Synchronous auditory nerve activity in the carboplatin-chinchilla model of auditory neuropathy

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Abstract: Two hallmark features of auditory neuropathy (AN) are normal outer hair cell function in the presence of an absent/abnormal auditory brainstem response (ABR). Studies of human AN patients are unable to determine whether disruption of the ABR is the result of a reduction of neural input, a loss of auditory nerve fiber (ANF) synchrony, or both. Neurophysiological data from the carboplatin model of AN reveal intact neural synchrony in the auditory nerve and inferior colliculus, despite significant reductions in neural input. These data suggest that (1), intact neural synchrony is available to support an ABR following carboplatin treatment and, (2), impaired spike timing intrinsic to neurons is required for the disruption of the ABR observed in human AN.

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PACS numbers: 43.64.Bt, 43.64.Pg, 43.64.Qh, 43.64.Ri [Q-JF]

Date Received: April 29, 2010 **Date Accepted:** May 20, 2010

1. Introduction

Auditory neuropathy (AN) is characterized by variable hearing loss (from normal to profound), and a speech comprehension impairment that is disproportionate to that hearing loss (Berlin *et al.* 2010; Starr *et al.*, 1991, 1996; Zeng *et al.*, 2005). AN patients exhibit normal otoacoustic emissions (OAEs) and cochlear microphonics (CMs), suggesting normal outer hair cell (OHC) function. On the other hand, AN patients show absent or abnormal auditory brainstem responses (ABRs) (e.g., Kraus *et al.*, 2000; Huang *et al.*, 2009). It is likely that the source of the ABR abnormality contributes to the speech comprehension deficit. Clinically, normal OAEs and/or CMs concurrent with abnormal or absent ABRs are used as hallmark features in the diagnosis of AN (Zeng *et al.*, 2005). This constellation of symptoms is ambiguous with respect to specifying the locus of pathology to the inner hair cells (IHCs), the auditory nerve, the synapse

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between them, or some combination of the above (Starr *et al.*, 1996). The purpose of what follows is to use existing evidence to resolve that ambiguity.

2. Auditory neuropathy

ABR abnormality in patients with AN is often observed as early as 2 ms (i.e., wave I) after stimulus onset, suggesting the existence of a pathology very close to the auditory transduction process. AN patients do not exhibit an acoustic middle ear muscle reflex, yet retain the reflex following non-acoustic (e.g., tactile) stimulation (Starr *et al.*, 1996; Berlin *et al.*, 2005). Despite disruption of auditory nerve fiber (ANF) activity revealed by absent or abnormal ABRs, AN patients are typically not deaf, although speech discrimination is impaired in noise backgrounds (Kraus *et al.*, 2000). Correspondingly, AN patients may exhibit sustained (albeit delayed) cortical potentials and long-latency cognitive components, reflecting higher-level processing of sound information (Starr *et al.*, 1991; Michalewski *et al.*, 2009).

At least two approaches have been used to infer the site(s) of pathology. The variable efficacy of cochlear implantation, successful in approximately 85% of AN patients (Berlin *et al.*, 2010), has been used to make inferences about the primary lesion site (Miyamoto *et al.*, 1999). The argument is that patients who do not benefit from cochlear implants may have significant damage to spiral ganglion cells or ANFs, while in patients who benefit from implantation, the damage may include IHCs and/or their synapses. Starr *et al.* (2004) distinguished between Type I and Type II AN, differentiating them on the inference that the primary site of pathology in the former involves the spiral ganglion and/or auditory nerve axons, while Type II AN is characterized by more distal pathology. Note, however, that poor implant outcomes can arise from a suboptimal interface between the device and the auditory nerve array. This means that poor post-implant performance need not be definitive of lesion locus. A second approach to specifying lesion locus comes from a survey of cochlear and neural potentials in AN patients. Santarelli *et al.* (2008) described three groups of AN patients distinguished by patterns of cochlear potentials (esp. summing potential [SP] and compound action potential [CAP]), and their dependence on stimulus rate. The groups of patients differed in whether electrocochleography suggested involvement of IHCs, the distal portion of ANFs, processes central to the generation of the CAP, or a combination of the above.

At a strictly behavioral level, patients with AN systematically display deficits in the perception of sounds whose detection or discrimination is heavily dependent on neural timing (Starr *et al.*, 2003; Zeng *et al.*, 2005). AN patients exhibit temporal gap detection thresholds between two and five times higher than those for normal hearing controls. AN patients are able to perform sound localization tasks using interaural level differences, but not interaural time differences. Impaired frequency discrimination is observed at low frequencies (<4000 Hz) but not at high frequencies (>4000 Hz); the former, but not the latter, might be aided or mediated by timing cues in the form of phase-locked responses to signal periodicity. On the other hand, when tested at or above their pure-tone thresholds, patients are able to detect intensity differences with similar accuracy to normal hearing controls (Zeng *et al.*, 2005).

Selective disruption of time- but not intensity-related processing in AN is consistent with dys-synchronous ANF activity. This impaired neural timing affects both periodic responses (binaural beat detection) and transient ones (gap detection). This pattern of deficits could occur as a result of variably decreased conduction velocities in surviving ANFs, resulting in a temporally uncoordinated (i.e., dys-synchronous) input to the cochlear nucleus—a phenomenon that could be compounded more centrally. However, a reduction of neural input may similarly explain the phenomenology of AN, because even normally-timed cochlear output may be too weak to participate effectively in temporally-convergent projections if there are too few fibers to contribute to the convergence, or if those neurons' response strengths are too low. Further discussions of the physiological and behavioral observations showing that either reduced input or altered neural synchrony may account for AN are provided by Starr *et al.* (2003) and Zeng *et al.* (2005). The important point is that studies of human AN have been unable to resolve which of these two dysfunctions, reduced input or altered neural synchrony, account for the absence of an ABR and AN's psychophysical correlates. In light of this issue, we now con-

sider the carboplatin-treated chinchilla, from the previously dissociated perspectives of it being an animal model of human AN, and a venue providing data on neural timing in the presence of reduced cochlear output.

3. The carboplatin-chinchilla model of auditory neuropathy

Carboplatin is a platinum-group antineoplastic agent used clinically for the treatment of ovarian cancer. Patients treated with carboplatin may exhibit side effects including ototoxicity manifested as high-frequency hearing loss (e.g., [Kennedy *et al.*, 1990](#)). The ototoxic effects of carboplatin-related agents led to the development of an animal model of selective IHC loss in the chinchilla ([Wake *et al.*, 1994](#)). Following intravenous administration of carboplatin, [Wake *et al.* \(1994\)](#) reported increased pure-tone thresholds associated with a loss of between 54 to 83% of IHCs and only minor loss of OHCs. There may also be significant loss of ANFs ([El-Badry and McFadden, 2009](#)). Following carboplatin treatment resulting in up to 95% IHC loss, surviving ANFs retain normal thresholds and frequency tuning ([Wang *et al.*, 1997](#)), although both spontaneous and driven spike rates of the fibers were significantly lower than normal. This pattern of data is important, because it indicates that the cochlear transduction process is largely intact in the chinchilla model, but that cochlear output, and therefore neural input to the auditory nervous system, is reduced, both in terms of the number of ANFs that are available, and in the spike rates of the surviving fibers.

It was subsequently proposed that the carboplatin-treated chinchilla was a model of human AN ([Harrison, 1998](#)). Following carboplatin treatment, chinchillas showed relatively normal OAEs and CMs in the presence of increased thresholds for cochlear whole-nerve action potentials (CAPs) and ABRs ([Wake *et al.*, 1994](#); [Harrison, 1998](#)). Two points about the importance of elevated ABR thresholds in the chinchilla model warrant closer attention. First, threshold elevation is not the same thing as absent or disordered ABRs (delayed or missing components). Second, subsequent research using the carboplatin model has not always confirmed the magnitude of the ABR threshold elevation. At least one study has shown that despite extensive loss of IHCs (approximately 60% throughout the cochlea) and ANFs (85% in the habenula perforatae), ABRs tested at threshold levels can be similar in all respects to those observed before treatment; moreover, even with such extensive damage, only a modest increase (less than 6 dB) in threshold was observed post-treatment ([El-Badry and McFadden, 2009](#)). It might be speculated that the carboplatin-chinchilla is a model of Type II AN because so much of the damage is distal to the spiral ganglion. On the other hand, both Type I and Type II AN patients have abnormal or absent ABRs, while the chinchilla model does not.

The presence of a relatively intact ABR in the carboplatin-chinchilla model stands in direct contrast with human AN. AN patients exhibit either an absent or abnormal (delayed, or entirely missing, wave components) ABR, especially at threshold hearing levels, and the ABR is not recovered when stimulus intensity is increased to well above threshold levels ([Starr *et al.*, 1991, 2003](#); [Kraus *et al.*, 2000](#); [Huang *et al.*, 2009](#)).

How does the ABR remain intact in carboplatin-treated chinchillas exhibiting extensive IHC and ANF loss? Unreported in most studies of carboplatin-treated chinchillas is the timing of auditory nerve and central responses. This is an important point, because if the carboplatin-treated chinchilla is to serve as an animal model of human auditory neuropathy, then it behooves the model to display poor response timing in the auditory nerve and in more central structures. In this regard, the distinction between transient and periodic temporal responses is significant, because the mammalian forebrain largely preserves the former but not the latter, and this difference has demonstrable perceptual correlates in neurological patients ([Phillips and Hall, 1990](#)).

[Phillips *et al.* \(2001\)](#) studied response amplitude and response timing in both the auditory nerve and inferior colliculus (IC) of unanesthetized chinchillas using gross electrodes implanted directly in/on those structures. Following [Heil and Irvine \(1997\)](#), [Phillips *et al.* \(2001\)](#) studied evoked response amplitude and timing as a function of both the plateau level (in dB SPL) and rise-time (in ms) of noise-burst stimuli. These two stimulus parameters are often confounded in studies of intensity coding, in the sense that most studies hold the stimulus rise-

Fig. 1. Latency functions for the whole auditory nerve (left) and inferior colliculus potential (right). Each panel shows mean latency plotted as a function of rate of pressure change at stimulus onset in Pa/s. Upper panels are reproductions of the plots of the raw data provided in Phillips *et al.* (2001). Lower panels show the same data after correcting for small threshold shifts seen after carboplatin treatment. Data are shown separately for recordings obtained pre- (filled symbols) and post-carboplatin treatment (open symbols), and for stimuli of rise times specified in the Legends. Data are reproduced from Phillips *et al.* (2001, Sensitivity of unanesthetized chinchilla auditory system to noise burst onset, and the effects of carboplatin. *Hearing Research*, 155, 133–142), with permission of Elsevier.

time constant while varying the plateau level of the stimulus, and measure the response latency as a function of the latter. This design results in a covariation of stimulus plateau level and the rate of pressure change at sound onset (Heil and Irvine, 1996) so that it is unclear which stimulus parameter (plateau level, rate of pressure change at sound onset) actually drives the responses. It is the latter (Heil and Irvine, 1997; Heil, 1997). Phillips *et al.* (2001) confirmed this in the unanesthetized chinchilla, and further, showed that the same relationship held in the carboplatin-treated animal.

Figure 1 shows latency functions for auditory nerve and IC responses to noise-burst stimuli before and after carboplatin treatment. The data in the upper panels are from Figs. 4 and 7 of Phillips *et al.* (2001). In that study, latency was first plotted as a function of stimulus plateau level, separately for different rise-time conditions, and then the stimulus level was re-specified in units of Pa/s by dividing plateau level by rise time. The data were then re-plotted to produce the curves shown in the upper panels in Fig. 1. For each recording site, the latency functions pre- and post-carboplatin treatment follow the same trajectories, and the response latencies for the

same rates of pressure change have absolute values that are within 0.5 ms or so of each other, except for threshold-level responses (i.e., very low rates of pressure change at sound onset). For the present report, the data were then corrected for small (<10 dB) threshold elevations following the carboplatin treatment (by subtracting the threshold shift before conversion of the stimulus specification to Pa/s, so that pre- and post-carboplatin responses are matched for suprathreshold plateau level). The resulting latency functions now must be expressed in terms of *relative* rate of pressure change at sound onset, because the corrections for the threshold shifts essentially displace rise-time specific latency functions leftwards along the abscissa, and differentially so for responses to stimuli of different rise times. With this correction employed, the latency functions seen pre- and post-carboplatin treatment become even more similar, typically within 0.3 ms of each other over much of their ranges (Fig. 1, lower panels). In the original study, response amplitudes were smaller in the carboplatin-treated animals (not shown; see Phillips *et al.*, 2001), which is to be expected given the prior single-unit data of Wang *et al.* (1997). However, despite any poverty of response amplitude in the carboplatin-treated chinchilla, auditory nerve responses have very near-normal latencies, indicating preservation of the neural synchrony required for transient response timing.

Additional evidence for maintained neural synchrony following carboplatin treatment and extensive IHC loss comes from nearfield recordings in the chinchilla. Recordings of IC field potentials revealed only marginal increases in IC response thresholds and response latencies for click stimuli following carboplatin treatment. Mean latency shifts pre- and post-carboplatin reported by Burkard *et al.* (1997) were on the order of 50 μ s. Moreover, near-normal click response amplitudes were observed in the IC for click rates as high as 1000 Hz (Burkard *et al.*, 1997). These data are evidence in the carboplatin-chinchilla model for preserved neural synchrony of the form required for periodic responses. The findings of Burkard *et al.* (1997) and Phillips *et al.* (2001) on temporal responses are all the more remarkable when one considers that the carboplatin treatment likely resulted not only in the documented IHC loss, but also in significant ANF loss (after El-Badry and McFadden, 2009).

Although the carboplatin-chinchilla model fails to model a hallmark feature of human AN (specifically an absent or abnormal ABR), it may nonetheless be informative about the mechanism. Recall that both human physiological and psychophysical studies are unable to determine the relative importance of reduced input and neural synchrony in AN. Studies of the carboplatin-treated chinchilla have already provided a direct answer to this question. In the presence of dramatically reduced ANF input, neural timing of transient (Phillips *et al.*, 2001) and periodic stimuli (Burkard *et al.*, 1997) remains largely intact, as does the ABR (El-Badry and McFadden, 2009). Given the lack of an ABR in human AN, dys-synchrony intrinsic to the ANF or more central neurons appears to be critical for disruption of the ABR.

One potential objection to this proposal is that loss of the ABR in human AN may require an even greater reduction of input (e.g., $>85\%$ ANF loss) than that achieved in the carboplatin-chinchilla studies (El-Badry and McFadden, 2009). In this regard, in the one case of human AN coming to autopsy, there was nearly 95% spiral ganglion cell loss in both cochleae (Starr *et al.*, 2003). This issue awaits further empirical data. What is striking about the chinchilla model is that despite reduced response strength in the auditory nerve, stimulus/timing-response relationships are so normal in the nerve and more centrally (Burkard *et al.*, 1997; Phillips *et al.*, 2001). Even animals with near total IHC loss exhibit ABRs at increased thresholds (Wake *et al.*, 1994). Taken together, these data suggest that a very small number of healthy ANFs may sustain synchronous activity and generate an intact ABR.

4. Summary and conclusion

A number of different pathologies may contribute to AN, but the balance of current evidence suggests that IHC loss is an unlikely cause of the neural dys-synchrony seen in human AN. The presence of an intact summing potential (generated by IHCs) but poor compound action potential (generated by ANFs) in human AN provides converging evidence for normal IHC function (Huang *et al.*, 2009), though not necessarily normal function of the synapses between the IHCs and the ANFs. Finally, disruption of the ABR typically occurs as early as 2 ms after

stimulus onset (i.e., wave I). Taken together, these data suggest that the majority of AN cases arise not from loss of IHCs, but from damage either to the synapse between IHCs and ANFs, or to the ANF dendrites themselves (see also Huang *et al.*, 2009).

The most important finding of this review comes from studies of neural response timing in the carboplatin-chinchilla model. Both transient responses (latencies to noise burst onset: Phillips *et al.*, 2001) and periodic ones (entrainment to click stimuli: Burkard *et al.*, 1997) are nearly normal in animals with substantial IHC loss following carboplatin treatment. Response amplitudes are lowered in such animals (Wang *et al.*, 1997; Phillips *et al.*, 2001), which is to be expected given a reduced neural output from the cochlea. The preserved response timing in the face of such reduced input to the auditory nervous system suggests that the neural timing deficits (and their perceptual correlates) in human AN reflect response timing deficits intrinsic to neurons, rather than reduced cochlear output. This conclusion is entirely in keeping with that of Huang *et al.* (2009) who describe a gene mutation in patients with cranial nerve neuropathies capable in principle of affecting the timing of spike generation in the auditory nerve. Their general argument is that the demonstrated mutation of the OPA1 gene in human AN compromises the ability of the OPA1 protein to contribute normally to mitochondrial energy production, and thus affect spike generation or conduction in nerve fibers post-synaptic to IHCs (and by extension, post-synaptic to other neurons). The success of cochlear implants over hearing aids in some AN cases (Berlin *et al.*, 2010) may thus reflect the effectiveness of direct electrical stimulation in overcoming a metabolic insufficiency that would otherwise impede spike generation driven by IHC output. This argument is consistent with the review of putative animal models of AN by Cacace and Burkard (2009), who considered the IHC-loss carboplatin data to be a poor model for human AN, but extends it to make the case that the poor ABRs and poor speech comprehension seen in AN patients are likely to have their origin in response timing deficits intrinsic to auditory neurons.

Acknowledgments

C.D.C.-S. was supported by studentships from the Canadian Institutes of Health Research/Institutes de recherche en santé du Canada (CIHR/IRCS). R.N.D. was supported by a post-graduate scholarship from NSERC of Canada. D.P.P. is supported by research grants from NSERC of Canada. The original work was supported by the NIH-NIDCD Grant No. DC03600.

References and links

- Berlin, C. I., Hood, L. J., Morlet, T., Wilensky, D., Li, L., Mattingly, K. R., Taylor-Jeanfreau, J., Keats, B. J., John, P. S., Montgomery, E., Shalloo, J. K., Russell, B. A., and Frisch, S. A. (2010). "Multi-site diagnosis and management of 260 patients with auditory neuropathy/dys-synchrony (auditory neuropathy spectrum disorder)," *Int. J. Audiol.* **49**, 30–43.
- Berlin, C. I., Hood, L. J., Morlet, T., Wilensky, D., St. John, P., Montgomery, E., and Thibodaux, M. (2005). "Absent or elevated middle ear muscle reflexes in the presence of normal otoacoustic emissions: A universal finding in 136 cases of auditory neuropathy/dys-synchrony," *J. Am. Acad. Audiol.* **16**, 546–553.
- Burkard, R., Trautwein, P., and Salvi, R. (1997). "The effects of click level, click rate, and level of background masking noise on the inferior colliculus potential (ICP) in the normal and carboplatin-treated chinchilla," *J. Acoust. Soc. Am.* **102**, 3620–3627.
- Cacace, A., and Burkard, R. (2009). "Auditory neuropathy: Bridging the gap between basic science and current clinical concerns," in *Controversies in Central Auditory Processing Disorders*, edited by A. Cacace and D. McFarland, (Plural, San Diego), pp. 305–343.
- El-Badry, M. M., and McFadden, S. L. (2009). "Evaluation of inner hair cell and nerve fiber loss as sufficient pathologies underlying auditory neuropathy," *Hear. Res.* **255**, 84–90.
- Harrison, R. V. (1998). "An animal model of auditory neuropathy," *Ear Hear.* **19**, 355–361.
- Heil, P. (1997). "Auditory cortical onset responses revisited. I. First-spike timing," *J. Neurophysiol.* **77**, 2616–2641.
- Heil, P., and Irvine, D. R. F. (1996). "On determinants of first-spike latency in auditory cortex," *NeuroReport* **7**, 3073–3076.
- Heil, P., and Irvine, D. R. F. (1997). "First-spike timing of auditory-nerve fibers and comparison with auditory cortex," *J. Neurophysiol.* **78**, 2438–2454.
- Huang, T., Santarelli, R., and Starr, A. (2009). "Mutation of OPA1 gene causes deafness by affecting function of auditory nerve terminals," *Brain Res.* **1300**, 97–104.
- Kennedy, I. C. S., Fitzharris, B. M., Colls, B. M., and Atkinson, C. H. (1990). "Carboplatin is ototoxic," *Cancer Chemother. Pharmacol.* **26**, 232–234.

- Kraus, N., Bradlow, A. R., Cheatham, M. A., Cunningham, J., King, C. D., Koch, D. B., Nicol, T. G., McGee, T. J., Stein, L. K., and Wright, B. A. (2000). "Consequences of neural asynchrony: A case of auditory neuropathy," *J. Assoc. Res. Otolaryngol.* **1**, 33–45.
- Michalewski, H. J., Starr, A., Zeng, F. G., and Dimitrijevic, A. (2009). "N100 cortical potentials accompanying disrupted auditory nerve activity in auditory neuropathy (AN): Effects of signal intensity and continuous noise," *Clin. Neurophysiol.* **120**, 1352–1363.
- Miyamoto, R. T., Kirk, K. I., Renshaw, J., and Hussain, D. (1999). "Cochlear implantation in auditory neuropathy," *Laryngoscope* **109**, 181–185.
- Phillips, D. P., and Hall, S. E. (1990). "Response timing constraints on the cortical representation of sound time structure," *J. Acoust. Soc. Am.* **88**, 1403–1411.
- Phillips, D. P., Hall, S. E., Guo, Y., and Burkard, R. (2001). "Sensitivity of unanesthetized chinchilla auditory system to noise burst onset, and the effects of carboplatin," *Hear. Res.* **155**, 133–142.
- Santarelli, R., Starr, A., Michalewski, H. J., and Arslan, E. (2008). "Neural and receptor cochlear potentials obtained by transtympanic electrocochleography in auditory neuropathy," *Clin. Neurophysiol.* **119**, 1028–1041.
- Starr, A., Isaacson, B., Michalewski, H. J., Zeng, F. G., Kong, Y. Y., Beale, P., Paulson, G. W., Keats, B. J. B., and Lesperance, M. N. (2004). "A dominantly inherited progressive deafness affecting distal auditory nerve and hair cells," *J. Assoc. Res. Otolaryngol.* **5**, 411–426.
- Starr, A., McPherson, D., Patterson, J., Don, M., Luxford, W., Shannon, R., Sininger, Y., Tonakawa, L., and Waring, M. (1991). "Absence of both auditory evoked potentials and auditory percepts dependent on timing cues," *Brain* **114**, 1157–1180.
- Starr, A., Michalewski, H. J., Zeng, F. G., Fujikawa-Brooks, S., Linthicum, F., Kim, C. S., Winnier, D., and Keats, B. (2003). "Pathology and physiology of auditory neuropathy with a novel mutation in the MPZ gene (Tyr145 > Ser)," *Brain* **126**, 1604–1619.
- Starr, A., Picton, T. W., Sininger, Y., Hood, L. J., and Berlin, C. I. (1996). "Auditory neuropathy," *Brain* **119**, 741–753.
- Wake, M., Takeno, S., Ibrahim, D., and Harrison, R. (1994). "Selective inner hair cell ototoxicity induced by carboplatin," *Laryngoscope* **104**, 488–493.
- Wang, J., Powers, N. L., Hofstetter, P., Trautwein, P., Ding, D., and Salvi, R. (1997). "Effects of selective inner hair cell loss on auditory nerve fiber threshold, tuning and spontaneous and driven discharge rate," *Hear. Res.* **107**, 67–82.
- Zeng, F. G., Kong, Y. Y., Michalewski, H. J., and Starr, A. (2005). "Perceptual consequences of disrupted auditory nerve activity," *J. Neurophysiol.* **93**, 3050–3063.