PROJECT SUMMARY (See instructions):

For nearly a decade functional analysis of brain circuitry has relied on tools that allow neuronal activity to be perturbed in an intact brain with cell type-specificity. Genetically-encoded neuron actuators have ranged from chimeric G-protein coupled receptors (GPCRs) with orthogonal ligands to light-gated inotropic channels. While these tools have helped uncover cellular substrates of cognitive and behavioral states, significant limitations remain. Optical fiber implantation is destructive, and illumination is limited by mechanical constraints and the requirement that target site be identified in advance. GPCRs are inefficient, display poor temporal control, and can produce long-term functional changes in neurons. We propose to develop and test a neuronal activator that embodies the strongest features of existing approaches. Based on the purinergic P2X receptor, this inotropic channel will display high unitary conductance, negligible desensitization, and tunable gating. Its small molecule ligand will readily cross the blood-brain barrier. Complementary modifications in channel and ligand structure will help generate a family of orthogonal receptor-ligand pairs for independent control over multiple cell populations within the brain while eliminating crosstalk with endogenous factors. The strength of this and other pharmacogenetic approaches is that the locations of target neurons need not be known *a priori*; however, should precise temporal regulation be needed, the ligands can be chemically disabled (caged), enabling brief localized photoactivation. We are confident that our novel synthetic purinergic activator (SPArk) will advance functional brain mapping, providing robust control over discrete neuronal populations that represent known neurochemical classes or are selected using pioneering activity-based molecular-genetic methods. SPArk is a timely, highly efficient and flexible alternative to existing approaches; it is essential for continued progress in *in vivo* mechanistic interrogation of neuronal signaling pathways.

RELEVANCE (See instructions):

Mapping functional connectivity within the brain is predicated on the ability to alter neuronal activity irrespective of cell number or anatomical locations using genetically targeted heterologous proteins that are otherwise absent from the central nervous system. In this respect, pharmacogenetic tools have an advantage over optogenetic ones: (1) neurons of interest may be distributed throughout the brain, making optical methods impractical; (2) cell locations may not be known ahead of time, as would be the case if activity-based labeling techniques were employed to identify them; and (3) optogenetic light guides produce spatially restricted illumination, potentially missing many cells of interest, and can damage neuronal connection—both drawbacks for behavioral analyses of cell function. In addition, where the experimental readout is optical or the behavioral assay incorporates a visual component, light stimulation can be counterproductive. Finally, control over multiple, potentially intermingled cell populations using light has proven elusive.

This pilot effort to develop a powerful and flexible alternative to optical methods stems from a collaboration between 2 laboratories in the departments of Integrative Biology and Neuroscience in the College of Natural Sciences at UT Austin. Boris Zemelman is a pioneer in the design and implementation of optogenetic and pharmacogenetics techniques; Andrew Ellington is renowned for engineering biological systems that regulate fundamental cellular processes. We envision a panoply of tools that will be deployed brain-wide across species to control distinct ensembles of neurons, uncovering circuit connectivity and signaling hierarchies. We see these tools as central to the success of functional brain mapping and congruent with stated goals of the BRAIN Initiative to support the development of techniques for unbiased whole brain characterization.

This proposal embodies the rational design, high throughput screening and *in vitro* characterization of novel neuronal actuators. The starting point for our endeavor is an inotropic channel that launched the optogenetic revolution. Following thorough analysis, the selectivity and efficacy of all candidate actuator-ligand pairs will be tested *in vivo*. We are confident that the highly original and comprehensive development scheme we have outlined will yield a new set of transformative tools for functional brain analysis.