SUMMARY STATEMENT

(Privileged Communication)

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Release Date: **11/09/2018**
Revised Date:

Application Number: **1 F30 MH118865-01A1**

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Review Group:	**ZRG1 F02B-D (20)** **Center for Scientific Review Special Emphasis Panel** **Fellowships: Sensory and Motor Neuroscience, Cognition and Perception**
Meeting Date:	**10/25/2018**
Council:	**JAN 2019** ***PCC:*** **7K-TGMCF**
Requested Start:	**04/01/2019**

Project Title:	**Sources of Cholinergic Modulation of Cortical Microcircuits**
	***Requested*: 4 Years**
Sponsor:	**BARTH, ALISON L**
Department:	**Biological Sciences**
Organization:	**CARNEGIE-MELLON UNIVERSITY**
City, State:	**PITTSBURGH PENNSYLVANIA**
SRG Action:	**Impact Score:24 Percentile:11**
Next Steps:	**Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects:	**10-No human subjects involved**
Animal Subjects:	**30-Vertebrate animals involved - no SRG concerns noted**

1F30MH118865-01A1 Myal, Stephanie

RESUME AND SUMMARY OF DISCUSSION:
In this resubmitted MD/PhD predoctoral fellowship application, the candidate proposes training while characterizing the cholinergic modulation of mouse somatosensory cortical circuits using anatomical, optogenetic and electrophysiological approaches. The candidate was viewed as talented and driven, with good grades, strong previous research experience and excellent productivity. Her career plan is well motivated, although not all reviewers felt that it was clearly explained how this specific training experience fits into her goal to be a physician scientist. The sponsor is a highly accomplished investigator with records of high impact publication productivity, good funding, and mentoring success. A detailed and comprehensive training plan demonstrates her enthusiastic commitment to the candidate, and several collaborators with specific relevant expertise will also contribute to the training. The University of Pittsburgh and Carnegie Mellon will provide a stimulating, collaborative broader environment that includes an outstanding neuroscience community and resources for professional development. Reviewers remained highly positive about the exciting and innovative questions being addressed in the research plan. The experiments are hypothesis driven and systematically laid out, they build nicely on previous work in the sponsor's lab, and potential pitfalls are thoughtfully addressed. The candidate has responded well to elements of the previous critiques, improving the clarity of the rationale for studying the S1 circuit. Some minor weaknesses remained, however, including the view that the research plan was densely written and somewhat difficult to follow. Moreover, reviewers felt that it was lacking a general functional framework tying the experiments together that would help explain their clinical and translational relevance. The work was again seen as highly ambitious for a 2 year period, although many reviewers saw this aspect positively. Overall, the panel saw very high training potential for this promising candidate, even if the work is not all completed as written, as she will gain new experience in a wide spectrum of cutting-edge technical approaches in a stellar environment. As a result, after discussion, reviewers were unanimous in their high ratings for this predoctoral training opportunity and the excellent foundation it will provide for a future career as a clinician-scientist.

DESCRIPTION (provided by applicant): The neurotransmitter acetylcholine (ACh) is important for attention and implicated in the cognitive symptoms of schizophrenia. In the cerebral cortex, rapid release of ACh acts as an attention signal, activating a disinhibitory circuit that "frees" projection neurons to receive input from the thalamus and transmit information to other brain areas. Our lab uses the primary somatosensory cortex (S1) of mice to model basic, highly conserved aspects of cortical circuitry. In a recent paper, we showed that endogenously released ACh strengthens intra-cortical excitation in a cell type- and synapse-specific way in mouse S1. We also find intrinsic responses to ACh in different cell types, mediated distinctly by nicotinic and muscarinic receptors. To fully understand how ACh modulates circuits underlying cognition, we need to know the sources of ACh modulation of cortical excitability. The main source of cortical ACh is the basal forebrain. However, there are also cortical ACh neurons of unknown function which could potentially influence cholinergic signaling in a sparse, targeted manner. In Aim 1, we propose to anatomically assess the relative contribution of basal forebrain and cortically-derived ACh to cholinergic innervation of four neuron types, and functionally assess the contribution of these inputs to postsynaptic ACh responses using whole-cell recordings with optogenetics in brain slices. We hypothesize that cortical cholinergic cells get basal forebrain inputs and selectively target interneurons in deeper cortical layers, in contrast to the spatially diffuse, cell type-nonspecific inputs from the basal forebrain. We suspect that ACh selectively mediates thalamocortical excitation as well. In a recent paper, we found that endogenous Ach strengthens intra-cortical excitatory synapses onto somatostatin, but not PV, interneurons. However, activating cholinergic receptors pharmacologically strengthens both types of synapses, suggesting that physiologic ACh release is spatially segregated to target certain synapses. Prior pharmacological work suggests that thalamic inputs from the ventral posterior medial nucleus (VPM, a lower-order sensory

thalamic nucleus) express nicotinic receptors, but it is unclear whether these inputs are strengthened by endogenous ACh, or whether inputs from a higher-order thalamic nucleus (the posterior medial nucleus, POm) could also be facilitated. Thalamic inputs show plasticity after attention-based learning, and we suspect that ACh may shift cortical circuits to favor different thalamic inputs during states of attention. In Aim 2, we will anatomically assess whether ACh release sites are located on VPM and POm thalamic inputs, and functionally assess whether endogenous ACh strengthens VPM and POm inputs using whole-cell recordings with dual-color optogenetics in brain slices. We hypothesize that ACh release sites are more commonly associated with POm inputs (to layers 2 and 5) than VPM inputs (to layer 4), and that endogenous ACh release has time-variable, facilitating effects on POm and VPM synapses.

PUBLIC HEALTH RELEVANCE: Patients with schizophrenia have lifelong attentional and cognitive deficits that impair daily functioning and are poorly treated by antipsychotics. Acetylcholine, a neurotransmitter important for attention, fine-tunes circuits in the cerebral cortex to achieve a complex balance of excitation and inhibition that is disrupted in schizophrenia. Using the mouse somatosensory cortex as a simplified model of cortical architecture, we will use in vitro anatomic, electrophysiologic and optogenetic techniques to test the effects of acetylcholine from diverse sources on four major neuron types and their thalamic inputs.

CRITIQUE 1

Fellowship Applicant: 3
Sponsors, Collaborators, and Consultants: 2
Research Training Plan: 1
Training Potential: 2
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit: Excellent revised application from a motivated MD/ PhD student proposing to use cutting edge transgenic and optogenetic tools to differentiate effects of acetylcholine released from basal forebrain local cholinergic neurons on interneurons and pyramidal neurons in barrel cortex. Applicant's response carefully addressed concerns of previous reviewers. Training potential is considerable as should be the impact of the anticipated findings on the field of neural mechanisms regulating sensory processing. Excellent match between sponsor's expertise and applicant's research interests. Collaborators bring additional expertise in thalamocortical neuroanatomy and 3D reconstruction. Outstanding neuroscience community and opportunities for professional development and networking in Pittsburgh are additional strengths.

1. Fellowship Applicant:

Strengths

- Middle author on a Neuron paper and 1st author on poster presentation at SFN
- Extensive post bac research experience in labs focused on cortex.

Weaknesses

- Applicant has finished only year 1 of medical school; concerns about completion of MD portion of the training.
- Fails to clearly explain how this specific training experience fits into her career goal to be a physician scientist.

2. Sponsors, Collaborators, and Consultants:

Strengths

- Sponsor is a highly respected electrophysiologist using state of the art optogenetic and viral tools to study cortical circuits.
- Excellent record of publishing in top tier, highly competitive neuroscience journals speaks to the quality of the PI's contributions and the high standards of the training environment.

Weaknesses

- Limited record of mentoring trainees who go on to careers as independent faculty.

3. Research Training Plan:

Strengths

- Hypothesis driven and builds on previous work from the lab.
- Established technical feasibility for a superb, albeit highly challenging, project—all of the necessary tools, resources and expertise are in place to test model.
- Outstanding job of considering limitations including an extensive and refreshing critical analysis of possible alternate outcomes
- Plan for alternative strategies.

Weaknesses

- Lacking a clinical/translational component

4. Training Potential:

Strengths

- Applicant will learn an array of cutting edge optogenetic, imaging and electrophysiological approaches.
- PI's training plan is individualized, and explicitly details areas that will be focused on in mentoring this applicant's experimental and professional development.

Weaknesses

- None noted

5. Institutional Environment & Commitment to Training:

Strengths

- Outstanding neuroscience community offers numerous excellent opportunities for scientific and professional development and networking

Weaknesses

- None noted

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

YES, all criteria addressed

Biohazards:

Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- Acceptable

Comments on Subject Matter (Required):

- Acceptable

Comments on Faculty Participation (Required):

- Acceptable

Comments on Duration (Required):

- Acceptable

Comments on Frequency (Required):

- Acceptable

Applications from Foreign Organizations:

Not Applicable

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 2
Research Training Plan: 2
Training Potential: 2
Institutional Environment & Commitment to Training: 3

Overall Impact/Merit: This is a resubmission of a proposal concerned with characterizing the source of cholinergic innervation of the cortex and whether it enhances thalamocortical transmission. The overall

score is based on the exceptional qualifications of the applicant, the quality of the training plan, and its potential for providing a good foundation for a career in clinical research. The training plan is well designed. The research uses contemporary techniques (optogenetics, cell-specific labeling, and slice neurophysiology) that systematically analyze the anatomical basis of cholinergic innervation of the cortex and its impact on thalamocortical transmission. This is a significant scientific and clinical problem that has implications for the treatment of Alzheimer's disease and other neurocognitive diseases. The applicant addressed the criticisms that appeared in the critiques of the first submission, and no significant weaknesses could be identified in this proposal.

1. Fellowship Applicant:

Strengths

- Very strong undergraduate academic record with virtually all A's
- Extensive research experience at University of Maryland, University of Arkansas, and the National Institute of Drug Abuse
- Impressive list of awards and honors
- Applicant's commitment to medical neuroscience research is evident from participation in numerous activities: assessing DBS outcomes, helping a neurosurgeon develop a business model for a chronic pain tracker, activities in medical education
- Research experience relevant to present proposal: whole cell recordings in brain slices, optogenetics, cholinergic neuropharmacology.
- First author or co-author on several peer-reviewed publications; participation in several conferences.
- Letters of recommendation are extremely strong; all view applicant as strategic in her thinking and noted her high level of achievement and productivity

Weaknesses

- None noted

2. Sponsors, Collaborators, and Consultants:

Strengths

- Dr. Alison Barth, the sponsor, is an outstanding neuroscientist with experience using whole-cell patch clamp electrophysiology, neuronal tracing, cellular identification, and other techniques relevant to this proposal.
- Sponsor has a productive research record; published 19 papers in the last 5 years, including several using techniques described in this proposal.
- Sponsor has trained a half dozen predoctoral students and 5 postdoctoral associates, most of whom have successfully pursued a scientific career.
- The applicant's research interests are closely aligned with the research conducted by Dr. Barth, and the applicant has already co-authored a Neuron paper with Dr. Barth
- Dr. Barth is PI on three active research grants, including two that extend into 2020

Weaknesses

- None noted

3. Research Training Plan:

Strengths

- The research is based on two cohesive sets of experiments. Studies characterizing the source of cholinergic innervation of cortical neurons (basal forebrain vs cortical VIP neurons) are complemented by experiments designed to determine if these sources enhance thalamocortical transmission.
- The applicant proposes a systematic approach in examining cholinergic innervation of different types of cortical neurons from both a structural and physiological perspective.
- The research plan is feasible; it contains controls to aid data interpretation; alternative outcomes and pitfalls are discussed.
- Cholinergic innervation of cortical circuitry is a significant scientific and clinical problem that matches with the applicant's goal of conducting translational research on the neural basis of cognition and its demise in certain neurological disorders.
- The sponsor provided a comprehensive description of specific training activities that extend beyond the research. Includes a focus on developing applicant's training in data analysis, scientific communication, and grant writing.

Weaknesses

- None noted.

4. Training Potential:

Strengths

- The applicant will expand her technical abilities in dual-color optogenetic stimulation, confocal microscopy, cholinergic neuropharmacology, and data analysis.
- The applicant will interact with several advanced trainees in the Barth lab as well as other faculty who provide technical and intellectual support.
- The proposed research seems likely to lead to a number of high quality publications
- Overall, the training plan provides a solid foundation for an independent research career as a clinical research scientist

Weaknesses

- None noted.

5. Institutional Environment & Commitment to Training:

Strengths

- The Institution provides a number of seminars, regular workshops, and other opportunities to enhance the applicant's training.
- The applicant has already taken institutional MSTP courses and related workshops focused on Professional Development, Scientific Writing, Reproducibility in Science, Biomedical Ethics, as well as activities and instruction aimed at integrating clinical responsibilities with scientific research
- The facilities and resources needed for the proposed research are provided by the sponsor's laboratory and the research cores (e.g., breeding facility) of the institution.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

YES, all criteria addressed

- use and treatment of animals are appropriate □□□□□

Biohazards:

Not Applicable (No Biohazards)

Resubmission:

- The applicant and sponsor addressed concerns raised in the previous review

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- roundtable discussions,

Comments on Subject Matter (Required):

- appropriate - ethics, COI, human research, authorship, scientific misconduct□□□□□

Comments on Faculty Participation (Required):

- RCR training is also discussed and provided by mentor and other faculty □□□□□

Comments on Duration (Required):

- 10 week MSTP course, 8 week course in CMU Biology program□□□□□

Comments on Frequency (Required):

- repeated ongoing discussions in lab meetings and journal clubs

Applications from Foreign Organizations:

Not Applicable

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Acceptable

- All experiments and results are freely shared□□□□□

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3

Fellowship Applicant: 2
Sponsors, Collaborators, and Consultants: 2
Research Training Plan: 3
Training Potential: 2
Institutional Environment & Commitment to Training: 2

Overall Impact/Merit: The present F30 proposal describes a training opportunity for a promising MD/PhD student to study microcircuits for acetylcholine (ACh) within S1 of the mouse. The applicant has demonstrated excellent scholastic performance and has very good prior research experience leading to multiple peer-reviewed publications. The sponsor is an established investigator with a research focus on neuromodulator circuits, good prior mentorship experience, expertise on the frameworks used here, and is joined by several collaborators with specific expertise on relevant topics. The institution is an excellent place for this research and all of the necessary equipment is available. The research topic addresses an important aspect of cognitive function: how acetylcholine is used within extrinsic and intrinsic circuits to amplify synaptic products. The paradigm proposed combines multiple modern techniques (patch clamp recording, fluorescence, optogenetics) to tease apart these circuits, stimulating inputs from specific sources and examining their influences on specific neuronal subtypes within S1. This over-arching framework challenges traditional wisdom that ACh acts via diffuse action, and may represent an excellent path to explaining functional variation observed in neurotypic and psychiatric populations. This is exciting. Slightly diminishing enthusiasm is the complexity of the exposition in the proposal, which is difficult to follow, the lack of a clear and coherent over-arching model, and a feeling that the project may be too ambitious even for such a highly-recommended applicant. This is a revision. The response to the previous critique addressed the main issues, but those raised about the complexity of the proposal and its ambitiousness unfortunately remain. Nonetheless, given the strengths of the applicant, sponsor, institution, and the interesting and thorough nature of the project described, this represents a high-quality training opportunity overall.

1. Fellowship Applicant:

Strengths

- 2nd year MD/PHD student, graduated college early at 19 and worked as fellow at NIDA and a few research laboratories before going to graduate/medical school
- Very good prior research experience with strong record of productivity, 1 1st author paper, several middle author papers on a variety of topics (including 1 from current lab), and several abstracts.
- Some prior work addresses neuromodulators, providing the applicant with useful practical background information in the context of the current proposal
- Excellent grades

Weaknesses

- None noted

2. Sponsors, Collaborators, and Consultants:

Strengths

- Sponsor is an established researcher with expertise in neuromodulation using the techniques proposed here.
- Adequate funding for the proposed work
- Sponsor has good mentoring history: 6 predocs, 5 postdocs have continued on to good positions.

Weaknesses

- None noted

3. Research Training Plan:

Strengths

- The research topic challenges more traditional wisdom that ACh influences via diffuse action – an exciting alternative idea is advanced here, and the appropriate methods deployed to test it.
- If the hypotheses are correct, the results will attract broad interest and be publishable in high quality journals.

Weaknesses

- There are lots of experimental measures are cross-checks that are proposed; however, due to the complexity and conciseness of exposition it is very difficult to keep track of all of the proposed examinations. There are some potential pitfalls acknowledged ("we are aware of the difficulty…") but no clear plan for determining how these pitfalls will be detected in cross-check and rectified.
- Missing from the exposition here is some general functional framework (or set of frameworks) that ties all of the experiments together. The information to be collected sounds like it would be useful, but it is not clear how all of these different pieces form a picture of a larger puzzle. Identifying such frameworks is going to be crucial to translating the research to clinical applications.

4. Training Potential:

Strengths

- Training in a wide spectrum of approaches and techniques is proposed, including path clamp recording, optogenetics, fluorescence imaging and histological reconstruction with modern methods.
- Subject matter appears nicely related to applicant's clinical rotations and future aims as a clinician-scientist

Weaknesses

- The proposed work appears to be very, very ambitious in scope. Experimental timeline specifies work starting in April of 2018. By the start of the funding period, Aim 1 should therefore be completed?

5. Institutional Environment & Commitment to Training:

Strengths

- Stimulating collaborative environment with other researchers at Pitt/CMU
- All equipment appears to be in place

Weaknesses

- None noted

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

YES, all criteria addressed

- Looks good

Biohazards:

Acceptable

Resubmission:

- The applicant has done a good job of addressing the concerns raised in the prior critique, including clarifying the purpose behind studying the targeted circuit (S1). I thought the revision did not respond to Rev 3's critique that the proposal was difficult to read. The complexity and presence of hypotheses stated in abstract or not operationalized terms (e.g., "cell type-specific and rapid effects") make the logic of the proposal difficult to follow.

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- Multiple courses: departmental seminars, multiple courses for MD/PhD and MD programs

Comments on Subject Matter (Required):

- Extensive list of topics, covers all recommended material

Comments on Faculty Participation (Required):

- Faculty-led seminars

Comments on Duration (Required):

- More than adequate

Comments on Frequency (Required):

- Yearly

Select Agents:

Acceptable

Resource Sharing Plans:

Acceptable

- Excellent

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMALS: ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 F30 MH118865-01A1; PI Name: Myal, Stephanie E.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

Center for Scientific Review Special Emphasis Panel
CENTER FOR SCIENTIFIC REVIEW
Fellowships: Sensory and Motor Neuroscience, Cognition and Perception
ZRG1 F02B-D (20)
10/25/2018 - 10/26/2018

Notice of NIH Policy to All Applicants: Meeting rosters are provided for information purposes only. Applicant investigators and institutional officials must not communicate directly with study section members about an application before or after the review. Failure to observe this policy will create a serious breach of integrity in the peer review process, and may lead to actions outlined in NOT-OD-14-073 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-073.html and NOT-OD-15-106 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-106.html, including removal of the application from immediate review.

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