USING SiNAPS
to Uncover Cochlear Neuropathy

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Groundbreaking advances in the regeneration of cochlear hair cells and auditory neurons hold promise for transforming the clinical focus from treating hearing loss to restoring normal hearing.

Groundbreaking advances in the regeneration of cochlear hair cells and auditory neurons hold promise for transforming the clinical focus from treating hearing loss to restoring normal hearing. Bramhall et al (2014), for example, recently discovered that supporting cells within the cochlea of mice may be pharmacologically induced to differentiate into new hair cells. Other recent work on hearing loss in animal models by Diensthuber et al (2014) has uncovered stem cells within the spiral ganglion region of the auditory nerve that could be targets for neural regeneration therapies.

The emerging research indicating that peripheral auditory nerve degeneration (cochlear neuropathy) is a potential consequence of exposure to even moderate levels of noise (Kujawa and Liberman, 2009; Lin et al, 2011; Furman et al, 2013), and is expected with aging (Sergeyenko et al, 2013), makes regenerative treatments even more urgent. There is also a pressing need for clinical tests with the capability of detecting and characterizing the extent of cochlear neuropathy. This condition has been called “hidden hearing loss” by Schaette and McAlpine (2011) because it is not detected with common, threshold-focused audiological tests.

The long-term research goal in the EARLAB at the University of Cincinnati is to develop an electrophysiologic test for the diagnosis of cochlear neuropathy that can guide treatment approaches and provide a method to gauge efficacy of regenerative treatments in the future.

Techniques for Differential Diagnosis of Auditory Nerve Pathology

Supra-threshold techniques using auditory-evoked potentials (AEPs) have been developed to improve differential diagnosis of acoustic neuromas and auditory neuropathy (Don et al, 2005, 2009; Riazi and Ferraro, 2008) and offer a prognosis to cochlear implant recipients (Smith and Simmons, 1983; Hall, 1990; Gibson and Sanli, 2007). Chertoff (2004) and Chertoff et al (2008) used a gerbil model to investigate the utility of the compound action potential (CAP), the laboratory equivalent of the action potential (AP) component of clinical electrocochleography and Wave I of the auditory brainstem response (ABR), in identifying auditory nerve fiber degeneration associated with sensorineural hearing loss. Their approach was to quantify the magnitude (amplitude) and other morphologic metrics of CAP waveforms, in addition to the traditional measures of threshold and latency. Lichtenhan and Chertoff (2008) also found that CAPs recorded from human participants were reduced in magnitude, relative to those recorded prior to noise
exposure, during periods of temporary noise-induced hearing loss.

In a mouse model of noise-induced neural degeneration, Kujawa and Liberman (2009) found that the amplitude of Wave I of the ABR predicted the extent of synaptic pathology, while threshold did not. These results and those of others (Lin et al, 2011; Furman et al, 2013) who observed the same outcome in noise-exposed guinea pigs align well with the much earlier animal work of Schuknecht and Woellner (1955) showing that destruction of up to 75 percent of auditory nerve fibers did not elevate behavioral hearing thresholds. Yuan et al (2013), using a mouse model of ouabain-induced auditory neuropathy, also observed the superior sensitivity of ABR Wave I amplitude to detect neural synapse pathology.

Using a gerbil model of partial auditory nerve degeneration, Earl and Chertoff (2010) found that the amplitude of CAPs, evoked with high-level tone burst stimuli, was strongly correlated with overall auditory nerve survival. High stimulus intensities, however, trigger firing across a large range of nerve fibers and thus confound identification of the precise location of neural pathology. A location-specific estimate of auditory nerve damage may be possible using high-level broadband stimuli to evoke CAPs while varying the bandwidth of high-pass masking noise to systematically limit the region of contributing nerve fibers.

**Novel Approach to Fill Gap in Clinical Diagnosis of Auditory Nerve Survival**

Using masked CAPs, here called signal-in-noise action potentials (SiNAPs), to derive amplitude growth functions is an approach that can potentially fill the gap in clinical techniques for predicting the location and extent of auditory nerve pathology. A schematic of an uncoiled cochlea in **Figure 1** (left panel) and the accompanying plot (**Figure 1**, right panel) illustrate the theory behind the approach. A high-intensity stimulus that triggers synchronous neural excitation along the majority of the cochlea (e.g., chirp) is used to evoke CAPs while the high-pass cutoff frequency of the masking noise is changed from low frequencies encoded in the apex to high frequencies encoded in the base. In a normal cochlea with a full complement of nerve fibers, CAP amplitude increases as progressively more neurons are allowed to contribute to the CAP (black line in **Figure 1**, right panel). In a cochlea with inner hair cell and/or neural damage in the base, normalized CAP amplitude would increase faster than normal because the middle and apical nerve fibers would contribute relatively more overall to the CAP. The cumulative amplitude functions (CAFs) would then

**FIGURE 1.** Left panel: Theoretical representation of SiNAPs technique that uses multiple bandwidths of high-pass noise to systematically limit the region of auditory nerve fibers that can contribute to chirp-evoked CAPs. Right panel: The cumulative amplitude function (CAF) for a cochlea with neural degeneration in the basal high frequency region (e.g., red curve) would theoretically be abnormal due to the absence of neural firing that is normally produced by functional afferent neurons in that region of the cochlea (e.g., black curve). The location and width of the CAF plateau is hypothesized to correspond with the cochlear location and extent of neural pathology.
plateau when the high-pass noise cutoff frequency moves into the region of damage because there are no additional nerve fibers to contribute to the response. The location and width of the plateau is hypothesized to correspond with the underlying anatomical location where inner hair cells and/or neurons are non-functional or absent.

Research to Verify Theory Behind the SiNAPs Technique

Preliminary research by Earl and Chertoff (2012) using the SiNAPs technique in normal-hearing animals suggested that high-level (90 dB SPL) chirp stimuli triggered synchronous neural firing throughout the majority of the cochlea. Earl and Chertoff (2012) also found that high-level CAFs were not altered by gentamicin-induced hearing loss, suggesting that high-level functions could provide an estimate of neural survival that is independent of cochlear outer hair cell pathology.

Follow-up animal experiments were subsequently designed to verify the sensitivity of the SiNAPs technique to neural-specific lesions using an infrared laser (Earl and Chertoff, 2013). The laser was used to induce lesions in the dendritic projections within the spiral lamina in the basal portion of the cochlea of gerbils. CAFs were constructed with broadband chirps at 90 dB SPL during simultaneous masking noise high-passed in 1/3 octave intervals between 0.4 and 50 kHz.

FIGURE 2 presents representative data from a group of gerbils with laser-induced neural lesions. In the majority of animals, regions of missing hair cells also were observed, presumably due to heat “splatter” secondary to the laser focus on the neural dendrites. This splatter into the hair cell region likely accounts for the threshold elevations observed (FIGURE 2, left panel). High-level CAFs in normal-hearing gerbils grew gradually as a function of high-pass masker cutoff frequency. In lesioned gerbils, the amplitude functions included regions of steep growth before plateauing in regions that generally corresponded to the cochlear location at which the laser was directed (FIGURE 2, middle and right panels). Confocal images of fluorescent-stained cochlea revealed circular lesions through regions of the osseous spiral lamina and missing hair cells.

The SiNAPs technique also reveals changes in neural firing following acoustic overexposure.

FIGURE 2. Left panel: CAP threshold elevation in two representative animals (GB940, GB950) demonstrates the common observation of high frequency threshold elevation corresponding to the basal turn of the cochlea at which the laser was directed. Middle panel: Post-lesion CAP amplitude growth (CAF) roughly follows the pattern of CAP threshold elevation and overlaps with location of anatomical damage (right panel). Right panel: Physiologic plateaus (CAF plateaus) generally overlap with neural damage and hair cell damage. Representative animals (GB940, GB950) are highlighted with boxes to facilitate comparisons with the data in the left and middle panels.
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Hair cells. Light micrographs of osmium-stained cochleae revealed regions of severed neurons and lighter staining consistent with myelin damage (FIGURE 3).

The SiNAPs technique also reveals changes in neural firing following acoustic overexposure (Earl et al, 2014). The post-noise, chirp-evoked CAFs differed significantly from the normal range and exhibited unique growth abnormalities for mid- versus low-frequency exposure (120-124 dB SPL, two to three hours). The CAF plateaus (FIGURE 4, bottom panel) generally overlapped with regions of missing outer and inner hair cells, as did the patterns of CAP threshold elevations (FIGURE 4, top panel). Light micrographs of the same cochleae stained with osmium, however, did not reveal neural damage at the level of the myelinated processes of peripheral auditory nerve fibers, with one exception.

The results from both the laser lesion and the acoustic overexposure experiments indicate that the location and extent of the plateaus observed in CAP-derived cumulative amplitude functions generally correspond to the location of cochlear damage, including hair cells and auditory neurons. Experiments are ongoing in the EARLAB to test the performance of the SiNAPs technique in detecting neural-specific lesions induced with moderate-to-high level noise exposure protocols similar to Furman et al (2013).

**Stimulus Characteristics and Masking Paradigms**

The use of chirp stimuli to evoke AEPs has been described as a form of “input compensation” for the cochlear traveling wave delay while the use of masking to isolate particular regions of neural activity has been called “output compensation” (Don et al, 2009). The SiNAPs technique exploits the benefits of both approaches to infer regional neural integrity throughout the majority of the cochlea. Chirp stimuli (i.e., rising frequency tone-bursts) theoretically evoke synchronous neural firing throughout the majority of the cochlea by compensating for the cochlear traveling wave delay. The instantaneous frequency of rising chirp stimuli is swept from low to high frequencies according to a time constant that allows individual frequency components to arrive simultaneously at their characteristic place, leading to synchronous displacement along the basilar membrane (Shore and Nuttall, 1985). The findings of several studies (Shore and Nuttall, 1985; Fobel and Dau, 2004; Eiberling and Don, 2008; Don et al, 2005; Chertoff et al, 2010; Earl and Chertoff, 2012) indicate that chirp stimuli consistently yield larger electrophysiologic response amplitudes than clicks and traditional toneburst stimuli.

**FIGURE 3.** Laser-induced nerve damage in basal portion of gerbil cochlea (faded area) contrasts with the black, branch-like appearance of normal nerve fibers.

**FIGURE 4.** Representative CAP threshold elevation (top panel) and CAF abnormalities (bottom panel) following exposure to low-frequency (open circles) and mid-frequency (closed circles) pure tones at 120–124 dB SPL for two to three hours.
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The stacked-ABR technique is a well-known example of using masking noise to compensate for the temporally disperse neural output triggered by click stimuli. The stacked-ABR described by Don et al (2005) represents the sum of five derived-band ABR waveforms that have been temporally aligned according to the latency of Wave V. The masked ABRs are recorded during simultaneous masking with broadband noise that is high-pass filtered with five different cutoff frequencies representing octave intervals between 0.5 and 8 kHz. Relative to MRI detection of small tumors, Don et al (2005) found that the stacked-ABR technique identified 100 percent of the tumors with a specificity of 50 percent. With a slightly lower sensitivity of 95 percent, the technique reached a specificity of 88 percent.

The animal research of Evans and Elberling (1982) aimed to validate the use of high-pass masking techniques for deriving narrow-band CAP responses. They recorded click-evoked single-fiber activity and round window CAPs simultaneously during high-pass noise masking at various cutoff frequencies. Decreases in single-fiber activity coincided with decreases in CAP amplitude, suggesting that a high-pass masking paradigm could provide an accurate survey of regional neural firing along the basilar membrane.

Burkard and Sims (2002) applied the idea of using a high-pass masking paradigm during high-level click ABR recordings to specifically test whether masking-induced changes in electrophysiology could account for the decline in speech-in-noise difficulty among elderly individuals. Interestingly, their results indicated that the Wave I component of the ABR, which like the CAP originates from the peripheral processes of the auditory nerve, was significantly reduced in amplitude compared to Wave I amplitude in a group of young adults with normal hearing thresholds. The majority of the individuals in the elderly group also had normal hearing thresholds, with the others having no more than a mild high-frequency hearing loss. Speech-in-noise ability was not measured in the study.

The current work in the EARLAB at the University of Cincinnati is focused on continuing the development of the SiNAPs technique as a clinical test of cochlear neuropathy. Because low- to mid-level SiNAPs are sensitive to cochlear hair-cell pathology, the technique also could be used to detect hearing loss related to hair-cell pathology and gauge the efficacy of sensory cell regeneration therapies. Clinical studies are being conducted in parallel with the work in the laboratory to determine the normal range of cumulative amplitude functions (CAFs) derived from chirp-evoked electrocochleography (ECochG) recordings. Preliminary results in normal-hearing adult participants show the amplitude of ECochGs evoked with high-level chirps to be 2.2 times larger on average than those evoked with clicks. This finding aligns well with the initial chirp-evoked ECochG study in humans completed by Chertoff et al in 2010. The ECochG-derived CAFs also show the general pattern that has been observed in previous animal and human studies of increasing amplitude as the high-pass masker cutoff frequency increases.

**Significance of a Clinical Measure of Cochlear Neuropathy**

The latest research in noise-induced cochlear neuropathy is pertinent for likely millions of individuals who have difficulty understanding speech in noisy listening situations, despite having normal hearing sensitivity.
to account for auditory nerve survival when prescribing amplification via hearing aids, programming the stimulation characteristics of cochlear implants, or eventually, specifying the target for delivery of therapeutic agents that can promote hair cell and neural regeneration.

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References


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