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Review

Assessment of the AAV-mediated expression of channelrhodopsin-2 and halorhodopsin in brainstem neurons mediating auditory signaling ☆

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ARTICLE INFO

Article history:
Accepted 14 October 2012
Available online 23 October 2012

Keywords:
Channelrhodopsin
Halorhodopsin
Dorsal cochlear nucleus
Chop2
HaloR
Auditory
Hearing
Gene expression
Transfection
ABR
Auditory brainstem response

ABSTRACT

The physiology and circuitry associated with dorsal cochlear nucleus neurons (DCN) have been well described. The ability to remotely manipulate neuronal activity in these neurons would represent a step forward in the ability to understand the specific function of DCN neurons in hearing. Although, optogenetics has been used to study the function of pathways in other systems for several years, in the auditory system only neurons in the auditory cortex have been studied using this technique. Adeno-associated viral vectors with either channelrhodopsin-2 fused with GFP (ChR2–GFP) or halorhodopsin fused with mCherry (HaloR–mCherry), capable of expressing light sensitive cation channels or chloride pumps, respectively, were delivered into the dorsal cochlear nucleus (DCN). One to 18 months later, expression of ChR2 and HaloR was observed throughout the DCN. Rhodopsin distribution within the DCN was determined to be within several cell types identified based on morphology and location within the DCN. Expression of ChR2–GFP and HaloR–mCherry was found at both the injection site as well as in regions receiving projections from the site. Wavelength appropriate optical stimulation *in vivo* resulted in neuronal activity that was significantly increased over pre-stimulation levels with no return to baseline levels during the time of the light exposure. We also examined the effects of optically driven neuronal activity on subsequent tone driven responses in the DCN. In the DCN 75% of the 16 electrode sites showed decreased neuronal activity in response to a tone immediately following light stimulation while six percent were decreased following tone stimulation and 19% of the electrode sites showed no change. This is in contrast to tone driven neuronal activity prior to the light exposure in which the majority of electrode sites showed increased neuronal activity. Our results indicate that expression and activation of rhodopsin within neurons involved in auditory processing does not appear to have deleterious effects on hearing even

☆Contract grant sponsor: Ralph Wilson Foundation (to A.G.H), Capita Foundation (to A.G.H), R03 DC007733 (to A.G.H) and P30 Grants (Anatomy and Cell Biology, Wayne State University and Kresge Hearing Research Institute, University of Michigan).
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http://dx.doi.org/10.1016/j.brainres.2012.10.030

18 months following expression. In addition, virally targeted rhodopsins may be useful as tract tracers to delineate as well as modulate the activity of pathways and specific neurons. In the future rhodopsins can be targeted to specific subpopulations of auditory neurons. Ultimately, photostimulation may provide a physiologically relevant method for modulating the function of auditory neurons and affecting hearing outcomes.

This article is part of a Special Issue entitled Optogenetics (7th BRES)

Contents

1. Introduction

Microbial rhodopsins have been cloned and when functionally expressed were found to form light-gated cation channels (ChR2) and chloride pumps (HaloR) (Boyden et al., 2005; Han and Boyden, 2007; Nagel et al., 2003; Zhang et al., 2007). Recently, several studies have been able to take advantage of these rhodopsin proteins. Once ChR2 and HaloR are inserted into the cell membrane a neuron can be depolarized or hyperpolarized remotely via specific wavelengths of light (Han and Boyden, 2007; Lima et al., 2009; Zhang et al., 2007). Even ectopic expression of one of these microbial rhodopsins, channelrhodopsin-2 (ChR2) or HaloR, was able to restore visual responses in mice with photoreceptor degeneration (Bi et al., 2006; Zhang et al., 2009).

With the proliferation of cochlear prosthetic devices that are implanted at various levels of the auditory system becoming a routine treatment for profound deafness in both young people and adults, developing the best possible device for implantation as well as providing the best possible platform for the reintroduction of hearing by understanding the effects of implantation and stimulation becomes critical. In the auditory system, restoration of hearing with cochlear prostheses is achieved through spread of electrical currents to neurons that are in direct contact with the implanted electrodes. The spatial sensitivity of this approach is limited by the spread of current. Recent approaches using lasers (Richter et al., 2008; Wenzel et al., 2009) to stimulate auditory neurons are able to activate specific regions of the cochlea thereby controlling the spread of activity providing discrete control of groups of neurons.

In the current study our goals were to determine whether constructs containing ChR2 and HaloR would be expressed across cell types in the dorsal cochlear nucleus (DCN); how long would the expression persist; whether rhodopsin expression was restricted to ascending and descending pathways related to the injection site and whether appropriate wavelengths of light could activate neurons *in vivo*.

In the future, transfection of neurons with ChR2 and HaloR will allow us to take advantage of a non-contact approach for neuronal stimulation and will provide us with the advantage of being able to target specific cell types with designer