Overview of Response

Thank you again to the reviewers for their thorough, helpful critique of this revised proposal, and a promising impact score of 24 (11th %ile). Overall, enthusiasm for the proposal seemed very high. However, reviewers requested more discussion of the project's fit for my career goals, its translational relevance, and an overall functional framework for our potential findings. I am pleased to discuss these remaining comments below.

Application of training to physician-scientist career

As a physician-scientist, I specifically want to treat patients with severe cognitive deficits. In parallel to my clinical work, I ultimately plan to apply the techniques and experimental philosophy I am learning from my PhD mentor, Alison Barth, to disease-based research in these populations. During residency and fellowship, I hope to study human circuit pathology at the cellular level in postmortem tissue obtained from brain banks, and possibly return to animal models of neuropsychiatric disease as an independent investigator. I chose to study cholinergic regulation of *healthy* sensory cortex for my PhD thesis because I believe that, as disease models keep evolving, learning fundamental circuit physiology will help me pose poignant experimental questions across many different disease models in a lifelong translational science career.

The proposed work is a superb foundation for excellence in the evolving field of experimental psychiatry. New circuit-based models are constantly emerging to explain, diagnose and treat diseases once thought to be "too complex" to approach medically, such as personality disorders and schizophrenia. Initiatives like the NIMH RDoC[1] and National Neuroscience Curriculum[2,3] are spurring the field toward functional, mechanistic approaches to disease management, all necessarily predicated on studies of healthy brains and simplified model species. There is currently a standstill in FDA approval of new drugs for schizophrenia, and the key to ending that standstill may lie in studies of normal brain plasticity and neuromodulation. I see this project as Phase One of a lifelong scientific path, beginning with disease-relevant basic research in a strong training environment for patient-centered research and patient care.

Translational relevance

We seek to understand baseline principles of cholinergic connectivity that may be disrupted in numerous neuropsychiatric diseases, including depression, autism, ADHD, dementia, bipolar disorder and schizophrenia. We propose to quantify targets of cortical acetylcholine (ACh) release by comparing inputs from two different ACh sources onto cortical neurons, and testing ACh effects at thalamocortical synapses, in a well-studied model of cortical circuitry: the mouse somatosensory cortex.

ACh dysfunction and schizophrenia are *associated* on many levels, from behavioral to genome-wide studies, though causal evidence remains sparse[4,5]. Schizophrenia also appears to have an attentional component that manifests in difficulty reading, following conversations, and attending to socially appropriate stimuli. Evidence for related pan-cortical pathology is abundant and includes impaired mismatch negativity, impaired working memory, and altered EEG signals measured across sensory, motor and prefrontal areas.

ACh has complex effects on cortical inhibition, and this complexity may explain why generalized treatment approaches such as cholinesterase inhibitors have not been beneficial in schizophrenia. An elegant paper by Koukouli et al. (2017) shows one aspect of this complexity that could be amenable to drug development. Here, the authors replaced the mouse alpha-5 nicotinic receptor gene with a polymorphic gene found in humans with schizophrenia. This resulted in decreased activity of cortical excitatory neurons and "schizophrenia-like" social withdrawal behavior[6]. Knocking out the alpha-5 receptor *only in VIP cells* was sufficient to replicate this neural phenotype, implicating VIP neurons (and their alpha-5 receptors) as possible therapeutic targets. Some VIP neurons are cholinergic themselves, and we propose to study this unique population in Aim 1.

Functional framework and overall model

In Aim 1 we will compare basal forebrain and cortical sources of ACh to understand how, and under what conditions, each one may alter cortical activity. One key difference between these sources is that basal forebrain ACh projections are enormous (with single neurons capable of projecting across the entire mouse somatosensory cortex), whereas cortical ACh projections are localized and sparse. This suggests that basal forebrain activity could induce modality-specific attention (for instance, activation of auditory but not visual cortex) *necessary* to perceive a stimulus, but probably cannot produce the stimulus-specific activation *sufficient* to discriminate that stimulus from others nearby. That stimulus-specificity likely emerges from the cortex, via combined cortical, thalamic, and neuromodulatory inputs to a dynamic local circuit.

We believe this is why, despite dense basal forebrain ACh input, small cortical ACh neurons with small arbors and localized projections could still be useful. Because they are *local interneurons*, cortical ACh cells are well-positioned to let microcircuit activity gate their ACh release. If they also get input from the basal forebrain (Aim 1.1), their activation could provide a localized, activity-dependent "boost" to basal forebrain ACh signaling. If not, their ACh release may be independently controlled by nearby activity.

We also hypothesize that thalamocortical inputs may be selectively associated with ACh release sites, allowing ACh to gate presynaptic sensory inputs from the thalamus (Aim 2.2). Whether this happens at lower-order, higher-order, or both types of thalamic inputs is important to determine, as lower-order somatosensory thalamus (VPM) carries "pure" spinothalamic sensory information, whereas higher-order thalamus (POm) is thought to integrate cortical and subcortical input, creating "loops" of ongoing, secondary activity in the brain. If ACh release favors strengthening VPM inputs, this would suggest that ACh biases the cortex toward the encoding of pure sensory stimuli. If it favors POm inputs or both, this might suggest a more nuanced role of ACh in maintaining some broader pattern of cortico-thalamocortical communication.

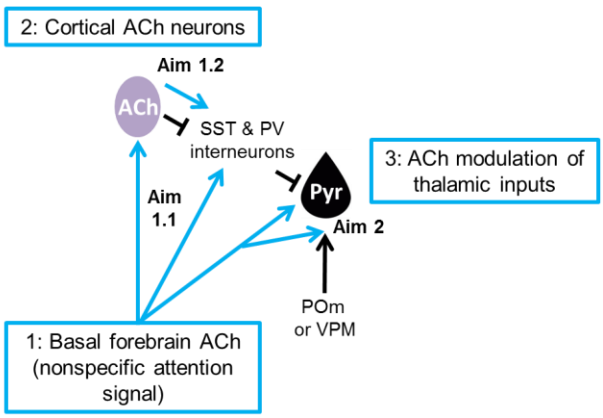

Fig. 1. Three potential sites where ACh release may directly alter cortical information flow. **1)** Basal forebrain ACh release onto processes of inhibitory and excitatory neurons (Aims 1.1 and 1.2). **2)** Cortical ACh neuron input to other inhibitory neurons (Aim 1.2). **3)** ACh input to thalamic presynaptic boutons, Aims 2.1 and 2.2.

Experimental progress

We are making steady progress toward Aims 1 and 2. We have discovered that uptake of AAV1.2- and AAV1.8-encoded viral vectors is poor in cortical ACh cells, yielding poor fluorophore and opsin expression. We have dealt with these reagent limitations by altering our approach as follows:

Aim 1.1: to label cortical ACh cells, we are now breeding ChAT-Cre mice crossed with Ai3 (YFP) for transgenic fluorescent labeling, bypassing the need for viral injections.

Aim 1.2: To test output of cortical ACh neurons, we are doing paired whole-cell recordings in Ai3-labeled ChAT neurons and nearby interneurons. I am already proficient in this approach, which I used in a prior publication in *Neuron*. Since cortical ACh cells are VIP-expressing, we are also attempting to isolate the cholinergic component of VIP neuron activity using VIP-Cre x Ai32 mice (VIP cells expressing transgenic channelrhodopsin) to record postsynaptic responses from neighboring cells under $GABA_A$ receptor blockade.

Adapting to these experimental pitfalls and anticipating new ones has been a valuable training experience. We are still confident that the experiments proposed can be completed, and that my training plan will produce a substantial body of work within the allotted time to PhD completion (i.e., summer 2020).

Again, I thank the reviewers and NIMH for their time, thoughtful review, and interest in this proposal.

Work Cited

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