure alters the reward pathway and result in addictive behaviors. Opioid drugs of abuse can cross cell membranes. We will test whether these drugs act on MORs in newly-discovered intracellular compartments, called Golgi satellites (GSats) and whether this activation subsequently alters their effects on synaptic and circuit behaviors. We will perform experiments first in cultured neurons and then in live slices from mouse brain exposed to opioids to test these hypotheses.

What makes this project high-risk/high-reward and groundbreaking? The social urgency for improved understanding of OUD cannot be overstated. Here, we propose new paradigms by which opioids interact with and regulate MORs, and lead to reward pathway changes. While opioid signaling is assumed to arise from cell surface MORs, all opioid drugs of abuse are membrane permeable and can activate MORs located in intracellular "endomembranes" of neurons. We have preliminary data that MOR-containing endomembranes are Golgi "satellites" (GSats), a newly discovered, small, mobile organelle. Previously, GSats were found to regulate the trafficking of other receptors at synapses (Figure.1). We hypothesize that activation of intracellular MORs in GSats results in significant changes in synaptic protein trafficking, which in turn underlies modifications to synaptic activity and plasticity in reward circuitry. If correct, intracellular MORs in GSats would represent a novel target for OUD therapeutics. However, our assumptions that opioids activate intracellular MORs located in GSats are counter to longstanding dogma in the field. Because of this, additional findings from our Catalyst grant proposal are needed to establish feasibility before applying for long-term NIH funding.

Application Statement: *Description of proposed research*

We will examine how MOR exposure to membrane-permeable opioid drugs differs from exposure to membrane-impermeable opioid peptides to modify their cellular trafficking and subsequent effects on synaptic signaling. To do this, we will characterize how the different opioids alter the recycling and cellular distributions of MORs, and how this modulates synaptic transmission in the VTA-NAc reward circuit. From these findings, we will establish the link between activity of intracellular MORs and their sorting by Golgi satellites, and long-term changes to synaptic plasticity underlying opioid addiction.

μ-opioid receptors and opioid abuse: Among the families of opioid receptor, the μ receptors (MORs) most directly underlie OUD (Johnson and North, 1992). MORs are transmembrane $G_{i/o}$-linked G-protein coupled receptors (GPCRs). Upon ligand binding, they release Gβγ subunits that mediate acute local cellular inhibition, and accelerate β-arrestin feedback that causes active MORs to internalize into intracellular organelles (Allouche et al., 2014). These receptors are expressed on GABAergic and cholinergic neurons that regulate dopaminergic neurons of the ventral tegmental area (VTA) and its primary target, the NAc shell (Johnson and North, 1992; Svingos et al., 2001), which together are the core of the reward pathway where drugs of abuse act to drive addictive behaviors.

In contrast to the endogenous membrane-impermeant opioid peptides, drugs of abuse such as morphine can diffuse freely into intracellular compartments of cells and bind intracellular opioid receptors. A recent study demonstrated that membrane permeable opioids interact freely and functionally with MORs in intracellular compartments (Stoeber et al., 2018). This finding is contrary to the assumption that GPCR signaling is restricted to GPCRs on the cell surface and implies that *in vivo* chronic exogenous opioid exposure activates MORs in endomembranes. Moreover, exogenous opioids are weak bases that are protonated and concentrate in the acidic GSats (Govind et al., 2017).

Golgi "satellites": Previous work from the Green lab has determined that increased neuronal activity causes novel changes in the intracellular architecture of neurons (Govind et al., 2021). Activity "fragments" the neuronal Golgi apparatus (Thayer et al., 2013) and redistributes small Golgi membranes, Golgi satellites (GSats), throughout dendrites and axons (Govind et al., 2021). GSats were not observed previously because standard Golgi markers used to identify somatic Golgi in dendrites are not present in these dispersed membranes. With activity changes, the addition of new GSats throughout dendrites modifies local secretory pathways at postsynaptic sites (Figure 1). GSats are released from the endoplasmic reticulum (ER) at ER release sites (ERESs) with increased synaptic

activity, and are distinct from “Golgi outposts”, which form via fission from pre-existing Golgi membranes (Quassollo et al., 2015). ERESs are found in the ER of dendrites at postsynaptic membranes, where synaptic activity regulates GSat formation locally at sites of protein translation, as shown in Fig. 1. MORs are N-glycosylated and sorted by way of Golgi membranes (Lemos Duarte and Devi, 2020), suggesting that GSats in the vicinity of synaptic membranes would modify their distribution, and may serve as a platform for intracellular functional MOR signaling. Moreover, we have collected preliminary data suggesting that MOR overlap with sialyltransferase-3, an enzyme that labels GSats. However, the presence of Golgi in the neuronal periphery, and signaling of receptors located there, remain controversial, and a physiological role for such a mechanism requires additional study.

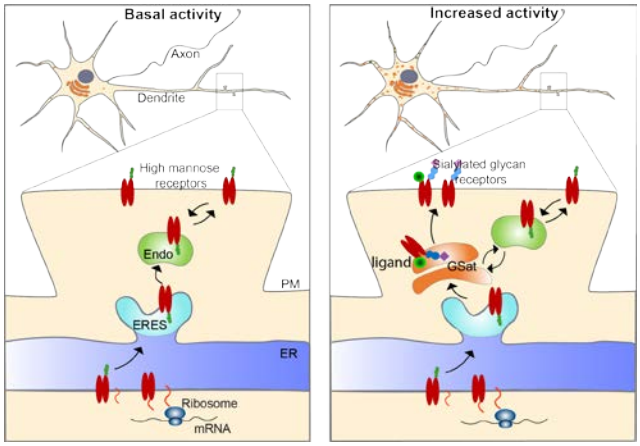

Figure 1. Model of activity-induced GSat function in dendrites. In neurons at rest, locally synthesized proteins in dendrites appear to bypass the Golgi, trafficking through endosomes to the cell surface. With increased neuronal activity, GSats (right panel, orange) arise from local ERES. Membrane proteins, including receptors that can be activated by membrane permeant ligands then traffic through GSats before reaching the surface, having their N-linked glycans modified from mannose to sialic acid, resulting in changes to membrane insertion and/or localization.

In the following Aims we will determine MOR localization, trafficking and synaptic effects *in vitro* and *in vivo* using several cutting-edge innovations. First, a combination of a genetically encoded biosensor for fluorescent tracking of activated MORs, M-SPOTIT1.1 (Kroning and Wang, 2021), with MOR and GSat tagged-constructs designed by ourselves, will allow imaging in live and fixed samples of the distributions of GSats, total MORs and activated MORs in neuronal processes. Second, our ability to section rodent brains at non-orthogonal angles with sufficient precision to preserve intact circuitry permits GSats and MOR movement to be visualized in live *ex vivo* tissue dissected from the reward circuit. Finally, lattice light sheet microscopy (LLSM) (Chen et al., 2014) has improved live imaging resolution from *in vitro* and *ex vivo* tissue sufficiently to allow tracking of subcellular compartments the size of GSats, permitting their movement to be robustly assessed in real time. LLSM also allow us to record high temporal resolution of neurotransmitter release at single synapses (Church et al., 2022).

Aim 1: Characterize the role of GSats in regulating MOR distribution and trafficking and assay changes caused by endogenous vs membrane permeable opioids. Here, we will use simultaneous MOR (Kroning and Wang, 2021) and GSat (Govind et al., 2021) live cell imaging in a medium spiny neuron culture model to visualize how their subcellular distribution is affected by opioids.

1.1. Assay localization and overlap of intracellular MORs and GSats comparing DAMGO-, morphine-treated, and untreated cells in dendrites and axons with attention to pre- and postsynaptic domains.

1.2. Compare active intracellular MORs (Kroning and Wang, 2021) relative to total MOR numbers using a fluorescent biosensor to localize and quantify intracellular MOR activation in the neuron following 24-48 hour exposure to membrane permeable and impermeable opioid agonists.

1.3. Assay MOR endocytosis and recycling to plasma membrane through early endosomes and GSats to contrast the effects of impermeable DAMGO and permeable morphine on their distribution.

Aim 2: Investigate the effects of intracellular opioid agonism on distribution of MORs, GSats and synaptic function in the reward pathway. Here we will test how either *in vivo* exposure of the VTA-NAc circuit to opioids regulates GSat-dependent distribution in fixed mouse brain sections, and movement of MORs in live *ex vivo* brain slices.

2.1. Image MOR/Gsat movement and overlap in live, non-orthogonal VTA-NAc mouse brain slices in response to challenge by exogenous (permeable) or endogenous (impermeable) opioids

2.2 Examine changes in MOR distribution in *ex vivo* VTA-NAc circuit after *in vivo* exposure to opioid agonists and membrane permeable/impermeable blockers by intracranial infusion in live animals.

2.3. Compare effects of long-term (up to 5 day) morphine exposure in mice on the overlap of GSats with MORs in the reward pathway, and the downstream effects on synaptic activity as quantified by the fluorescent reporter of neurotransmitter release "iGABASnFR" (Marvin et al., 2019).

Nature of inter-institutional collaboration and how the success of the Catalyst project requires the expertise of each PI. This proposal will formalize a preliminary collaboration between three groups from two CBC institutions. The expertise of each group is critical for carrying out the proposed aims.

Christian J. Peters – Assistant Professor, University of Illinois Chicago. Dr. Peters is a junior faculty member at UIC studying how activity in midbrain circuitry drives withdrawal symptoms using mouse behavior and neurophysiological recordings from *ex vivo* tissue. His lab has expertise in cell culture models (including from native tissues), stereotaxic brain surgeries for viral infection and implantation of cannulas and *ex vivo* slice electrophysiology. They have developed methodology for non-orthogonal brain slicing and will perform viral infection in mice and implantation of cannulas for intracranial infusions, tissue culture from those mice and imaging of GSats/MOR (in collab with S. Alford).

William N. Green – Professor, University of Chicago. Dr. Green is a senior faculty member at UC who has developed a toolbox for the study of intracellular protein trafficking and protein posttranslational modifications in cellular organelles *in vitro* and has developed highly sensitive optical labels capable of tracking satellites during live cell and tissue imaging. His lab has previously shown that long-term nicotine exposure *in vivo* in mice increases nicotinic receptor-containing GSats in VTA dopamine neurons. Using cell culture and confocal imaging, his lab will examine whether opioid exposure in cultured neurons causes similar effects on MORs and GSats.

Simon T. Alford – Sweeney Professor and Department Chair, University of Illinois Chicago. Dr. Alford is a senior faculty member at UIC specializing in the use of high-resolution live tissue imaging techniques to questions of synaptic signaling and plasticity, with a specific focus on synaptic roles of GPCRs. His lab has adapted the lattice light sheet microscopy technology for the specific use in small particle tracking and synaptic transmission in *ex vivo* brain preparations. This method overcomes previous challenges in resolution and tissue photodamage to allow fine structures such as GSats and fluorescent-labeled MOR to be tracked accurately in real time in live cells and tissues.

Criteria for measuring success of the proposed project: We will examine links between opioid exposure, GSat formation, cellular redistribution of MORs and synaptic function. Landmarks for how the work is progressing will be measured by success in determining the importance of intra- and extracellular MOR signaling in both cell culture and *in vivo* systems as well as the importance of this signaling for opioid effects on synaptic function. We will determine:

1) How opioid exposure causes surface and/or endomembrane MOR activation to affect MOR distribution and their endocytosis into GSats.

2. How opioid exposure alters MOR-containing GSat numbers and distribution in neurons.

3. Whether these changes in MOR and GSat distributions correlate with changes to opioid-induced synaptic activity and plasticity in neurons of the VTA-NAc circuit following opioid exposure *in vivo*.

Long-term funding plan for continuation beyond the Catalyst Award, including funding institution that will be solicited, grant category (i.e. R01, P series, U01) and tentative timeline.

Our expected progress on these aims over the 1-year period of the Catalyst award will allow us to apply for a multi-investigator R01 application from NIH/NIDA immediately afterwards. The primary objective of the “Catalyst” period is to generate compelling preliminary data to establish that MOR presence and functional activity in a peripheral Golgi-like organelle contributes to alterations to signaling in the brain’s reward circuitry associated with OUD. By providing strong experimental evidence for this cellular mechanism, we will address the most significant impediments to funding through more traditional funding avenues, which are the contradictions between our proposed mechanism and the longstanding preconceptions among the neuroscience community regarding cellular Golgi distribution and GPCR function. Our future grant proposals will pursue three crucial lines of inquiry, all built on the preliminary findings developed from this Catalyst award: 1. Cellular mechanism by which surface glycans and their processing in GSats regulate processing of MOR protein in the presence of chronic opioids; 2. Functional crosstalk between intracellular MOR G-protein signaling on GSats and synaptic physiology and plasticity, and 3. Consequences of intracellular opioid signaling on live rodent models of addiction behavior.

Cited Scientific References:

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Program Director/Principal Investigator (Last, First, Middle): Peters, Christian

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 6/1/2023 THROUGH 5/31/2024

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

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Peters, Christian	PD/PI	.24			116,127	2,323	801	3,124
Xiu Ming Wong	Res. Scholar	7.2			53,760	32,256	11,128	43,384
Shana Netherton	Technician	6			45,000	22,500	7,763	30,263
SUBTOTALS →						57,079	19,692	76,771

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

Picospritzer III, a precise solution applicator, used to equip Lattice Light Sheet Microscope for acute focal application of opioid solutions to neurons and slices in situ. ($7461 at Parker.com) 7,461

SUPPLIES *(Itemize by category)*

Laboratory supplies: AAV viruses ($2000), brain slicing consumables ($250/mo = $3000), stereotaxic surgery sterile materials and consumables and pharmaceutical reagents ($250/mo = $3000), laboratory reagents and chemicals including designer fluorophores ($2000) 10,000

TRAVEL

Travel for PI and Research Scholar to attend one meeting 1,000

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*

Animal facility per diems (housing) $11,289 Publication fees $500 11,789

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 107,021
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 107,021

Program Director/Principal Investigator (Last, First, Middle): Peters, Christian

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	76,771				
CONSULTANT COSTS					
EQUIPMENT	7,461				
SUPPLIES	10,000				
TRAVEL	1,000				
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	11,789				
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	107,021				
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	107,021				

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 107,021

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

All surgical infection of mouse models, mouse husbandry and circuit specific tissue dissection and recording will be performed in the Peters laboratory, and data acquisition in these tissues will be performed by Peters lab personnel in the neighboring Alford laboratory. In concert with Green lab, we will also use commercial vendors to generate AAV reagents (developed in Green lab) to infect neurons to label GSats (as well as MORs) in mouse tissues and cultures.

PERSONNEL

Dr. Christian J. Peters, Ph.D., Principal Investigator, 0.24 calendar months - Salary is requested at 2% for the proposed budget period. Dr. Peters is a tenure track assistant professor in the Department of Anatomy and Cell Biology at UIC and will oversee animal-based and imaging experiments as part of this proposal. His role also includes experimental setup specific to this project, training a scholar and technician specifically for the project, hands-on lab experimentation in concert with Peters lab members and the inter-institutional collaborators, as well as literature review and analysis of results. His laboratory carries equipment appropriate to this project, including all apparatus required for animal surgeries, tissue dissection and molecular biology.

Budget Justification, Peters (continued)

Additional Personnel

Xiu Ming Wong, Ph.D., Research Scholar, 7.2 calendar months.

Dr. Wong is a newly hired Research Scholar who began working in the Peters lab in March 2022. She is an expert microscopist, electrophysiologist and molecular biologist who has been receiving additional training in developing tissue slice preparations for lattice light sheet in coordination with Drs. Alford and Potcoava. She will perform animal surgeries, tissue dissections and live and fixed tissue imaging and cellular electrophysiology (the latter in concert with Alford lab). She also brings expertise in molecular cloning and genetics and will coordinate with the Green lab to generate virus preparation for labeling constructs for use in imaging of MORs and GSats in mouse models. She will lead the data analysis and presentation of all acquired data. Salary is requested for Year 1 of the budget period at 60% effort for 12.00 calendar months.

Shana Netherton, M.Sc., Technician, 6 calendar months. Ms. Netherton is an experienced animal technician and laboratory scientist who joined the Peters lab in July 2019. She also has considerable specific expertise and skill in implementing animal models of drug exposure gained in the Peters lab. She will be responsible for mouse breeding, colony maintenance for all aims, as well as assisting with mouse surgeries and tissue processing, and in inventory and supplies management for all materials including designer fluorophores and virus material used for imaging experiments. Salary support is requested at 50% effort.

Program Director/Principal Investigator (Last, First, Middle): Green, William Nathan

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH
	6/1/2023	5/31/2024

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

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Green, William	PD/PI	0.24			223,988	4,480	1,133	5,613
Govind, Anitha	Staff Scient	6			67,236	33,618	8,505	42,123
TBN	Technician	6			40,498	20,249	5,123	25,372
SUBTOTALS →						58,347	14,761	73,108

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* 1. Microscopy supplies ($3,000); 2. Glycosylation reagents ($2,000); 3.Tissue culture supplies ($3,000); 4. Transfection reagents, recombinant DNA work ($2,000); 5. General lab supplies ($3,387).	13,387
TRAVEL Travel for PI	1,000
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Light microscopy core facility ($5,000); Timed pregnant mice for neuronal cultures ($5,000); Publication costs ($500)	10,500

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$** 97,995
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 97,995

Program Director/Principal Investigator (Last, First, Middle): Green, William Nathan

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	73,108				
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	13,387				
TRAVEL	1,000				
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	10,500				
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	97,995				
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	97,995				

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 97,995

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

The Green lab will perform all experiments that involve primary culture of embryonic mouse neurons or HEK cells as an in vitro environment for MOR imaging. They will perform imaging and analysis of labeled MORs in cultured neurons and HEK model cells expressing recombinant MORs (themselves generated in the Green lab) and their overlap with markers of GSat location and MOR activity.

PERSONNEL

William N. Green, PhD Investigator (Professor of Neurobiology at the University of Chicago). He will devote 2% effort (0.24 calendar months) to the project by planning and overseeing experiments in the Green lab, supervising the activities of the personnel below in the Green lab and helping to perform some experiments. He will also coordinate with the Peters and Alford laboratories the data sharing, discussions, and the writing of papers.

The inclusion of faculty salary in this budget reflects the policy of the Division of the Biological Sciences (BSD) and the Pritzker School of Medicine. (to be continued, see attached)

Budget Justification, Green (continued)

William N. Green, PhD Investigator (continued)
It is expected that investigators will recover reasonable and appropriate salary support from grants and contracts proportionate to the fraction of their time and effort devoted to the project. BSD faculty appointments are made on a twelve-month basis and the requested salary is based upon the investigator's total University compensation. If an award is made in a reduced amount, the investigator will be expected to retain an appropriate amount of salary support in the budget

Anitha Govind, PhD Assistant Research Professor, (50% effort, 6 Calendar Months): Dr. Govind joined my laboratory as a postdoctoral scholar about 10 years ago. She received her Ph.D. in 2003 from Mahatma Gandhi University in India and was a postdoc at the University of Rochester before joining my lab. She has extensive experience in protein biochemistry, subcellular fractionation, receptor pharmacology, microscopy and molecular techniques to assay receptor trafficking. Dr. Govind has overseen the work of a number of undergraduates and graduate students in the lab, and will conduct or oversee all experiments from this project that are performed in the Green lab.

TBN, Research Technician, (50% effort, 6 Calendar Months): Green lab: The technician will help facilitate the experiments in all aims by maintaining cultures, helping with imaging and analysis and with the running of gels for MOR glycosylation analysis.

Lab Supplies:

1. Light Microscopy: Primary and fluorescent secondary antibodies, other fluorescent dyes, tubing supplies for perfusion system, cell experimental solutions and pharmacological drugs: **$3,000/yr**

2. Glycosylation reagents for analysis of MOR glycosylation changes: **$2,000/yr**

3.Transfection reagents, recombinant DNA work: The largest expenses are i) the reagents and columns used for preparing large scale preparations of the receptor subunit cDNAs in expression vectors (spun columns for plasmid preps and DNA gel extraction kit from Qiagen) and transfection quality and ii) lipofectamine reagent for neuronal transfection. Also needed are standard reagents for subcloning, sequencing, PCR (restriction enzymes, ligases, nucleases, phosphatases, polymerases, kinases): **$3,000/yr**

4. Tissue culture reagents and material (plasticware, growth media, animals):
Includes growth media, sera, B-27, glutamine, glutamax, antibiotics, dissection and neuronal cell isolation reagents (papain, DNAse, etc), cell line (HEK293) maintenance (trypsin, 10% fetal bovine). Plasticware (petri dishes, sterile pipettes, coverslips): **$2,000/yr**

5. General laboratory supplies includes multiwell plates, cryotubes, Pasteur pipettes, plastic pipettes, (1, 2, 5, 10, 25 ml), centrifuge tubes (15, 50 ml), and filter units; for bacterial culture: petri dishes and culture tubes; and for general lab use: microcentrifuge tubes (0.5, 1.5 ml), micropipette tips, and aerosol tips for PCR work. Chemicals and reagents include salts, buffers, detergents, phospholipids, electrophoresis reagents (acrylamide, bis, agarose, DNA and protein markers, gel enhance, stain, ect.), Bacto agar, tryptone, yeast extract, phenol and ethidium bromide. Items such as glassware (bottles for media and solutions, beakers, flasks ect). Funds are requested for the occasional purchase of small laboratory equipment items that break or need updating such as pipetmen, benchtop centrifuge, and computer software. Additional supplies are also requested for increased culture and lab maintenance will be needed later.

Program Director/Principal Investigator (Last, First, Middle): Alford, Simon

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 6/1/2023 THROUGH 5/31/2024

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

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Simon Alford	PD/PI	.18			327,345	4,910	1,694	3,124
Mariana Potcoava	R.Asst.Prof.	3.6			80,000	24,000	8,280	43,384
Shana Netherton	Technician	6			45000	22500	7763	30,263
SUBTOTALS →						57,079	19,692	76,771

CONSULTANT COSTS

EQUIPMENT *(Itemize)*
Picospritzer III, a precise solution applicator, used to equip Lattice Light Sheet Microscope for acute focal application of opioid solutions to neurons and slices in situ. ($7461 at Parker.com) 7461

SUPPLIES *(Itemize by category)*
Laboratory supplies for lattice light sheet imaging ($3000) 3,000

TRAVEL
Travel for PI 1,000

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*
Animal facility per diems (housing) $11,289 Publication fees $500 500

CONSORTIUM/CONTRACTUAL COSTS | DIRECT COSTS

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* **$** 107,021

CONSORTIUM/CONTRACTUAL COSTS | FACILITIES AND ADMINISTRATIVE COSTS

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD **$** 107,021

Program Director/Principal Investigator (Last, First, Middle): Alford, Simon

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	76,771				
CONSULTANT COSTS					
EQUIPMENT	7461				
SUPPLIES	3,000				
TRAVEL	1,000				
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	500				
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	107,021				
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	107,021				

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 107,021

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Dr. Simon Alford (PI, 0.18 calendar months) - Dr Alford, PI, will oversee imaging experiments. He is experienced with electrophysiological and imaging experiments and along with Dr Potcoava, has implemented a custom Lattice Light Sheet Microscope in his laboratory that will be used for this project. His laboratory combines a variety of fluorescence microscopy approaches in combination with electrophysiology in the central nervous system. He is also experienced in the use of both field recordings from mouse brain slices as well as intracellular recordings under microelectrode and patch clamp conditions, paired cell recording, voltage clamp and in combination with imaging microfluorimetry.

Dr. Mariana Potcoava (Research Assistant Professor, 3.6 calendar months) - Dr Potcoava is trained as a physicist specializing in optical imaging techniques, with a particular recent emphasis on Lattice Light-Sheet Microscopy as well as digital holography. She has built the UIC lattice light sheet microscope (LLSM) that is now functional in the Alford laboratory. She is capable of maintaining and upgrading this system at all levels, from hardware to software, and will be responsible for its day-to-day operation and for aiding all investigators. She will provide software and hardware development support for expanding the use of LLSM technology. She

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: Christian Joseph Peters

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

POSITION TITLE: Assistant Professor of Anatomy and Cell Biology

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
Simon Fraser University	B.Sc. (Kin)	05/2005	Kinesiology
University of British Columbia	Ph.D.	05/2012	Physiology
University of California, San Francisco	Postdoc	04/2019	Physiology

A. Personal Statement

Over the course of my academic career, I have performed studies on membrane proteins and signaling from the level of biophysics of the proteins up to their effects on animal behavior. My deepest interest is to understand how the normal functions of membrane proteins are coopted by agents of disease. I know that the field of addiction neurobiology is one of the most compelling in this regard: drugs of abuse interact with native receptors in the brain and hijack normal cellular signaling to influence behavior in a maladaptive way. I first became interested in this field by studying chloride channel expression in the nicotine aversion/withdrawal circuitry and used the mentored period from a K99/R00 Pathway to Independence award to receive training in the use of mouse models to study nicotine addiction, including the use of conditioned place testing paradigms and of stereotaxic surgeries to inject drugs and implants.

For me, this proposal represents an extension of my interest in the cellular underpinnings of addiction, but to a subject (opioid use disorder) that is simply too pressing for me not to wish to address. It was inspired by a long series of productive brainstorming conversations with Dr. William Green of the University of Chicago and Simon T. Alford of UIC that evolved into this collaborative Catalyst grant proposal. The Green lab has established an anti-dogmatic but compelling model of nicotinic receptor redistribution in cultured neurons, involving vesiculation of the Golgi apparatus, which has significantly informed my own lab's ongoing investigations of how nicotinic receptors are reassembled in the midbrain in response to chronic nicotine exposure. The ventral midbrain is also host to a high expression of the mu-opioid receptor (largely in GABAergic neurons that regulate the VTA). How the redistribution of this group of receptors are regulated by their exogenous ligands represents a similar pathophysiological hijacking of normal physiology in addiction. Moreover, recent work has highlighted that intracellular mu opioid receptors are still functional after agonist-induced internalization, an observation which begs for further mechanistic understanding and examination in acute tissue slices and live animals. It is these questions, and how such processes may underlie opioid addiction physiology, that underlie this project. The expectation from the Catalyst phase is to generate the crucial pieces of preliminary data needed to support this theory (despite long established paradigms in cell biology which it contradicts) and make it feasible for NIH-level grant funding.

I have over 15 years of experience in using cultured and primary cells and *ex vivo* tissue preparations to study transmembrane protein properties in excitable membranes (especially ion channels). Furthermore, I have extensive expertise in using molecular cloning, protein biology and pharmacology to study membrane proteins,

from the submolecular properties at the level of primary sequence up to the macromolecular properties of protein complexes, to delineate how these properties drive cellular physiology. Thanks to my K99/R00 funding, I also have over 5 years of experience in performing stereotaxic injections and tissue imaging in mouse brains, as part of two studies in preparation for submission. I have conducted and supervised projects by trainees from the undergraduate up to the postdoctoral level in applying these methods in cell culture and model organism paradigms to achieve experimental goals. For this study and the future directions to be pursued thanks to its support, all this experience will be brought to bear to examine how opioid receptor trafficking is regulated to drive opioid use disorder. I have built a capable research group in the College of Medicine at the University of Illinois at Chicago with mentees well-trained (by myself) in cell culture, electrophysiology, mouse neuroanatomy, behavior and *in vivo* imaging. I have a strong track record of publication, as an author/co-author of 16 peer-reviewed publications to date. I will be responsible for conducting and overseeing experimental design for both aims and preparing a newly hired post-doctoral research scholar with the specific skills required of this project. I will also oversee data analysis while maintaining a specific focus on rigorous data handling and processing. Finally, I will assist in dissemination of all results through publication in peer reviewed journals and presentation at international conferences.

Recently completed projects that I would like to highlight include:
R00 DA041500 Peters (PI) 9/30/2019-9/30/2022
Neurons of the medial habenula regulate behavioral responses to nicotine in mouse
The purpose of this study is to probe the physiology and responses to nicotine of the neurons of the medial habenula, and to examine how these properties underlie the role of these cells in regulating behavioral phenotypes associated with nicotine aversion and withdrawal.
Role: PI

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022 *Ad hoc* member, NIH Center for Scientific Review BBM Study Section
2019- Assistant Professor, Department of Anatomy and Cell Biology, University of Illinois at Chicago
2012-19 Postdoctoral Scholar, Department of Physiology, UCSF

Other Experience and Professional memberships

2007- Member, Biophysical Society
2011- Member, Society for Neuroscience

Honors

2022 UIC Anatomy and Cell Biology Rising Star Award
2022 Brain Research Foundation Seed Fellowship Award
2012-14 Heart & Stroke Foundation Research Fellowship
2008-10 UBC First Tier University Graduate Fellowship
2008-09 British Columbia Pacific Century Graduate Scholarship
2006-08 Michael Smith Foundation Junior Graduate Studentship
2006 UBC Graduate Entrance Scholarship
2002-05 Simon Fraser University Undergraduate Open Scholarship
2001-03 Hy Aisenstat Scholarship

C. Contributions to Science

1. **Intrinsic and extrinsic protein domain interactions during gating of *Shaker* family potassium channels.** In this group of studies, I investigated how the voltage-dependent activation gates of *Shaker* family (Kv1) potassium channels are linked to the voltage-sensing apparatus as well as downstream gating mechanisms. Because the gate region also lines the permeation pore for potassium ions, it interacts both allosterically with other protein domains, and directly with permeating ions and pore blocking compounds. Mutations within the region, such as occur in some episodic ataxia type-1 cases, can have profound effects on both steric and allosteric interactions, making specific contributions of each difficult to parse. Similarly, the

beta-subunits of the channel complexes can contribute to multiple steps of gating. I adapted the voltage clamp fluorimetry technique, in collaboration with the lab of David Fedida (also of UBC), to probe the gating of the channel separately and simultaneously with the ionic current, to isolate and study these processes independently. In these studies, I was able to answer questions regarding the opening and closing properties of these channels underlying normal physiology in neurons and elsewhere, and to describe how a human disease mutant in the gating region manifests as the disease episodic ataxia type-1 and regulates the molecular pharmacology of a commonly used episodic ataxia treatment, 4-aminopyridine, which we proposed should be contraindicated by some underlying molecular etiologies. These findings have direct application to future drug development with regards to Kv channels.

a. Peters, C.J., Vaid, M., Horne, A.J., Fedida, D., Accili, E.A. The molecular basis for the actions of Kvbeta1.2 on the opening and closing of the Kv1.2 delayed rectifier channel. Channels. 3 (2009) pp. 314-322. PMID: 19713757
b. Peters, C.J., Werry, D., Gill, H.S., Accili, E.A., Fedida, D. Mechanism of accelerated current decay caused by an episodic ataxia type-1 associated mutant in a potassium channel pore. J. Neurosci, 31 (2011) pp. 17449-17459. PMID: 22131406
c. Peters, C.J., Fedida, D., Accili, E.A. Allosteric coupling of the inner activation gate to the outer pore of a potassium channel. Sci Rep. 3 (2013) doi: 10.1038/srep03025.

2. **Structure-function dissection of TMEM16A family calcium-activated chloride channels and their interactions with small molecules.** This work centered on investigating the TMEM16A channel, a calcium-activated chloride channel (CaCC) of widespread physiological importance. As this protein was only determined to be a CaCC in 2008, fundamental questions of how they conduct ions, are activated by calcium and voltage gradients, and are modified by inhibitory small molecules were still major unanswered basic questions and roadblocks to deeper inference into how they behaved physiologically. My goal was to combine structural and molecular biology techniques combined with electrophysiology to elucidate channel function in the TMEM16A channel and published several peer-reviewed studies on the molecular basis of calcium-dependent binding and channel topology, the permeation of chloride ions, and the mechanisms of activation gating in a high-resolution structure. In articles a, b and d, I used mutagenesis, compound screening and pharmacology experiments to examine calcium-binding, permeation and activation gating respectively and predict and evaluate the attendant changes to ion channel function. In article c, I was responsible for optimizing and validating protein-expression constructs for their fidelity to native function, and for interpreting the high-resolution electron cryomicroscopic data to identify and test the regions of tertiary structure that drive ion permeation and ion-dependence of gating.

a. Tien, J.*, Peters, C.J.*, Wong, X.M., Cheng, T., Jan, Y.N., Jan, L.Y., Yang, H. A comprehensive search for calcium binding sites critical for TMEM16A calcium-activated chloride channel activity. Elife. (2014) doi: 10.7554/eLife.02772. (* denotes equal contribution)
b. Peters, C.J., Yu, H., Tien, J., Jan, Y.N., Li, M., Jan, L.Y. Four basic residues critical for the ion selectivity and pore blocker sensitivity of TMEM16A calcium-activated chloride channels. Proc Natl Acad Sci U S A. 112 (2015) doi: 10.1073/pnas.1502291112.
c. Dang, S.*, Feng, S.*, Tien, J.*, Peters, C.J., Bulkley, D., Lolicato, M., Zhao, J., Qi, L., Chen, T., Jan, Y.N., Minor Jr., D.L., Cheng, Y., Jan, L.Y. Electron cryo-microscopy structures of the TMEM16A calcium-activated chloride channel. Nature. 552 (2017) doi: 10.1038/nature25024. (*denotes equal contribution)
d. Peters, C.J., Tien, J., Bethel, N.P., Qi, L., Chen, T., Wang, L., Jan, Y.N., Grabe, M., Jan, L.Y. The sixth transmembrane segment is a major component of gating in the TMEM16A calcium-activated chloride channel. Neuron. 97 (2018) doi: 10.1016/j.neuron.2018.01.048.

3. **Mechanistic roles of ion channels in proliferative cell types.** An emerging area of interest in biology is how ionic currents and the channels that carry them are important to the physiology of dividing cells, such as tumor cells or developmental progenitors. Previously, I was able to contribute to two specific projects in this area and several others are ongoing in my lab at the moment. In article a, we investigated the implications of TMEM16A expression and function in prostate cancer lines, where duplication or hypermethylation of the 11q13 genomic region where the channel gene is found leads to more aggressive cancer types, and found that the channel interacts with EGFR, primarily to increase cell migration. Over the course of this study, I tested the effects of applying known TMEM16A inhibitory compounds on the membrane properties of pancreatic cancer cells using electrophysiology and fluorescent imaging methods. In article b, we studied the

function of the Drosophila sodium channel *Paralytic*, whose expression is critical for asymmetric division of two different neuroblast lineages. I experimentally tested the mechanism by which known loss-of-function mutants, which reduced rates of neuroblast division *in vivo*, inhibited the channel's function, and then designed and validated a normal expressing but functionally deficient *de novo* channel mutant as a reagent in larval rescue of asymmetric division. This study found that active sodium current permeation was a critical determinant of asymmetric division, likely suggesting that transient voltage depolarizations are a prerequisite for cell cycle progression.

a. Crottès, D., Lin, Y.T., Peters, C.J., Gilchrist, J.M., Wiita, A.P., Jan, Y.N., Jan, L.Y. TMEM16A controls EGF-induced calcium signaling implicated in pancreatic cancer prognosis. Proc Natl Acad Sci U S A. 116 (2019) doi: 10.1073/pnas.1900703116.
b. Piggott, B.J., Peters, C.J., He, Y., Huang, X., Younger, S., Jan, L.Y., Jan, Y.N. Paralytic, the Drosophila voltage-gated sodium channel, regulates proliferation of neural progenitors. Genes Dev. 33 (2019) doi: 10.1101/gad.330597.119.

4. **Ion channels underlie activity of CNS neurons driving aggression and addiction behaviors.** A major research focus has become to investigate how function and cellular regulation of membrane proteins (especially ion channels) in neuronal populations drives or modifies impulsive and maladaptive behaviors in rodent models. Areas of interest include the medial habenula, a small and understudied nucleus where high expression of the TMEM16A-CaCC overlaps with Nicotinic Acetylcholine Receptors (ligand-gated ion channels that are the primary targets of the drug nicotine), as well as the lateral septum, where we identified the presence of TMEM16B-CaCC. The medial habenula has garnered recent attention for its role in nicotine associated behaviors, and in a series of recent experiments, I demonstrated that conditional knockout of TMEM16A blunts the effects of nicotine application on neuronal firing and diminishes the expression of nicotine withdrawal symptoms from live mice in well-established paradigms designed to induce behaviors that emulate human "cravings", one of the key roles ascribed to medial habenula neurons *in vivo*. The findings from this research were developed into a successful application for K99/R00 funding from NIDA and are in preparation for submission, and we expect that upon publication of that manuscript, TMEM16A may emerge as a potential drug target for nicotine cessation therapy. In a separate study of lateral septum physiology (involving many of the same participants), a graduate student under my direct mentorship demonstrated that TMEM16B regulates aggression behavior in male animals, using brain slice recordings and *in vitro* and *in vivo* optogenetics.

a. Wang, L., Simms, J., Peters, C.J., Tynan-La Fontaine, M., Li, K., Gill, T.M., Jan, Y.N., Jan, L.Y. TMEM16B calcium-activated chloride channels regulate lateral septum activity and aggression in male mice. J Neurosci. 39 (2019) doi: 10.1523/JNEUROSCI.3137-18.2019.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/18EU6Cdmd5gkn/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: William N. Green

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

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
University College, University of Toronto	B.Sc	06/1978	Physics & Zoology
Cornell Univ. Grad. School of Med. Sciences	Ph.D.	06/1986	Physiology & Biophysics
Yale University, Dept, Cell. & Molec. Physiology	Postdoctoral Fellow	06/1990	Physiology & Neurobiology
Yale University, Dept, Cell. & Molec. Physiology	Res. Asst Prof.	12/1992	Physiology & Neurobiology

A. Personal Statement

I am interested in the cell biology of neurons, specifically, how synapses form, are maintained and change with activity and disease. We focus on the neurotransmitter receptors, nicotinic acetylcholine receptors (nAChRs) and NMDA- and AMPA-type glutamate receptors, which are responsible for the rapid postsynaptic excitatory responses. Our goal is to understand how neurons assemble these receptors, traffic them to and from synapses and how they are involved in synapse formation and plasticity.

A major focus of my research is to understand at the cell and molecular level how nicotine exposure causes nicotine addiction. This work began with our work characterizing nicotine upregulation (1) and continued with the completed R01DA035430, “Different components of nicotine-induced upregulation of nicotinic receptors”, which examined the different components of nicotine-induced upregulation of $\alpha4\beta2$-type nAChRs ($\alpha4\beta2$Rs) and the mechanisms underlying the upregulation. These studies led to the discovery that the anti-smoking drug, varenicline (Chantix), is selectively trapped as a weak base within acidic vesicles in neurons that contain $\alpha4\beta2$Rs. Slow release of varenicline from the vesicles causes nAChR desensitization at the plasma membrane (2) that counters $\alpha4\beta2$R functional upregulation. Our results provide a new paradigm for how varenicline causes smoking cessation and provide new insights into the cellular distribution of the nAChRs weak base ligands used in PET imaging and new models of parameters involved in measuring nicotine addiction. These finding led to the current grant R01 DA044760-01 “PET imaging of $\alpha4\beta2$ nicotinic receptor upregulation and smoking cessation”, which is examining how our discovery of the trapping of weak base $\alpha4\beta2$R ligands in acid vesicles affects the imaging of $\alpha4\beta2$Rs using PET probes and to use PET probe imaging to examine how nicotine causes $\alpha4\beta2$Rs upregulation and how varenicline alters upregulation.

Through this work, we discovered new mechanisms by which the Golgi apparatus (GA) in neurons give rise to the intracellular acidic vesicles that trap varenicline, which we are now calling “Golgi satellites” (GSats). GSats were not observed previously because they lack standard Golgi markers. We identified GSats using Golgi enzymes that modify glycans, such as sialotransferases (3). GSats are found throughout dendrites and axons and both increases in synaptic activity and long-term nicotine exposure increased their formation, thereby altering the trafficking of locally translated and endocytosed proteins. The effects of nicotine exposure on GSats required $\alpha4\beta2$R expression while increases in synaptic activity, which had the same effects, were independent

of $\alpha 4\beta 2$R expression. Our findings revealed novel activity- and drug-dependent functions mediated by GSat formation (4) that will be further explored in this proposal. Here, we apply them to opioid addiction by examining how opioid exposure alters GSats and their trafficking of μ-opioid receptors at synapses.

1. Vallejo, Y., B. Buisson, D. Bertrand and W. N. Green. 2005. Chronic Nicotine Exposure Upregulates Nicotinic Receptors by a Novel Mechanism. *Journal of Neuroscience* **25**:5563-5572. PMCID: 2246082.

2. Govind A.P., Y. Vallejo, J. R. Stolz, J. Z. Yan, G. T. Swanson and W. N. Green. 2017. Selective and regulated trapping of nicotinic receptor weak base ligands and relevance to smoking cessation. *Elife* 6:e25651. PMID:8718768.

3. Govind, A. P., O. Jeyifous, T. A. Russell, Z. Yi, A. V. Weigel, A. Ramaprasad, L. Newell, W. Ramos, F. M. Valbuena, J. C. Casler, J-Z. Yan, B. S. Glick, G. T. Swanson, J. Lippincott-Schwartz, and W. N. Green. 2021. Activity-dependent Golgi satellite formation in dendrites reshapes the neuronal surface glycoproteome. *eLife* 10:e68910. PMID: 34545811.

4. Zhang, HJ, Kao, C-M, Zammit, M, Govind, AP, Mitchell, M, Holderman, N, Bhuiyan, M, Freifelder, R, Zhuang, X, Mukherjee, J, Chen, C-T, Green, WN. 2022. Trapping of nicotinic acetylcholine receptor ligands assayed by in vitro cellular studies and by in vivo PET imaging. *Journal of Neuroscience* JN-RM-2484-21. PMID: 36028313.

B. Positions, Scientific Appointments, and Honors

Positions and Employment:
2009-present: Professor, Department of Neurobiology, University of Chicago
2007-present: Member of the Yale University/NIDA Neuroproteomics Center.
2007-2009: Associate Professor, Department of Neurobiology, University of Chicago
2002-present: Principle/Whitman Investigator, Marine Biological Laboratory, Woods Hole, MA
2000-2007: Associate Professor, Department of Neurobiology, Pharmacology & Physiology, University of Chicago
1993-2000: Assistant Professor, Department of Neurobiology, Pharmacology & Physiology, University of Chicago

Other Experience and Honors:
Scholarships to Enhance and Empower Diversity *(SEE-Diversity*) Board member 2019 - present
Member, *Scientific Reports* Editorial Advisory Panel and Editorial Board 2017 – present
Member of Science Council at the MBL, 2013-present, Chair 2017-2020
Chair of the Whitman Center Summer Research Steering Committee at the MBL, 2011-16
Associate (2020 – present) & Reviewing Editor (2009 – 2020), *Frontiers in Synaptic Neuroscience*
Member and Chair, NIH ZRG1 MDCN R15 study section, 2010 – 2015
Member, NIH MNPS study section, 2006 – 2010
Member, NIH CNNT study section, 2001 – 2005
Member, *Journal of General Physiology* Editorial Board, 2000 – 2010
Herbert W. Rand and the Colwin Endowed Summer Research Fellowship from the MBL, 2011
Stephen W. Kuffler Research Award from the Marine Biological Laboratory (MBL), 2010
Distinguished Research Visitor Award from the University of Auckland, 2006.
Albert & Ellen Grass Faculty Award from the Grass Foundation, 2002 - 2005
The Osserman/McClure Postdoctoral Fellowship, Myasthenia Gravis Foundation, 1989 - 1990.

C. Contributions to Science

1. My early publications from my Ph.D. thesis work addressing questions about the detailed workings of voltage-gated Na channels using single-channel methods. These studies began before the development of patch-clamp techniques and assayed Batrachotoxin-modified Na channels in planar lipid membranes.

a. Green, W. N., L. B. Weiss, and O. S. Andersen. 1987. Batrachotoxin-modified sodium channels in planar bilayers. Ion conduction and block. *Journal of General Physiology* **89**:841-872.

b. Green, W. N., L. B. Weiss, and O. S. Andersen. 1987. Batrachotoxin-modified sodium channels in planar bilayers. Characteristics of saxitoxin- and tetrodotoxin-induced channel closures. *Journal of General Physiology* **89**:873-903.

c. Chabala, L.D., B.W. Urban, L.B. Weiss, W. N. Green and Andersen, O.S. 1991. Steady-state activation properties of batrachotoxin-modified sodium channels in lipid bilayers. *Journal of General Physiology* **98**:197-224.

d. Green, W. N. and O. S. Andersen. 1991. Surface charge and ion channel function. *Annual Review of Physiology* **53:**341-359.

2. My publications from my postdoctoral work and after starting my faculty position addressed questions about the cell biology of the muscle-type nicotinic acetylcholine receptors (nAChRs). To study muscle-type nicotinic acetylcholine receptors, we stably transfect a multi-subunit receptor into a mammalian cell line for the first time. Subsequent studies characterized the trafficking, phosphorylation and subunit assembly of muscle-type nAChRs and nAChRs in mammalian brain.

a. Claudio, T., W. N. Green, D. S. Hartman, D. Hayden, H. L. Paulson, F. J. Sigworth, S. S. Sine, and A. Swedlund. 1987. Genetic reconstitution of functional acetylcholine receptor-channels in mouse fibroblasts. *Science* **238**:1688-1694.

b. Green, W. N., A. F. Ross and T. Claudio. 1991. Acetylcholine receptor assembly is stimulated by phosphorylation of its alpha subunit. *Neuron* **7**:659-666.

c. Green, W. N. and T. Claudio. 1993. Acetylcholine receptor assembly: subunit folding and oligomerization occur sequentially. *Cell* **74**: 57-69.

d. Drisdel, R. C. and W.N. Green. 2000. Neuronal α-Bungarotoxin receptors are homomers composed of five $\alpha 7$ subunits. *Journal of Neuroscience* **20**: 133-139.

3. At the University of Chicago, we have developed new methods to assay the posttranslational modification of palmitoylation, acyl-biotin exchange (ABE), which are quantitative and more sensitive than previous methods. We used these methods to develop proteomic tools and to assay nAChRs, PSD-95 and a number of proteins linked to neurodegenerative diseases.

a. Drisdel, R. C. and W.N. Green. 2004. Labeling and Quantifying Sites of Protein Palmitoylation. *BioTechniques*. **36**:276-285. PMID: 14989092.

b. Drisdel, R. C., E. Manzana and W.N. Green. 2004. The role of palmitoylation in functional expression of nicotinic α7 receptors. *Journal of Neuroscience* **24**:10502-10510. PMID: 15548665.

c. Kang, R., J. Wan, P. Arstikaitis, K. Huang, A. F. Roth, R, Drisdel, W. N. Green, J. R. Yates, N. G. Davis, A. El-Husseini. 2008. Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. *Nature* 456:904-9. PMCID: PMC2610860.

d. Antinone, S. E., G. D. Ghadge, L. W. Ostrow, R. Roos and W. N. Green. 2017. -acylation of SOD1, CCS, and a stable SOD1-CCS heterodimer in human spinal cords from ALS and non-ALS subjects. *Scientific Reports*: **7**:41141. PMID: 28120938.

4. At the University of Chicago, we have developed FRET-based, single-molecule/super-resolution and other techniques to characterize the trafficking of glutamate receptors and adaptor/scaffold proteins to and from excitatory synapses.

a. Jeyifous, O, M. Schubert, C. G. Specht, C. L. Waites, E. Lin, S. Fujisawa, J. Marshall, C. Aoki, J. M. Montgomery, C. C. Garner and W. N. Green. 2009. SAP97 and CASK mediate sorting of N-Methyl-D-Aspartate Receptors through a previously unknown secretory pathway. *Nature Neuroscience* **12**:10111019 PMCID: PMC2779056.

b. Zheng, N., O. Jeyifous, C. Munro, J. Montgomery and W. N. Green. 2015. Synaptic Activity Regulates AMPA Receptor Trafficking Through Different Recycling Pathways. *Elife*. 4:e06878.

c. Jeyifous, O., E. I. Lin, X. Chen, S. E. Antinone, R. Mastro, R. Drisdel, T. S. Reese and W. N. Green. 2016. Palmitoylation regulates glutamate receptor distributions in postsynaptic densities through control of PSD95 conformation and orientation. *Proceedings of the National Academy of Sciences* 113:8482–8491.

d. Lee, S. H., C. Jin, E. Cai, P. Ge, Y. Ishitsuka, K. W. Teng, A. A. de Thomaz, D. Nall, M. Baday, O. Jeyifous, D. Demonte, C. M. Dundas, S. Park, W. N. Green, P. R. Selvin. 2017. Super-resolution Imaging of Synaptic and Extrasynaptic Pools of Glutamate Receptors with Different-sized Fluorescent Probes. *Elife* 6: e25651.

5. At the University of Chicago, we have characterized nicotine upregulation, which are the long-term effects of nicotine on $\alpha4\beta2$-type nicotinic receptors ($\alpha4\beta2$Rs) and the neurons expressing the receptors and is tied to nicotine addiction. We have discovered that nicotine exposure to neurons expressing $\alpha4\beta2$Rs, causes Golgi dispersal and the appearance of Golgi satellites (GSats) in dendrite and axons. Furthermore, GSat formation is also regulated by changes in synaptic activity, and when containing $\alpha4\beta2$Rs, trap $\alpha4\beta2$R ligands such as varenicline and the PET ligand 2-FA.

a. Vallejo, Y., B. Buisson, D. Bertrand and W. N. Green. 2005. Chronic Nicotine Exposure Upregulates Nicotinic Receptors by a Novel Mechanism. *Journal of Neuroscience* **25**:5563-5572.

b. Govind A.P., Y. Vallejo, J. R. Stolz, J. Z. Yan, G. T. Swanson and W. N. Green. 2017. Selective and regulated trapping of nicotinic receptor weak base ligands and relevance to smoking cessation. *eLife* 6:e25651. PMID:8718768.

c. Govind, A. P., O. Jeyifous, T. A. Russell, Z. Yi, A. V. Weigel, A. Ramaprasad, L. Newell, W. Ramos, F. M. Valbuena, J. C. Casler, J-Z. Yan, B. S. Glick, G. T. Swanson, J. Lippincott-Schwartz, and W. N. Green. 2021. Activity-dependent Golgi satellite formation in dendrites reshapes the neuronal surface glycoproteome. *eLife* 10:e68910. PMID: 34545811.

d. Zhang, HJ, Kao, C-M, Zammit, M, Govind, AP, Mitchell, M, Holderman, N, Bhuiyan, M, Freifelder, R, Zhuang, X, Mukherjee, J, Chen, C-T, Green, WN. 2022. Trapping of nicotinic acetylcholine receptor ligands assayed by in vitro cellular studies and by in vivo PET imaging. *Journal of Neuroscience* JN-RM-2484-21. PMID: 36028313.

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BIOGRAPHICAL SKETCH

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NAME: Alford, Simon

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

POSITION TITLE: Professor and Head

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 Bristol, Bristol, UK	B.Sc.	07/1984	Zoology
University of London, London, UK	Ph.D.	05/1988	Physiology
Karolinska Institutet, Stockholm, Sweden	Postdoctoral Fellow	1989	Neurophysiology
Universities of Bristol & Birmingham, Bristol/Birmingham, UK	Postdoctoral Fellow	1991	Neuropharmacology

A. Personal Statement

My work bridges cellular and systems neuroscience with an emphasis on the mechanisms and effects of synaptic plasticity and has been published amongst others in *Science, Nature, Neuron, Nature Neuroscience, PNAS,* and the *Journal of Neuroscience*. From the late 1980s I have developed new approaches to live cell imaging in neuronal tissue. I was the first scientist to combine patch clamp recording with confocal imaging (Alford et al J Physiol. 1993 469:693-716) and used this approach to demonstrate the critical role of Ca^{2+} entry through NMDA receptor during the induction of LTP. I have developed processes for high-speed imaging in presynaptic terminals (Cochilla and Alford S. Neuron 1998 20:1007-16) and for using fluorescence anisotropy for measuring real time protein-protein interaction in lipid bilayers Zurawski J. Biological Chemistry (2017) 292:12165-12177. My laboratory utilizes simultaneous electrophysiological recording and live cell imaging of neurons both as a determinant of neuronal activity (Smetana et al Nat Neurosci. 2010 13:731-8) but also looking at ionic fluxes, synaptic vesicle turnover and dynamic protein-protein interactions at the level of synapses (Hamid et al J Neurosci. 2014 34:260-74) to determine pathways involved in synaptic plasticity. My research has utilized various approaches to live cell imaging of ions in synapses, axons and dendrites and relating this to both synaptic plasticity and behavior. We have also developed novel imaging approaches for vesicular transport (Kay et al Neuron. 1999 24:809-17) and for protein-protein interaction in vitro and in situ (Zurawski et al *Sci Signaling* 12, 569, eaat8595). More recently, we have routinely started using defined system fusion assays to investigate modulation of synaptic vesicle fusion. We have also designed and implemented a version of Lattice Light Sheet microscopy specifically to enable high-resolution live cell imaging in situ. This minimizes photodamage and high-speed volumetric imaging of synaptic function and we have used this system to visualize single Ca^{2+} channels in lamprey presynaptic terminals and single vesicle quantal release of glutamate using genetically engineered sensors.

A particular focus of my laboratory has been on the role that the G proteins play in synaptic modulation. This work is important to understand how plasma membrane MORs alter synaptic transmission. We discovered how $G\alpha_{i/o}$ coupled GPCRs dynamically modulate neurotransmitter release by effects of $G\beta\gamma$ at the SNARE complex. We originally demonstrated this effect with the SNARE complex fusion machinery in lamprey, but we and others have expanded findings to the mammalian brain and more recently to endocrine vesicle fusion. We

have also employed and continue to work on defined systems to understand lipid vesicle fusion mechanisms. This work has led to the development of live cell imaging resources for synaptic function.

For this application, our resources for imaging the Ca^{2+} entry and neurotransmitter release at single presynaptic terminals are extremely important. It is particularly important to investigate these pathways in intact and live neurons because this is where we observe maladaptive plasticity as part of broader circuitry. This was the principal reason for our use of Lattice Light-Sheet Microscopy which provides submicron resolution, very little bleaching and imaging rates sufficient to resolve responses from even single presynaptic Ca^{2+} channels and functional organic or genetically expressed sensors in tissue *in situ*. This system is designed for straightforward imaging of intact tissue during electrophysiological stimulation. We have demonstrated examples of the extraordinary resolution of this machine imaging Ca^{2+} dyes in (Ramachandran et al J Neurosci. 2022 Jan 18; doi: 10.1523/ JNEUROSCI .2207-21.2022) in which Ca^{2+} transients from just single Ca^{2+} channel openings are resolved, and we have also resolved glutamate release from just single quantal release sites in hippocampus (Church et al J Neurosci. 2022 Feb 9;42(6):980-1000).

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Mechanisms of synaptic dysfunction in Parkinson's and other synuclein-linked diseases

R01NS111749
Hamm, Alford (MPI)
4/1/2019-3/31/2024
Regulation of exocytosis by direct Gβγ blockade of fusion

Citations:

1) In a collaboration with Dr Réjean Dubuc (University of Montreal), we have studied how descending motor command systems are modulated by G protein coupled receptor systems. The work has implications for recovery from injuries in descending command systems. An example from many publications: Smetana R, Juvin L, Dubuc R, Alford S. A parallel cholinergic brainstem pathway for enhancing locomotor drive. Nat Neurosci. 2010 Jun;13(6):731-8. PubMed PMID: 20473293; PubMed Central PMCID: PMC2881475. **(discussed in News and Views section of Nature Neuroscience - Tresch Nature Neuroscience 13:659-660; Faculty of 1000 recommended article)**
2) We have combined high speed high resolution presynaptic Ca^{2+} imaging with active zone cell attached recording to resolve single channel presynaptic events. This work uses Lattice Light Sheet microscopy (LLSM) to resolve single Ca^{2+} openings following single action potentials and highlights the extraordinary sensitivity of the LLSM Ramachandran S, Rodgriguez S, Potcoava M, Alford S. Single calcium channel nanodomains drive presynaptic calcium entry at lamprey reticulospinal presynaptic terminals. J Neurosci. 2022 42:2385-2403 PubMed PMID: 35063999. ***(J. Neurosci. Research Spotlight featured article)***
3) We discovered the SNARE target for Gβγ using Botulinum toxins: Gerachshenko T, Blackmer T, Yoon EJ, Bartleson C, Hamm HE, Alford S. Gβγ acts at the C terminus of SNAP-25 to mediate presynaptic inhibition. Nat Neurosci. 2005 May;8(5):597-605. PubMed PMID: 15834421. ***(News and Views - Nature Neuroscience – Sullivan Nature Neuroscience 8:542-544; Faculty of 1000 recommended article)***
4) We recently developed holographic technology to detect tomographic output from Lattice Light-Sheet microscopes. This gives us access to phase information in the volumetric data and allows us to capture 3D information with minimal movement of the objective lens and at much higher rates. This work has been published in a series of peer reviewed papers and conference papers in 2021. A representative manuscript is: Potcoava M, Mann C, Art J, Alford S (2021) Spatio-temporal performance in an incoherent holography lattice light-sheet microscope (IHLLS). Optics express 29:5. PMID: 34614645, PMC 8327923.

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

2022 Canadian Foundation for Innovation 2023 Innovation Fund, Expert Committee – Neuroscience (physiology)

2020 Special Emphasis Panel/Scientific Review Group, 2021/01 ZRG1 MDCN-R (04) M
2016 Special Emphasis Panel/Scientific Review Group, NIH ZRG1 MDCN-N (02) M
2015 Special Emphasis Panel/Scientific Review Group, NIH CSR ZRG1 MDCN M
2015 - Present Sweeney Professor of Basic Sciences and Head, University of Illinois at Chicago, Department of Anatomy and Cell Biology, Chicago, IL
2015 - Present Affiliate Professor, University of Illinois at Chicago, Department of Biological Sciences, Chicago, IL
2014 – 2019 Executive Committee Member, University of Illinois at Chicago, Graduate Program in Neuroscience, Chicago, IL
2013 - 2014 University of Illinois at Chicago, Graduate Program in Neuroscience, Director of Graduate Studies, Chicago, IL
2013 Member NIH special emphasis panel, NIH CSR ZRG1 MDCN-G(02) Molecular and Cellular Neurobiology
2013 Special Emphasis Panel/Scientific Review Group 2014/01 ZRG1 MDCN-R (04) M
2012 Member NIH peer review committee. Therapeutics for Neurotropic Biodefense Toxins and Pathogens (R21/R33)
2011-2012 Member NIH peer review committee ZRG1 MDCN Program Project review 2010-Present
Member NIH peer review committee F03B Fellowship study section
2009 National Science Foundation, Organization Review Panel 2007-2016 Ad hoc NIH peer review committee SYN study section
2005-2014 University of Illinois at Chicago, Graduate Program in Neuroscience, Co-Director 2004
2004 National Science Foundation, FIBR pre-review Panel
2004 Welcome Trust, UK, Reviewer
2004 Royal Society, UK, Reviewer
2004 National Science Foundation, Behavioral Neuroscience/Computational Neuroscience Panel.
2003 - 2015 Professor, University of Illinois at Chicago, Department of Biological Sciences
2003 – 2015 Professor, University of Illinois at Chicago, Department of Biological Sciences
1999 - 2003 Associate Professor, University of Illinois at Chicago, Department of Biological Sciences 1993-1999 Editorial Board, Neuropharmacology
1991 - 1992 Visiting Research, Universite de Montreal
1991 - 1999 Assistant Professor, Northwestern University, Department of Physiology 1989 - 1991Research Associate, University of Bristol/Birmingham, Birmingham 1988 - 1989 Postdoctoral Fellow, Karolinska Institutet, Stockholm
1987 - Present Member, Society for Neuroscience
1984 - 1988 Research Assistant, St George's Hospital Medical School, University of London

Honors

2014-2016 Whitman Fellow, Marine Biological Laboratory, Woods Hole, MA 2011
Researcher of the Year Award, University of Illinois at Chicago
1998 Dean’s Award for Teaching Excellence, Northwestern University Medical School 1988 Royal Society European Exchange Fellowship, Royal Society, London
1987 Rushton Fund Fellowship, Physiological Society, UK
1987 Grass Foundation Fellowship, Marine Biolgogical Laboratory, Grass Foundation

C. CONTRIBUTION TO SCIENCE

1. Development of live cell imaging approaches. As a postdoctoral fellow in Dr Graham Collingridge’s lab, I developed the first simultaneous patch clamp and live cell Ca^{2+} imaging approach in neurons in slices. I used this to demonstrate that Ca^{2+} influx through NMDA receptors drives LTP induction, but also that this Ca^{2+} triggers release of Ca^{2+} from internal stores. We have since expanded our use of live cell imaging approaches across numerous physiological systems – recently to quantify Ca^{2+} entry into subcellular structures such as presynaptic terminals. Most recently we have developed specific uses of lattice light sheet microscopy to allow live cell imaging in brain slices and intact tissue, combining this powerful approach with electrophysiology, but also to enable 3D holographic imaging during recording, removing the need to move the stage or objective.

a) Alford S, Frenguelli BG, Schofield JG, Collingridge GL. Characterization of Ca^{2+} signals induced in hippocampal CA1 neurones by the synaptic activation of NMDA receptors. J Physiol. 1993 469:693-716. PubMed PMID: 1143895.

b) Hamid E, Church E, Alford S. Quantitation of single action potential-evoked Ca^{2+} signals in CA1 pyramidal neuron presynaptic terminals eNeuro 2019; ENEURO.0343-19.2019; DOI: https://doi.org/10.1523/ENEURO.0343-19.2019. PMID: 31551250.

c) Potcoava M, Mann C, Art J, Alford S (2021) Spatio-temporal performance in an incoherent holography lattice light-sheet microscope (IHLLS). Optics express 29:5. PMID: 34614645, PMC 8327923.

d) Ramachandran S, Rodgriguez S, Potcoava M, Alford S. Single calcium channel nanodomains drive presynaptic calcium entry at lamprey reticulospinal presynaptic terminals. J Neurosci. 2022 42:2385-2403 PubMed PMID: 35063999. ***(J. Neurosci. Research Spotlight featured article)***

2. Descending motor command systems and modulation by G protein coupled receptors. This work has been in collaboration with Dr Réjean Dubuc (University of Montréal). It has implications in recovery from injuries in descending command systems resulting from spinal damage or stroke. We have made two broad recent discoveries in this area. The first was that muscarinic transmission from the mesencephalic locomotor region – a key region responsible for activated goal-directed locomotion – generates extraordinarily long bursts of activity. We discovered a new class of neurons showing sustained excitation, which maintain activation of the reticulospinal command neurons responsible for locomotor behaviors. We have also demonstrated that the lamprey homolog of the substantia nigra pars compacta sends descending projections to this brainstem command system to amplify descending command output. We have now demonstrated that this descending dopaminergic system is common to all vertebrates including mammals. This latter finding necessitates a reappraisal of the means by which dopamine contributes to motor tone during movements. Direct descending effects of outputs from the substantia nigra should be considered alongside classical ascending modulation.

a) Smetana RW, Alford S, Dubuc R. Muscarinic receptor activation elicits sustained, recurring depolarizations in reticulospinal neurons. J Neurophysiol. 2007 97(5):3181-92. PubMed PMID: 17344371, PubMed Central PMCID: PMC2397553.

b) Smetana R, Juvin L, Dubuc R, Alford S. A parallel cholinergic brainstem pathway for enhancing locomotor drive. Nat Neurosci. 2010 13(6):731-8. PubMed PMID: 20473293; PubMed Central PMCID: PMC2881475. ***(News and Views – Tresch Nat. Neurosci. 13:659-660; Faculty of 1000 recommended article)***

c) Ryczko D, Cone JJ, Alpert MH, Goetz L, Auclair F, Dubé C, Parent M, Roitman MF, Alford S, and Dubuc R. A descending dopamine pathway conserved from basal vertebrates to mammals Proc Natl Acad Sci U S A. 2016 113(17): E2440-9. PubMed PMID: 27071118; Pubmed Central PMCID: PMC4855556

d) Ryczko D, Grätsch S, Alpert MH, Cone JJ, Kasemir J, Ruthe A, Beauséjour PA, Auclair F, Roitman MF, Alford S, Dubuc R. Descending Dopaminergic Inputs to Reticulospinal Neurons Promote Locomotor Movements. J Neurosci. 2020 Oct 28;40(44):8478-8490. PMID: 32998974

3. G protein coupled receptor modulation of neurotransmitter release. I have maintained a long-standing collaboration with Dr Heidi Hamm, now at Vanderbilt. In the 2000s. we started to tease apart presynaptic inhibitory mechanisms of G protein coupled receptors. These effects are ubiquitous in the CNS and have implications for disorders ranging from Parkinson's disease to depression. This work began with two papers one published in the Journal of Physiology and a second in Science that first demonstrated that 5- HT mediated presynaptic inhibition does not modify presynaptic Ca^{2+} entry and then that this effect is mediated by an effect of $G\beta\gamma$ on the SNARE complex. In two papers in Nature Neuroscience we then demonstrated that this presynaptic modulation is mediated by a direct effect of $G\beta\gamma$ binding to the c- terminal region of SNAP25. This has proven since to be a broad presynaptic target for $G\beta\gamma$ demonstrated since by us and other groups to modify synpaptic transmission mediated by both α_{2a} and α_{2b} receptors and is found in a number of regions of the CNS, including cortex, hippocampus, amygdala and spinal cord.

a) Blackmer T, Larsen EC, Takahashi M, Martin TF, Alford S, Hamm HE. G protein betagamma subunit-mediated presynaptic inhibition: regulation of exocytotic fusion downstream of Ca2+ entry. Science. 2001 292(5515):293-7. PubMed PMID: 11303105.

b) Takahashi M, Freed R, Blackmer T, Alford S. Calcium influx-independent depression of transmitter release by 5-HT at lamprey spinal cord synapses. J Physiol. 2001 532(Pt 2):323-36. PubMed PMID:

11306653; PubMed Central PMCID: PMC2278557.

c) Blackmer T, Larsen EC, Bartleson C, Kowalchyk JA, Yoon EJ, Preininger AM, Alford S, Hamm HE, Martin TF. G protein betagamma directly regulates SNARE protein fusion machinery for secretory granule exocytosis. Nat Neurosci. 2005 8(4):421-5. PubMed PMID: 15778713. ***(News and Views - along with article (d) below – Sullivan Nat. Neurosci8:542-544; Faculty of 1000 recommended article)***

d) Gerachshenko T, Blackmer T, Yoon EJ, Bartleson C, Hamm HE, Alford S. Gbetagamma acts at the C terminus of SNAP-25 to mediate presynaptic inhibition. Nat Neurosci. 2005 8(5):597-605. PMID: 15834421. ***(News and Views - Sullivan Nat. Neurosci. 8:542-544; Faculty of 1000 recommended article)***

4. Targets of protein-protein interactions in presynaptic terminals. We therefore directly investigated the possible targets of Gβγ on the SNARE complex. We began by demonstrating that Gβγ competes with Synaptotagmin binding to the SNARE complex and then identified possible residues on the SNARE component SNAP-25 that may mediate this interaction. Finally, by comparing effects of removal of the c-terminal region of SNAP-25 in mammalian hippocampus we demonstrated that this mechanism of Gβγ and 5-HT_{1B} receptors is true in mammalian CNS as well as simple vertebrates such as the lamprey.

a) Hamid E, Church E, Wells CA, Zurawski Z, Hamm HE, Alford S. Modulation of neurotransmission by GPCRs is dependent upon the microarchitecture of the primed vesicle complex. J Neurosci. 2014 34(1):260-74. PubMed PMID: 24381287; PubMed Central PMCID 3866488

b) Zurawski Z, Rodriguez S, Hyde K, Alford S, Hamm HE. Gβγ Binds to the Extreme C Terminus of SNAP25 to Mediate the Action of Gi/o-Coupled G Protein-Coupled Receptors. Molecular pharmacology. 2016; 89(1):75-83. PMID: 26519224, PMCID 4702098

c) Zurawski Z, Page B, Chicka MC, Brindley RL , Wells CA, Preininger AM, Hyde K, Gilbert JA, Cruz-Rodriguez O, Currie KPM, Chapman ER, Alford S, Hamm HE. Gβγ directly modulates vesicle fusion by competing with synaptotagmin for binding to neuronal SNARE proteins embedded in membranes. J. Biological Chemistry (2017) 292(29):12165-12177 PMID:28515322, PMCID 5519367

d) Zurawski Z, Thompson Gray AD, Brady LJ, Page B, Church E, Harris NA, Dohn MR, Yim YY, Hyde K, Mortlock DP, Jones CK, Winder DG, Alford S, Hamm HE. Disabling the Gβγ-SNARE interaction disrupts GPCR-mediated presynaptic inhibition, leading to physiological and behavioral phenotypes. Sci Signal. 2019 12(569). doi: 10.1126/scisignal.aat8595. PubMed PMID: 30783011, PMCID 7758873

5. The physiological outcome of G protein-SNARE complex interactions. We have determined effects of Gβγ binding on vesicle fusion. Using dye loading of vesicles with FM1-43 we have shown that vesicles trap dye following GPCR mediated presynaptic modulation consistent with kiss and run fusion. We then showed this reduces the peak synaptic cleft neurotransmitter concentrations, to alter the balance of postsynaptic activation of NMDA and AMPA receptors. This implies a profoundly more complex synaptic response than if presynaptic receptors only modulated release probability. Using motor control as an example we show substantial effects on behavior. We have also demonstrated this in terminals of mammalian neurons.

a) Photowala H, Blackmer T, Schwartz E, Hamm HE, Alford S. G protein betagamma-subunits activated by serotonin mediate presynaptic inhibition by regulating vesicle fusion properties. Proc Natl Acad Sci U S A. 2006 103(11):4281-6. PubMed PMID: 16537522; PubMed Central PMCID: PMC1449684.

b) Schwartz EJ, Blackmer T, Gerachshenko T, Alford S. Presynaptic G-protein-coupled receptors regulate synaptic cleft glutamate via transient vesicle fusion. J Neurosci. 2007 27(22):5857-68. PubMed PMID: 17537956. PubMed Central PMCID: PMC6672243.

c) Gerachshenko T, Schwartz E, Bleckert A, Photowala H, Seymour A, Alford S. Presynaptic G-protein-coupled receptors dynamically modify vesicle fusion, synaptic cleft glutamate concentrations, and motor behavior. J Neurosci. 2009 29(33):10221-33. PubMed PMID: 19692597; PubMed Central PMCID: PMC2756137.

d) Church E, Hamid E, Zurawski Z, Potcoava M, Flores-Barrera E, Caballero A, Tseng KY, Alford S. Synaptic integration of subquantal neurotransmission by co-localized G protein coupled receptors in presynaptic terminals. J Neurosci. 2022 42(6):980-1000. PMID: 34949691. PubMed Central PMCID: PMC8824496.

Complete Work is list in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/simon.alford.1/bibliography/public/