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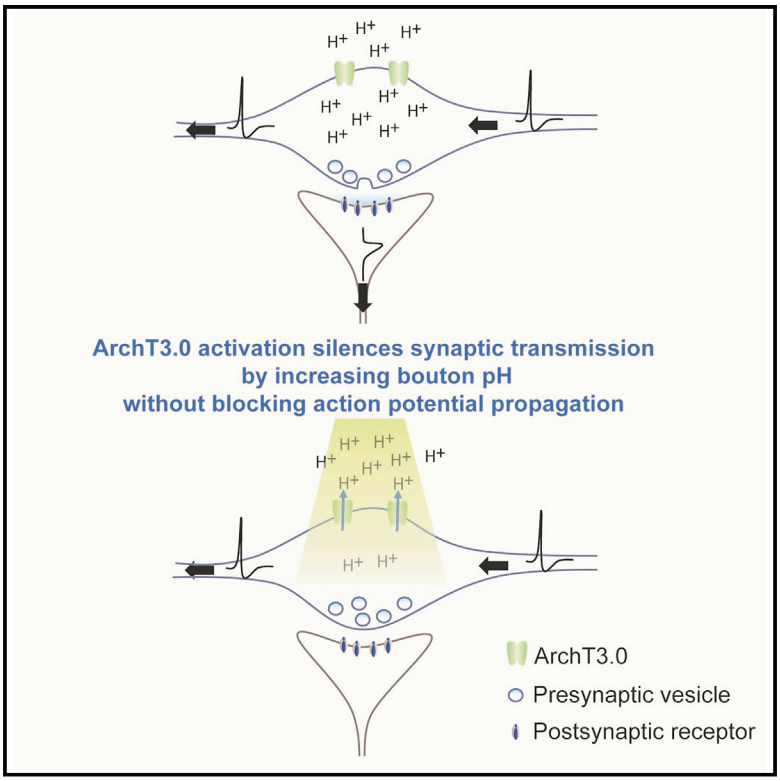
# Archaerhodopsin Selectively and Reversibly Silences Synaptic Transmission through Altered pH

## Graphical Abstract




## Highlights

- Archaerhodopsin selectively and reversibly silences synaptic transmission
- Archaerhodopsin silences synaptic transmission without blocking action potentials
- Archaerhodopsin mediates synaptic silencing through changes in pH
- Synaptic silencing reveals distinctions among CA3-CA1 synapses during learning

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## In Brief

El-Gaby et al. demonstrate that archaerhodopsin can acutely and selectively silence synaptic transmission through changes in pH rather than hyperpolarization. Application of this tool in behaving animals reveals a necessity for synapses from the left CA3 onto CA1 neurons, but not from the right CA3, in long-term memory performance.

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# Archaerhodopsin Selectively and Reversibly Silences Synaptic Transmission through Altered pH

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## SUMMARY

**Tools that allow acute and selective silencing of synaptic transmission in vivo would be invaluable for understanding the synaptic basis of specific behaviors. Here, we show that presynaptic expression of the proton pump archaerhodopsin enables robust, selective, and reversible optogenetic synaptic silencing with rapid onset and offset. Two-photon fluorescence imaging revealed that this effect is accompanied by a transient increase in pH restricted to archaerhodopsin-expressing boutons. Crucially, clamping intracellular pH abolished synaptic silencing without affecting the archaerhodopsin-mediated hyperpolarizing current, indicating that changes in pH mediate the synaptic silencing effect. To verify the utility of this technique, we used trial-limited, archaerhodopsin-mediated silencing to uncover a requirement for CA3-CA1 synapses whose afferents originate from the left CA3, but not those from the right CA3, for performance on a long-term memory task. These results highlight optogenetic, pH-mediated silencing of synaptic transmission as a spatiotemporally selective approach to dissecting synaptic function in behaving animals.**

## INTRODUCTION

Optogenetic tools have ushered in a new era in the quest to understand the neural basis of behavior. Through rapid, reversible, and cell-type-specific silencing of neuronal firing, light-driven hyperpolarizing ion pumps have enabled a hitherto unparalleled spatiotemporal precision in interrogating neuronal populations and their involvement in specific behaviors (Yizhar et al., 2011; Goshen et al., 2011; Shipton et al., 2014). Similarly, methods to silence synaptic transmission with high spatiotemporal precision would be of great value in assessing the roles of specific synaptic connections in behavior (e.g., Martin et al., 2000; Rolls, 2010; Larkum, 2013). Efforts to address this tool gap include the recent development of an optogenetic synaptic silencing method, termed chromophore assisted light inactivation (CALI; Lin et al., 2013). CALI acts via oxidative disruption of synaptic release machinery and has enabled light-mediated reduction of synaptic transmission in *Caenorhabditis elegans* and concomitant disruption of movement (Lin et al., 2013). However, the recovery of synaptic transmission after such a manipulation is incomplete and slow, requiring at least 24 hr for movement to re-emerge (Lin et al., 2013), precluding repeated use for multiple behavioral trials. Furthermore, manipulations with such prolonged effects may trigger compensatory changes at the subcellular, cellular, and network levels (Turrigiano, 2008; Goshen et al., 2011), which could confound the interpretation of synaptic silencing experiments. Thus, in order to fully harness the power of optogenetics for understanding the behavioral function of specific synapses, there is a need for rapidly reversible synaptic silencing tools.

A potentially promising approach for acute synaptic silencing makes use of the halorubrum-derived opsins, archaerhodopsin (Arch) and archaerhodopsin T (ArchT). These opsins respond to yellow or green light by pumping protons out of cells, thereby producing membrane hyperpolarization (Chow et al., 2010; Han et al., 2011). Compared to equivalent versions of other commonly used silencing opsins, archaerhodopsins have higher maximum photocurrents, increased light sensitivity, and strong expression in the axonal plasma membrane (Chow et al., 2010; Han et al., 2011), which are further enhanced for third-generation versions (Arch3.0 and ArchT3.0) by the addition of endoplasmic reticulum export motifs and neurite targeting sequences (Mattis et al., 2011). These factors make archaerhodopsins potentially suitable for acute silencing of axonal outputs. Furthermore, this approach builds on the successful use of optogenetics in mammalian systems, where the prevalence and complexity of compensatory mechanisms places a premium on manipulations with rapid onset and offset (Goshen et al., 2011). We therefore investigated the effects of axonal archaerhodopsin activation on synaptic transmission and the mechanisms through which archaerhodopsins may act at axonal projections. We demonstrate that archaerhodopsins are capable of robust, rapid, and reversible synaptic silencing. Surprisingly, this effect was mediated via changes in pH rather than hyperpolarization. Furthermore, we employ trial-limited, archaerhodopsin-mediated presynaptic silencing in vivo to demonstrate a differential requirement of distinct subpopulations of CA3-CA1 synapses in hippocampus-dependent learning.

## RESULTS

### Archaerhodopsin Produces Robust Reversible Silencing of Synaptic Transmission

To assess the ability of light-driven proton pumps to achieve silencing of synaptic transmission, we stereotactically injected