Selective Control of Synaptically-Connected Circuit Elements by Interluminescence

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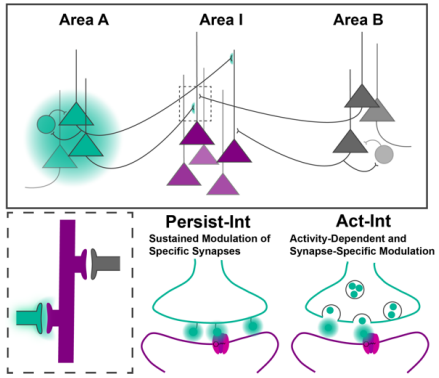
Introduction

A wealth of new tools can directly control output of specific neurons on fast (e.g., optogenetic) or sustained (e.g., chemogenetic) time scales. In contrast, almost no methods exist for selectively modulating communication between defined cells at the synaptic level, which is key to understanding how functional connectivity creates percepts, engrams and actions. Here, we advance a novel strategy for selectively modulating synaptic transmission, Inter-luminescence. This approach uses bioluminescent light from a presynaptic axon terminal, generated by a luciferase, to modulate an opsin in its postsynaptic target under experimenter-controlled introduction of a small molecule (luciferin). We developed two separate methods that target the luciferase to the synaptic cleft. To provide sustained and synapse-specific regulation, the 'Persist-Int' strategy places a luciferase in the synaptic cleft tethered to the presynaptic terminal, and an opsin in the opposing postsynaptic membrane. In this configuration, light generation creates sustained and activity-independent modulation. In the complementary 'Act-Int' strategy, luciferase is released into the synaptic cleft in response to presynaptic activity, a synapse-specific form of activity-dependent modulation.

Interluminescence



Two further steps are crucial to deliver a reliable toolset that can be readily adopted, making Interluminescence useful to a broad community. First, we will characterize in detail the impact of Interluminescence in individual neurons. To this end we will conduct experiments in cell culture (**A**) and brain slices (**B**), recording from individual postsynaptic neurons and endogenous brain circuits *in vitro*. Second, we need to examine the impact of Interluminescence *in vivo* (**C**). Here we will test Interluminescence in anesthetized and awake animals, in the well-characterized barrel cortex.

A) Systematically test Interluminescent modulation in synaptically connected pairs *in vitro*

These experiments will test the impact of Interluminescence on communication between single neurons in culture, the synapse specificity of bioluminescent activation, and the temporal window within which presynaptic activity drives postsynaptic modulation in Act-Int.

B) Systematically test the impact of Interluminescence on endogenous brain circuits *ex vivo*

These experiments will test synapse-specific Interluminescent modulation in the hippocampal brain slice. They extend the sensitivity and specificity studies begun in Aim IA to the well-studied CA3-CA1 projection, enabling validation of key Interluminescence properties under more physiologically relevant conditions.

C) Systematically test Interluminescent strategies *in vivo*

These experiments will test Interluminescent modulation on two distinct thalamic pathways that converge in the SI 'barrel' cortex. We will test these properties in the better-controlled anesthetized setting and in the awake-behaving animal, the latter during performance of a well-established sensory task. We will test sub- and suprathreshold cell-type specific impact using a suite of electrophysiological and imaging methods.

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