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Research Report

Optogenetic stimulation of the cochlear nucleus using channelrhodopsin-2 evokes activity in the central auditory pathways

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ABSTRACT

Optogenetics has become an important research tool and is being considered as the basis for several neural prostheses. However, few studies have applied optogenetics to the auditory brainstem. This study explored whether optical activation of the cochlear nucleus (CN) elicited responses in neurons in higher centers of the auditory pathway and whether it elicited an evoked response. Viral-mediated gene transfer was used to express channelrhodopsin-2 (ChR2) in the mouse CN. Blue light was delivered via an optical fiber placed near the surface of the infected CN and recordings were made in higher-level centers. Optical stimulation evoked excitatory multiunit spiking activity throughout the tonotopic axis of the central nucleus of the inferior colliculus (IC) and the auditory cortex (Actx). The pattern and magnitude of IC activity elicited by optical stimulation was comparable to that obtained with a 50 dB SPL acoustic click. This broad pattern of activity was consistent with histological confirmation of green fluorescent protein (GFP) label of cell bodies and axons throughout the CN. Increasing pulse rates up to 320 Hz did not significantly affect threshold or bandwidth of the IC responses, but rates higher than 50 Hz resulted in desynchronized activity. Optical stimulation also evoked an auditory brainstem response, which had a simpler waveform than the response to acoustic stimulation. Control cases showed no responses to optical stimulation. These data suggest that optogenetic control of central

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auditory neurons is feasible, but opsins with faster channel kinetics may be necessary to convey information at rates typical of many auditory signals.

1. Introduction

Optogenetic control of neural pathways has been used to investigate many neural systems including memory, olfaction, motor control, and the limbic system (Boyden et al., 2005; Ayling et al., 2009; Hira et al., 2009; Rolls et al., 2011; Stortkuhl and Fiala, 2011; Huff et al., 2013; Shimano et al., 2013). Optogenetics uses viral vectors (Boyden et al., 2005) or tissue-specific promoters (Zhao et al., 2011) to deliver light-sensitive microbial opsins into neural membranes and enable the neurons to respond to optical stimulation (Boyden et al., 2005; Han and Boyden, 2007; Chow et al., 2010). Channelrhodopsin-2 (ChR2) is the most widely used opsin in neuroscience research. This molecule, when delivered to neurons of the central nervous system, can be activated by pulses of blue light. ChR2 has been safely expressed and stimulated, without observed immune response, in vivo in multiple species, including non-human primates, over a period of months to years (Zhang et al., 2006; Wang et al., 2007; Bernstein et al., 2008; Han et al., 2009; Chan et al., 2010; Chow et al., 2010).

Only a few recent studies have applied optogenetics to the auditory system. In a pioneering study of the cochlear nucleus (CN), Shimano et al. (2013) introduced ChR2 into CN neurons and demonstrated local increases in activity in response to light. In a study of the cochlea of transgenic animals expressing ChR2, stimulation of the cochlea with light activated auditory-nerve fibers and higher centers in the auditory pathway (Hernandez et al., 2014). That study proposed the idea of an auditory implant based on optogenetics, an optical cochlear implant. The cochlear implant is an auditory prosthesis implanted into the inner ear and it successfully restores hearing in terms of comprehension of speech (Moore and Shannon, 2009; Colletti et al., 2012). Another auditory prosthesis potentially amenable to the use of optogenetics is the auditory brainstem implant (ABI; Otto et al., 1998). The ABI is an array of electrodes surgically placed on the surface of the CN, bypassing a damaged cochlea or auditory nerve in human patients who cannot benefit from a cochlear implant. The significant limitation of the ABI is that the majority of users, especially those who have had a vestibular schwannoma removed from the area, have poor speech comprehension when compared with users of the more successful cochlear implant (Colletti et al., 2012). There are reports, though, that some ABI users have good comprehension and they point out influencing factors such as the presence of a tumor (Colletti and Shannon, 2005), the type of processor (Behr et al., 2007) and the duration of deafness (Matthies et al., 2014). In addition, many ABI users experience side effects (e.g. tingling, facial twitching, dizziness, and sometimes pain) from the non-specific activation of neighboring nerves affected by electric current spread. Usually, the electrodes causing such side effects are turned off so that only a subset of the 22 electrodes of the implanted array are actually used to convey speech information. A revised design of the ABI with penetrating electrodes did not improve comprehension (Otto et al., 1998) and is no longer an option. New approaches to the ABI using optogenetics could be explored as a means to more effectively restore hearing to these deaf individuals, as optogenetics could potentially provide more specific activation of individual frequency regions by focusing light.

In the current study, the goal was to establish the response characteristics of neurons in higher centers following stimulation of ChR2-expressing CN neurons. We chose to record in the inferior colliculus (IC), a higher-order nucleus that receives direct projections from the CN, and from auditory cortex (Actx), which is several synapses above the IC. Of special interest is the temporal response to optical stimulation at high pulse rates, because the ChR2 ion channel has sluggish kinetics (Boyden et al., 2005), which may limit the ability to transmit fast temporal information when compared to acoustic stimulation. We also compared the far-field evoked response characteristics evoked by light and those evoked by sound. Given the complex arrangement of excitatory and inhibitory neurons in the CN (Nelken and Young, 1994), it is difficult to predict the responses of higher auditory centers. However, our results will be important for future the development of an auditory prosthesis based on optogenetics.

2. Results

2.1. *Expression of ChR2 in the cochlear nucleus*

Mice injected with ChR2 had ChR2-GFP immunolabeled neurons and axons throughout the three subdivisions of the CN (DCN, dorsal; PVCN, posteroventral and less label in the AVCN, anteroventral; Fig. 1A–C). For example, there was labeling in the fusiform cell layer of DCN (Fig. 1A). There was also labeling in neuropil and axons (arrowheads in Fig. 1A and B and inset images in Fig. 1D). The anterogradely labeled axons were observed in the exit pathways of the CN (dorsal and ventral acoustic stria); (Warr, 1966; Smith et al., 1993) and in the targets of these axons, the contralateral CN (Cant and Gaston, 1982; Alibardi, 1998; Brown et al., 2013) and contralateral IC (Oliver, 1985; Schofield and Cant, 1996; Malmierca et al., 2005). Although there was variability from animal to animal, all cases with labeling in the CN also had axonal labeling in at least 3 of these 4 pathways/targets. Cases with significant labeling (10 or more cells or axons) in the injected CN and upstream targets were defined as ChR2+ (e.g. Fig. 1A–C), whereas cases with no labeling in the CN were defined as ChR2− (e.g. Fig. 1E). For the most part, however, the density of extracellular immunofluorescence hindered identification of discrete cell types in the CN. In total, 18 of