

1 **Title:**

2 **Cochlear nucleus small cells use olivocochlear collaterals to encode sounds in noise**

3

4 Adam Hockley¹, Calvin Wu¹, Susan E Shore^{1,2,3}

5

6 ¹ Kresge Hearing Research Institute, Department of Otolaryngology, ² Department of Molecular
7 and Integrative Physiology, and ³ Department of Biomedical Engineering
8 University of Michigan, Ann Arbor, MI, 48109, U.S.A.

9

10 **Correspondence:**

11 Dr. Susan E. Shore,

12 Department of Otolaryngology, University of Michigan, 1150 West Medical Center Drive, Ann
13 Arbor, MI 48109

14 sushore@umich.edu

15

16 **Author contributions:**

17 A.H., C.W. and S.E.S. designed research; A.H. and C.W. performed research; A.H. and CW
18 analyzed data; A.H., C.W. and S.E.S. wrote the paper.

19

20 Number of Figures: 7

21 Number of Tables: 1

22 Number of words in abstract: 124

23 Number of words in introduction: 387

24 Number of words in discussion: 1164

25

26 **Conflict of Interest Statement:** The authors declare that the research was conducted in the
27 absence of any commercial or financial relationships that could be construed as a potential
28 conflict of interest.

29

30 **Acknowledgements:**

31 The authors would like to thank Mr. Dileepkumar for engineering support throughout this
32 project. This study was supported by National Institutes of Health grant R01-DC017119 (S.E.S.).

33

34 **ABSTRACT**

35 Understanding speech, especially in noisy environments, is crucial to social interactions. Yet, as
36 we age, speech processing can be disrupted by cochlear damage and the subsequent auditory
37 nerve fiber degeneration. The most vulnerable—medium and high-threshold—auditory nerve
38 fibers innervate various cell types in the cochlear nucleus, among which, the small cells are
39 unique in receiving this input exclusively. Here, we characterize small cell firing characteristics,
40 demonstrating superior temporal as well as intensity coding. We show that small-cell unique
41 coding properties are facilitated by direct cholinergic input from the medial olivocochlear
42 system. These results highlight the small cell–olivocochlear circuit as a key player in signal
43 processing in noisy environments, which may be selectively degraded in aging or after noise
44 insult.

45
46 **INTRODUCTION**

47 Speech perception in ever-changing soundscapes is key for communication. However, this
48 important task is degraded with age (Pichora-Fuller, 1997). Crucial to speech signal processing
49 are the temporal fine structures and envelope information (Shannon et al., 1995), and
50 difficulties in resolving these signals by the brain can produce speech-in-noise perceptual
51 difficulties (Bharadwaj et al., 2014). In the auditory periphery, the high threshold, low
52 spontaneous rate (SR) auditory nerve fibers (ANFs) may be particularly important as their best
53 dynamic range overlaps with normal levels of communicative signals. These specialized ANFs
54 project along with low threshold ANFs throughout the cochlear nucleus (CN). However, the
55 small-cell cap region receives exclusive input from low (and medium) SR ANFs (Liberman, 1991;
56 Ryugo, 2008).

57
58 Located between the granule-cell and magnocellular domains (Cant, 1993), the small-cell cap
59 (SCC), as the name suggests, is a region containing small cells (SCs; $<20\ \mu\text{m}$). SCs project either to
60 medial olivocochlear (MOC) neurons in the ventral nucleus of the trapezoid body (VNTB; Darrow
61 et al., 2012; De Venecia et al., 2005; Thompson & Thompson, 1991; Ye et al., 2000) or directly to
62 the medial geniculate body, the latter serving as short-latency relay to the auditory cortex
63 (Schofield, Mellott, et al., 2014; Schofield, Motts, et al., 2014). SCs, in turn, receive branched
64 collaterals from MOC neurons (Benson et al., 1996; Benson & Brown, 1990; Ryan et al., 1990),
65 whose major function is suppression of cochlear outer hair cell electromotility to alter cochlear
66 gain. MOC activation releases the cochlea from background masking by shifting its dynamic range
67 (Dolan & Nuttall, 1988; Kawase & Liberman, 1993). However, SCs remain largely unexplored.
68 Here, we hypothesize that MOC collateral projections to the CN, and especially onto SCs, may
69 counteract the effect of cochlear suppression, allowing SC firing to accurately represent stimulus
70 intensity.

71
72 In this study, we characterized SCs response properties that are consistent with its unique circuit
73 arrangement. We showed that SCs can more accurately encode intensity as well as temporal
74 information compared to other CN cell types. Further, we found both MOC activation and
75 inhibition of cholinergic input to the CN bidirectionally modulated SC firing properties. In the
76 presence of background noise, SCs maintained superior intensity coding. Taken together, these

77 findings reveal a special role for SCs in processing communicative signals and that its degradation
78 after selective auditory neuropathy may underlie speech-in-noise difficulties.

79 **RESULTS**

80 Using multichannel electrodes, we isolated 1523 single units from 83 CN electrode locations in
 81 26 guinea pigs. Unit distributions are shown in Table 1. We excluded onset units in subsequent
 82 analyses due to their low numbers.

83

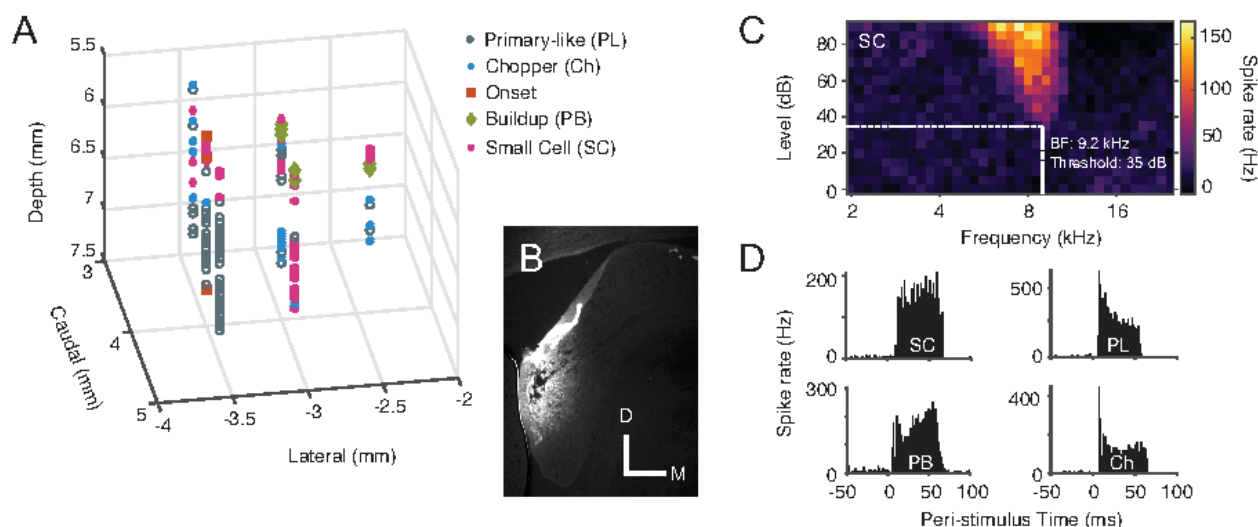
	PL	Ch	SC	On	B	Total
Control	722	247	310	57	187	1523
VNTB stimulation	260	96	139	15	75	585
Atropine infusion	20	36	34	4	8	102

84 *Table 1: Number of single CN units recorded. Cell types are Primary-like (PL), Chopper (Ch), Small*
 85 *cell (SC), Onset (On) and Buildup (B).*

86

87 Single-shank 32-channel electrodes with 50 μm spacing of electrode sites were positioned to
 88 simultaneously record units from the dorsal CN (DCN), SCC and ventral CN (VCN)(Fig 1). Units
 89 were classified by their temporal and frequency response patterns (Fig 1B)(Stabler et al., 1996;
 90 Winter & Palmer, 1995; Young et al., 1988). SCs, located dorsoventrally between DCN and VCN,
 91 displayed several unusual PSTH shapes, the most common being a ‘flat’ or bimodal PSTH shape
 92 (Fig 1B). SCs were therefore identified by a combination of their PSTH shapes and unique
 93 locations within the cochlear nucleus and by their distinct best frequency (BF) progressions with
 94 depth.

95

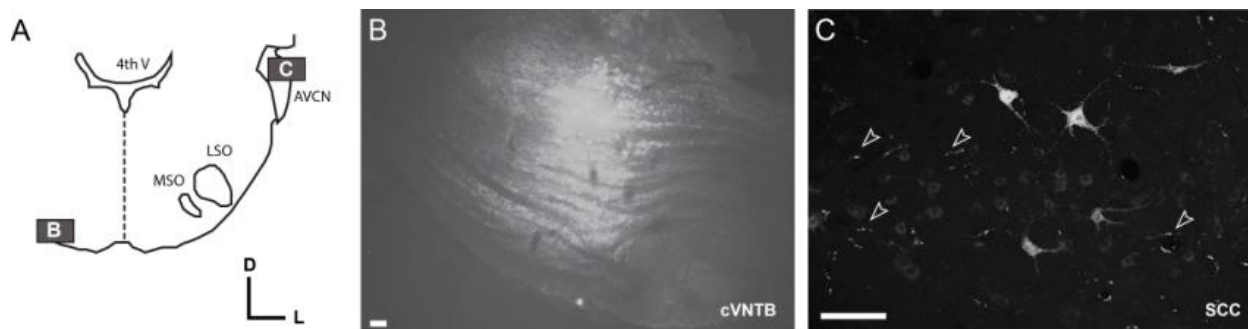


96
 97 **Fig 1: Validating recordings from CN small cells.** A) An example 3D plot of unit types within the
 98 left CN of one guinea pig. Cells exhibiting buildup PSTH shapes were found more medial and
 99 caudal, in the DCN. The VCN is more rostral and lateral, with the SCC units located between these
 100 two structures. B) Coronal section of the cochlear nucleus, containing a Fluorogold-marked
 101 electrode track in the SCC. (Bar = 0.5 mm) C) Receptive field of a CN small cell. D) Example PSTHs
 102 from 4 different CN cell types: small cell (SC) with flat, bimodal PSTH, primary-like (PL), buildup
 103 (PB) and chopper (CH).

104

105 To confirm reciprocal projections from SCs to MOC neurons in the VNTB, a tracer (FluoroEmerald)
106 was pressure-injected into the VNTB. One week later, retrograde and anterograde staining was
107 observed in the SCC (Fig 2), demonstrating, along with the physiological data, that SCC neurons
108 form a circuit with MOC neurons, confirming previous findings (Benson et al., 1996; Benson &
109 Brown, 1990; Ye et al., 2000).

110



111
112 Fig. 2: VNTB neurons project to and receive projections from the SCC. A) Schematic showing the
113 locations of photomicrographs in B (VNTB) and C (SCC). B) FluoroEmerald injection in the
114 contralateral VNTB (cVNTB). C) Anterograde labeling of puncta (arrowsheads) in SCC originating
115 in VNTB and retrograde labelling of SCC somata. Bar = 100 μ m.

116

117 **Small cell firing patterns resemble those of low/medium SR ANFs**

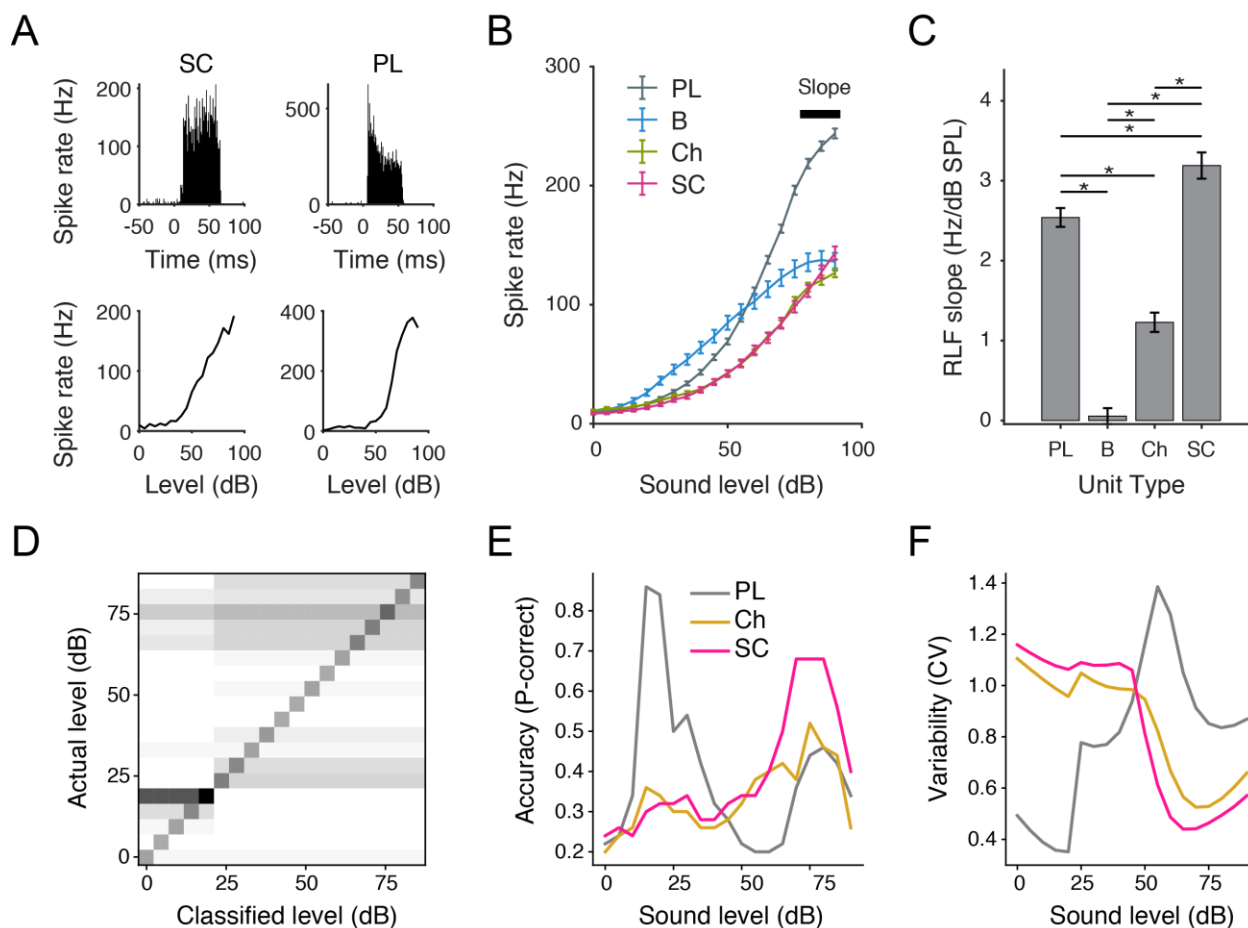
118 Afferent input to SCs is exclusively from low/med SR, high threshold ANFs, while SCs project to,
119 and receive input from MOC neurons. Due to this unique circuit configuration, we hypothesized
120 that SCs would have different patterns of intensity coding (rate-level functions) compared to
121 other CN cell types that also receive input from high SR, low threshold ANFs. SC response
122 characteristics were like their low/medium SR, high threshold ANF inputs, with low SRs, high
123 thresholds, and long first-spike latencies compared to other VCN cell types (Fig S1). SC receptive
124 fields contained inhibitory areas (Figs 1C S1D), signifying a currently unknown source of
125 inhibition. Like low SR ANFs, SCs had non-saturating rate-level functions (RLFs). Above 80 dB SPL,
126 SC RLFs slopes were significantly greater compared to all other CN cell types (Fig 3C).

127

128 To examine intensity coding at speech-relevant levels, we used a PSTH-based classifier to
129 compare the trial to trial reliability across cell types in response to different intensity stimuli
130 (Foffani & Moxon, 2004). Intensity coding fidelity is represented by accurate representations of
131 stimuli for each single-trial spiking response (Fig 3D). Primary-like units displayed better intensity
132 discrimination between 20-45 dB as well as very low variability even for misclassified trials, while
133 SCs were better at intensity coding above 60 dB (Fig 3E-F; ANCOVA $F=9.1$, $P=1.1e-4$, effect size
134 (partial eta squared) =0.006). The high-fidelity intensity coding and absence of RLF plateaus in
135 SCs is likely due to their input from low SR ANFs combined with excitatory input from MOCs at
136 high sound levels. The combined action of peripheral and central excitation would allow SCs to
137 better encode tone intensities at levels relevant for speech processing.

138

139



140
141

142 Fig 3: CN small cells show superior intensity coding at high sound levels. A) Example PSTH and
143 RLF for a CN small cell and a primary-like unit. B) Mean (\pm SEM) RLFs of 4 major CN cell types.
144 ‘Slope’ marks the region of the RLF used for slope quantification. C) Quantification of RLF slopes
145 between 80- and 90 dB SPL, calculated as the spike rate increase per dB SPL increase (mean \pm
146 SEM). Comparisons between groups were made using a Kruskal-Wallis with Tukey’s multiple
147 comparison test ($p < 0.05$). D) Confusion matrix for sound intensity classification across the
148 population (all cell types). Greyscale – white pixels: 0%, black pixels: 100%. E) Probability of
149 correct classification across unit-types. F) Trial to trial variability (coefficient of variation) of
150 classifier performance across unit-types.

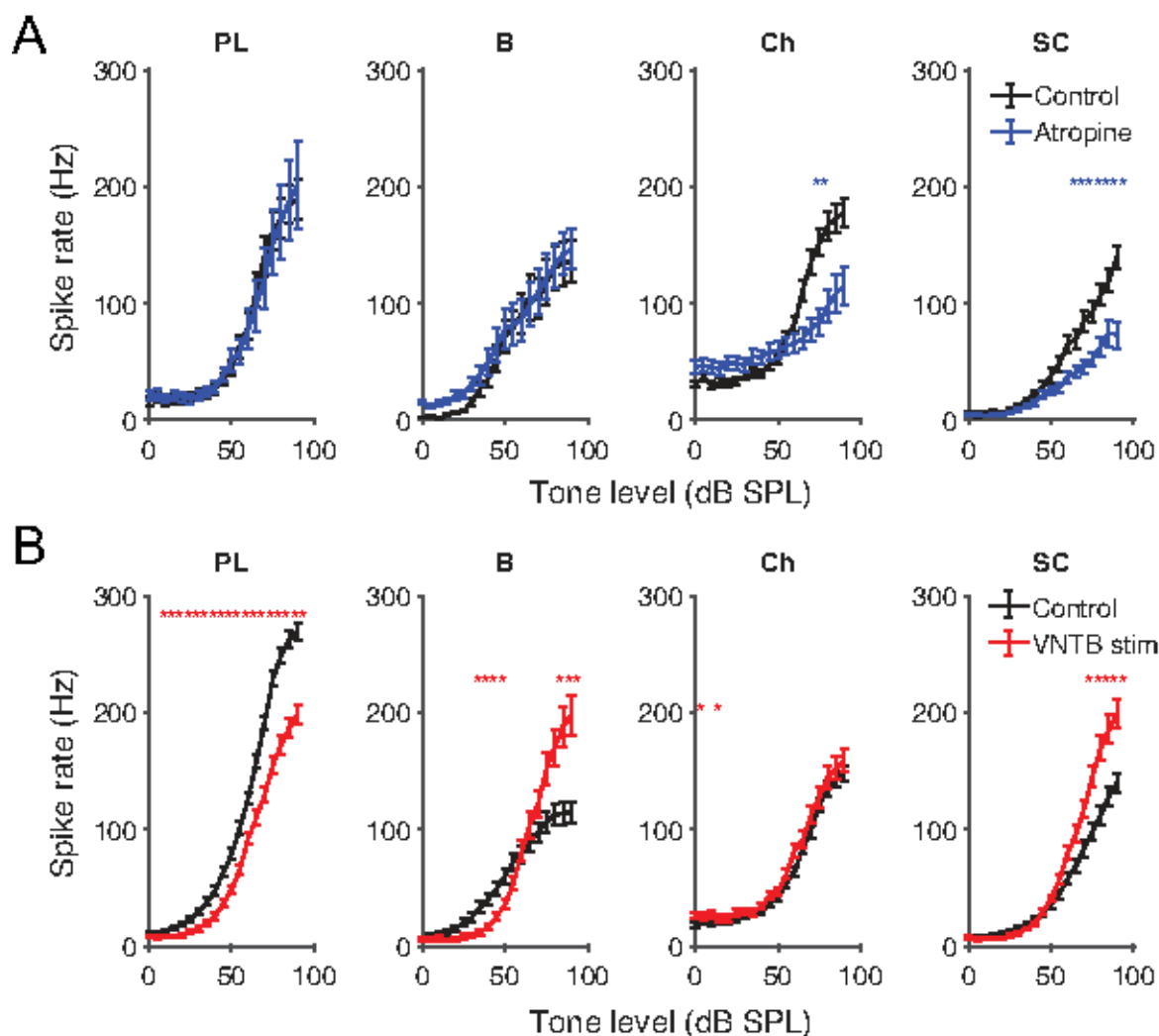
151

152 MOC neurons modulate small-cell excitability

153 Cholinergic MOC neurons send projections into the SCC (Benson et al., 1996; Benson & Brown,
154 1990; Ryan et al., 1990). Acetylcholine is known to act on CN cells through muscarinic receptors,
155 resulting in a cell-type specific excitation or modulation of neural plasticity (Fujino & Oertel, 2001;
156 Stefanescu & Shore, 2017). To investigate whether cholinergic MOC inputs mediate the steeper
157 RLF slopes in SCs, first, we applied the muscarinic acetylcholine receptor (mAChR) antagonist,
158 atropine using drug delivery probes. Atropine reduced SC tone-evoked activity above 60 dB SPL,
159 confirming a role of cholinergic input in the steepened SC RLF slopes at high tone intensities (Fig
160 4). Chopper cells, which also receive cholinergic input (Fujino & Oertel, 2001), likewise showed

161 reduced sound-evoked activity following atropine infusion, but over a narrow intensity range.
162 Atropine had no significant effect on the RLF slopes of primary-like or buildup cells. These findings
163 demonstrate that the primary action of cholinergic input on SCs and choppers is to increase
164 sound-driven responses above 60 dB, resulting in a widened dynamic range that is essential for
165 intensity coding.

166
167 Next, to determine whether the MOCs were the key cholinergic modulators of SCs, we electrically
168 stimulated MOC neuron somata in the VNTB while performing CN recordings. Successful
169 targeting of MOC cells was confirmed by the reduction of cochlear compound action potential
170 (CAP) amplitudes during electrical stimulation. MOC stimulation significantly increased SC firing
171 rates to tones above 70 dB SPL but not to lower intensity or sub threshold tones (Fig 4),
172 suggesting that cholinergic MOC input to CN small cells enhances ANF-evoked activity. As
173 expected, the overall effect of MOC activation on CN small cells was opposite to that achieved by
174 cholinergic blocking. In contrast, VNTB stimulation had no effect on chopper firing while
175 decreasing primary-like firing across the entire range of tone intensities. An unexpected finding
176 was an increase in fusiform cell firing rates with MOC stimulation but no effect by blocking
177 muscarinic receptors. The reciprocal findings of increased firing rates with MOC stimulation and
178 decreased firing rates with atropine in SCs confirm the role of MOC input to SCs in enhancing
179 sound-driven responses. Furthermore, SC excitation by MOC neurons can overcome the MOC
180 suppression of cochlear output. The failure of MOC stimulation to alter firing rates in choppers
181 suggests that the cholinergic effect observed originates from VNTB cells that are not MOCs.
182



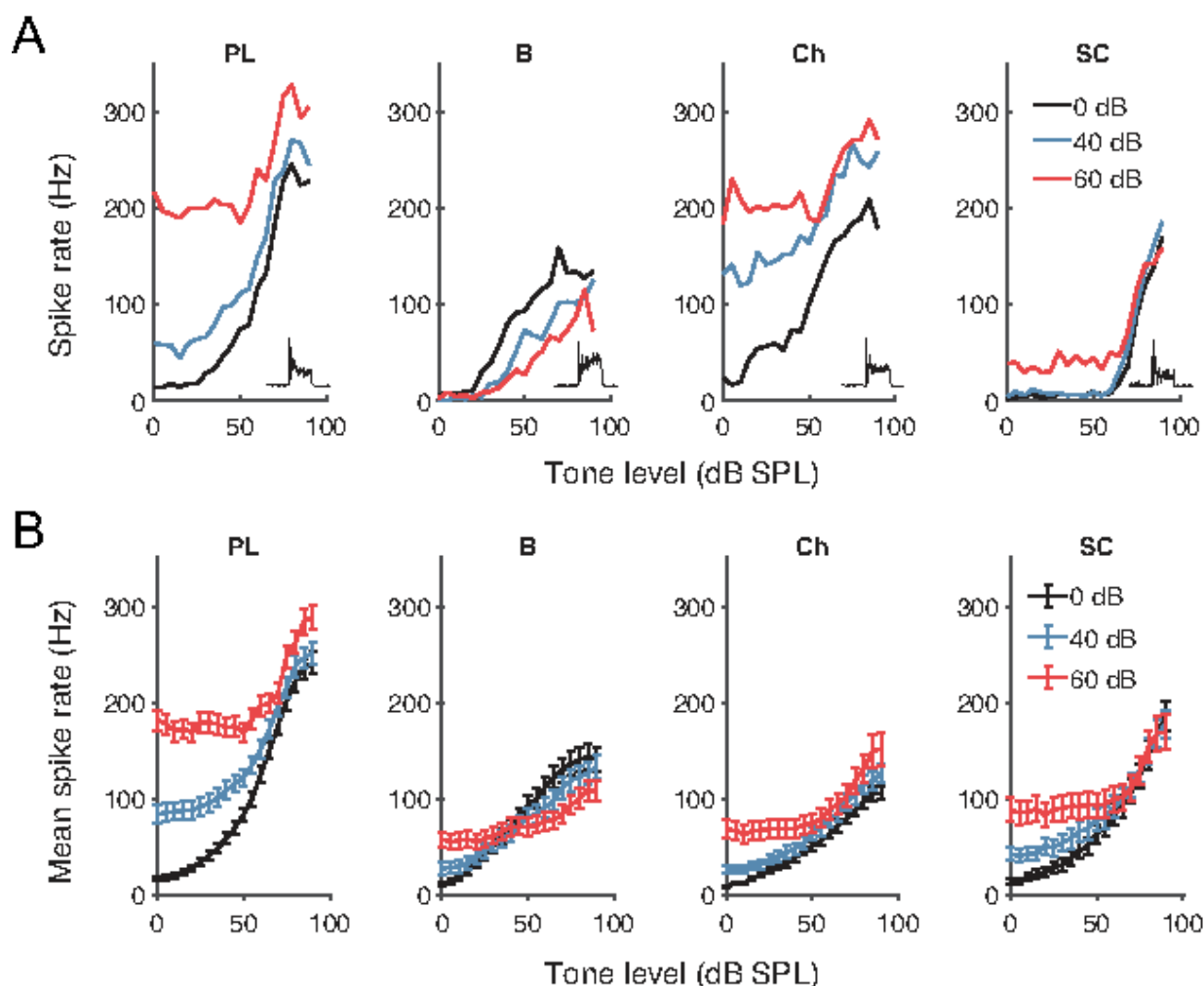
183
 184 Fig 4: CN small cells receive excitatory cholinergic input from MOCs. Top row: Blocking muscarinic
 185 cholinergic receptors with atropine suppresses CN small-cell and chopper responses to tones. Top
 186 row shows mean RLFs \pm SEM for primary-like, buildups, chopper cells and small cells before and
 187 after local application of atropine via a drug-delivery probe. Bottom row: Electrically stimulating
 188 MOC neurons increases tone-evoked responses in small cells but not choppers. Mean RLFs \pm SEM
 189 for primary-like, buildups, chopper cells and small cells are shown before and during MOC
 190 electrical stimulation. All statistical comparisons made using paired t-test with Bonferroni's
 191 multiple comparison correction ($\alpha = 0.00263$, stars signify $p < 0.05$).

192

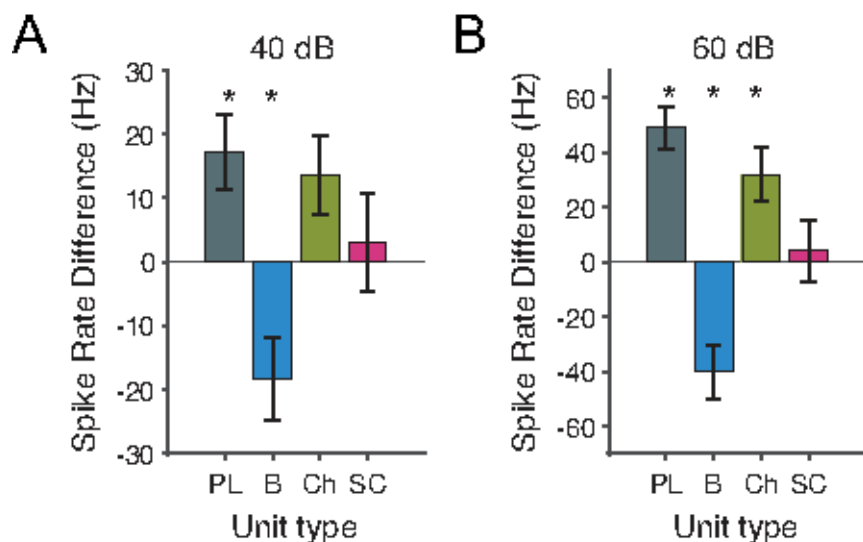
193 **Small cells use MOC input to precisely encode tones in background noise**

194 MOC projections to the cochlea suppress cochlear outer hair cell electromotility, thereby
 195 reducing cochlear amplification. A subsequent unmasking of sounds by background noise
 196 facilitates the detection of tones and speech in noise (Dolan & Nuttall, 1988; Kawase & Liberman,
 197 1993). Since SCs receive projections from MOC collaterals, we hypothesized that SCs are involved
 198 in encoding sounds in the presence of background noise. Signal-in-noise discrimination was
 199 tested in CN neurons by comparing firing rates to tones in silence with tones embedded in
 200 broadband noise.

201
202 As seen in individual and mean RLFs, SCs maintained their firing rates to tones even in the
203 presence of background noise (Fig 5). In fact, SC spike rates in response to BF tones in the
204 presence of 40- and 60 dB background noise were equivalent to their responses to tones in
205 silence (Fig 6). Chopper units also maintained their firing rates to BF tones in background noise
206 at 40, but not 60 dB SPL. In contrast, background noise degraded the responses to BF tones in
207 primary-like and buildup cells, which showed degraded firing rates in the presence of 40- and 60
208 dB SPL broadband noise. These differences were not due to thresholds as they were apparent for
209 cells of similar thresholds. Taken together, these results demonstrate that, vis-à-vis other CN cell
210 types, small cells reliably encode medium to high stimulus intensity in the presence of
211 background noise.
212



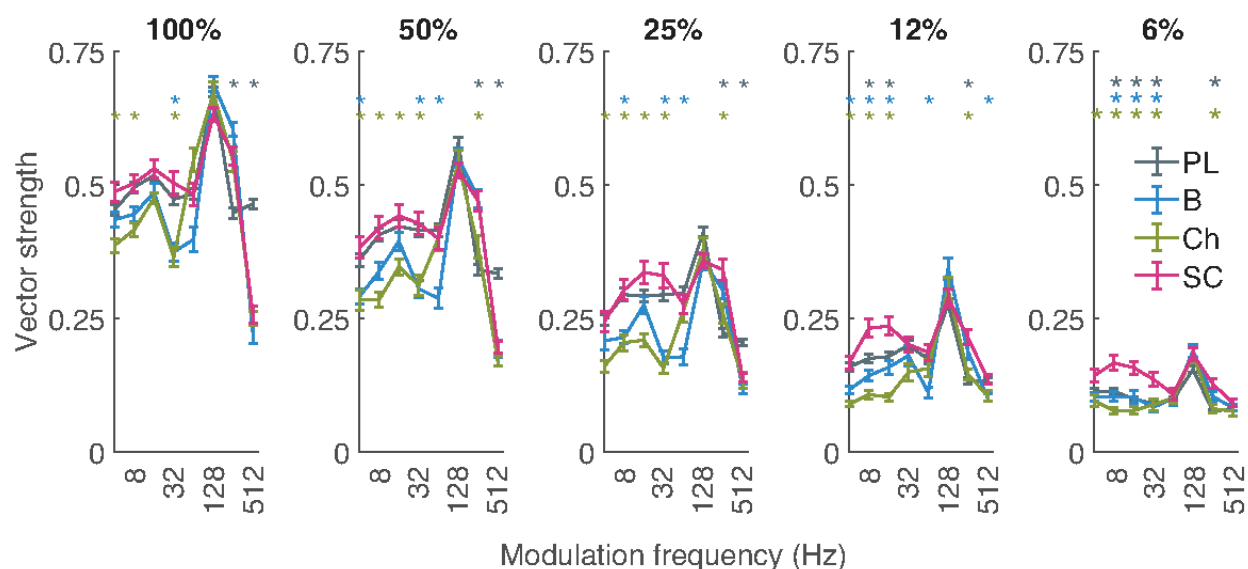
213
214 Fig 5: CN small cells maintain tone-level coding in the presence of background noise. A) Single
215 unit examples of RLFs in the presence of 0, 40- or 60 dB SPL background noise, for the 4 major
216 cell-type classifications (columns). Insets show PSTH shapes. B) Mean (\pm SEM) RLFs of 4 major CN
217 cell types in the presence of background noise.
218



219
220 Fig 6: Quantification of small cell firing rates in background broadband noise (BBN) noise
221 demonstrates maintenance of tone encoding. For a background sound level of either 40- (A) or
222 60- (B) dB SPL, the mean change in firing rate of each cell type between 60- and 90 dB SPL was
223 calculated ($\alpha = 0.0125$, $p < 0.05$; t-test with Bonferroni's correction).
224
225

226 **Small cells display precise envelope coding**

227 Tracking sound envelopes is important for decoding human speech or animal vocalizations
228 (Shannon et al., 1995). Here, we used amplitude modulated broadband noise (75 dB SPL) to
229 evaluate envelope coding in CN neurons. Figure 7 (A-C) shows an example vector strength
230 calculation for a single SC at a modulation frequency of 32 Hz and depth of 12%, (Goldberg &
231 Brown, 1969). This unit had a vector strength value of 0.43191, demonstrating accurate spike
232 timing to the amplitude modulation. Figure 7 D shows that mean vector strength of the different
233 VCN neurons peaked at a modulation frequency of 256 Hz, similar to that in previous studies
234 (Sayles et al., 2013). At low modulation frequencies and depths, SCs had significantly greater
235 vector-strength values than other VCN types (Fig 7) demonstrating superior envelope coding. The
236 enhanced coding of low-frequency amplitude modulation in CN cells may be important for
237 species-specific vocalization processing, as guinea pig vocalizations contain predominantly low
238 frequency amplitude modulation bursts (Berryman, 1976).
239



240
241 Fig 7: Small cells show more accurate amplitude-modulation coding than other CN cell types.
242 Modulation transfer functions show vector strength of spiking to sinusoidally modulated BBN at
243 5 different modulation depths (100-6%). CN small cells have significantly greater vector strength
244 of firing at modulation frequencies below 128 Hz and at lower modulation depths. Stars indicate
245 significance between CN small cells and other cell types (One-way ANOVA with Tukey's multiple
246 comparison test, $p < 0.05$).

247 248 **DISCUSSION**

249 SCs receive exclusive afferent input from high-threshold, low/med SR ANFs, positioning them to
250 encode sounds at levels well above threshold. Conversational speech (around 60 dB SPL) is
251 encoded by temporal cues within amplitude modulated signals. Here, we showed that SCs
252 encode both intensity and amplitude modulation with higher precision than other CN cell types.
253 For tones in background noise, SCs were the only CN cell type that maintained coding above 60
254 dB SPL. The unique properties of SCs are likely mediated by cholinergic, MOC input to SCs, which
255 provide excitation to SCs capable of overcoming the suppressive effect of MOCs on the cochlea,
256 thereby increasing their suprathreshold firing rates.

257
258 SCs exhibited low SRs, high thresholds and steep, unsaturating (up to 90 dB SPL) rate-intensity
259 slopes. These characteristics reflect the firing properties of the low/medium SR ANFs that
260 innervate SCs and are similar to those identified in cells in the marginal region of the cat CN
261 (Ghoshal & Kim, 1996, 1997). Those early studies also showed flat, bimodal or Unusual PSTH
262 shapes, signifying a heterogenous population, similar to that shown here for guinea-pig CN SCs.
263 However, in the present study, the difference between SC thresholds and other VCN cell types
264 (~5 dB) was less than the threshold difference between low/medium and high SR ANFs (~20 dB;
265 Liberman, 1978; Winter and Palmer, 1991), putatively due to the excitatory MOC cholinergic
266 input to SCs demonstrated here. Blocking muscarinic, cholinergic receptors with atropine
267 reduced SC spike rates while MOC stimulation, confirmed by CAP reductions, increased their
268 firing rates. Both of these effects are consistent with the presence of excitatory cholinergic MOC
269 input to the SCC. The greatest effect of blocking muscarinic cholinergic receptors was observed

270 during suprathreshold sound stimulation, which would be expected to also activate the sound-
271 driven, cholinergic projections from MOC collaterals to the SCC (Lee et al., 2006).

272
273 MOC collaterals to the CN can influence other cell types (Baashar et al., 2019; Mulders et al.,
274 2003, 2007) including T-stellate cells (planar multipolar cells with chopper responses) and D-
275 stellate cells (radiate multipolar cells with onset chopper responses). Previous studies showed an
276 increased firing rate in 94% of T-stellate cells following application of carbachol (AChR agonist) in
277 vitro (Fujino & Oertel, 2001). However, Baashar et al. (2019) used tract tracing and double
278 labelling to view contacts from MOC collaterals onto T- and D-stellate cells and demonstrated
279 that MOC input to these CN cells had a weaker innervation pattern than would be expected based
280 on the early electrophysiological studies. Some studies have suggested that there is inhibitory
281 MOC projection to the CN (Mulders et al., 2002), however the present study found no evidence
282 for inhibitory MOC or cholinergic effect on CN small cells or choppers. One possible reason for
283 this discrepancy is the more targeted stimulation of contralateral MOC neurons in the present
284 study, compared to midline stimulation, (Mulders et al., 2002), which would activate all crossed
285 olivocochlear fibers including the lateral olivocochlear system.

286
287 The present data reveal strong, excitatory MOC-collateral innervation of SCs in the SCC but weak
288 input to T-stellate cells. SC receptive fields in the present study had inhibitory sidebands,
289 revealing a currently unknown source of inhibition. This is likely to be the glycinergic, D-stellate
290 cells, which provide wideband inhibition to several cell types in the CN (Arnott et al., 2004; Oertel
291 et al., 1990). However, further studies are required to confirm this pathway. An alternative
292 source of inhibition is the L-stellate cell, which also responds to carbachol, suggesting cholinergic
293 input, and thus potentially of olivocochlear origin (Ngodup et al., 2020). However, prediction of
294 sources based on bath infusion of cholinergic modulators must be made with caution, due to
295 multiple sources of cholinergic modulation to the CN (Mellott et al., 2011).

296
297 We demonstrated that SCs more accurately encode tones-in-noise compared to other CN cell
298 types, putatively due to their restricted ANF inputs and unique input from MOC collaterals.
299 Activating the MOC pathway to the cochlea suppresses the cochlear amplifier, benefitting
300 speech-in-noise processing (de Boer et al., 2012; Winslow & Sachs, 1987). The function of MOC
301 collaterals to the SCC may be to further aid speech coding. SCs have the characteristics necessary
302 for superior suprathreshold sound encoding and accurately encode stimulus intensity (Figs 3&6).
303 Pre-emptive MOC-pathway enhancement protects the cochlea from synaptopathic acoustic
304 trauma (Boero et al., 2018), but whether increased MOC activation following synaptopathic noise
305 exposure could reverse speech-in-noise deficits is still unknown. Conversely, in humans with
306 tinnitus or hyperacusis, an increased contralateral suppression of otoacoustic emissions
307 (Knudson et al., 2014) suggests an over-responsive MOC system in this pathology. MOC neurons
308 are modulated by descending input from the inferior colliculus (Malmierca et al., 1996; Robertson
309 & Mulders, 2000; Schofield & Cant, 1999), auditory cortex (Coomes & Schofield, 2004; Mulders
310 & Robertson, 2000), and local inhibitory interneurons of the medial nucleus of the trapezoid body
311 (Torres Cadenas et al., 2020). Descending modulation may be a route for auditory attention to
312 affect peripheral processing, which may explain attentional facilitation of speech-in-noise
313 understanding (de Boer et al., 2012). Descending modulation of MOC activity would also affect

314 MOC collateral input to CN SCs, which accurately encode intensity, providing an additional action
315 pathway for auditory attention. The SCC in humans occupies a larger proportion of the CN than
316 in rodents, and is therefore poised to play a major role in central mechanisms of speech
317 perception (Moore & Osen, 1979).

318
319 Hearing damage in the absence of threshold shifts preferentially affects the higher threshold
320 ANFs (Furman et al., 2013) that provide the sole afferent input to SCs. Cochlear synaptopathy is
321 a potential major health issue in humans, as human temporal bones demonstrate widespread
322 synaptic damage (Viana et al., 2015). One downstream effect of cochlear synaptopathy may be
323 impaired speech perception, especially in the presence of background noise. This widespread
324 complaint has been attributed to cochlear synaptopathy, but this relationship is still unclear
325 (Plack et al., 2014). Human studies suggest that such perceptual deficits reflect difficulties in
326 coding temporal envelopes (Bharadwaj et al., 2014), expected to impair speech recognition
327 (Shannon et al., 1995). Temporal-coding deficits after synaptopathy are not apparent in ANFs
328 (Heeringa et al., 2020), suggesting their origin may be central. The enhanced intensity coding and
329 precise envelope coding shown here for SCs primes the SCC as an important subcortical area for
330 speech processing is expected to be preferentially targeted by cochlear synaptopathy. In humans,
331 the SCC occupies a large proportion of the CN and is therefore poised to play a major role in
332 central mechanisms of auditory neuropathy (Moore & Osen, 1979). Speech coding in human SCs
333 may be impaired following cochlear synaptopathy, and modulation of CN activity via the MOC
334 pathway may be a potential treatment for the associated speech-in-noise deficits.

335

336 **MATERIALS AND METHODS**

337 All animal experimental procedures were performed in accordance with the protocols
338 established by the National Institutes of Health (Publication 80-23) and approved by the
339 University Committee on Use and Care of Animals at the University of Michigan. Male and female
340 ($n = 11$ and 15 , respectively) pigmented guinea pigs weighing 280-800g obtained from Elm Hill
341 Labs were used. No differences in unit responses for animal's sex or age was found. Guinea pigs
342 were dual-housed on a 12/12 h light-dark cycle, with food and water readily available. Auditory
343 brainstem responses (ABRs) were used to confirm normal auditory thresholds of ≤ 20 dB SPL at 4-
344 20kHz in all animals. ABRs (0–90 dB SPL tone bursts; 5 ms duration, 1 ms rise/fall times, 21 Hz
345 presentation rate, 512 repetitions in 10 dB steps; Tucker-Davis Technologies RZ6) were recorded
346 at 4, 8, 12, 16 and 20 kHz.

347

348 **Surgery**

349 Guinea pigs were initially anaesthetised with ketamine (50 mg kg^{-1} ; Hospira Inc., Lake Forrest, IL,
350 USA) and xylazine (5 mg kg^{-1} ; Akorn Inc.; Lake Forrest, IL, USA). Anaesthetic depth was maintained
351 using 10 mg kg^{-1} ketamine and 1 mg kg^{-1} xylazine supplements. Atropine (0.05 mg kg^{-1}) was
352 administered during the initial surgery to reduce bronchial secretions. Animals were placed in a
353 stereotaxic frame (Kopf; Tujunga, U.S.A.) within a sound-attenuating and electrically shielded
354 double-walled chamber. The skull was exposed, and a midline incision made, temporalis muscle
355 removed, and a craniotomy performed to allow access to the left CN. The dura mater was
356 removed and the exposed brain surface was kept moist by regular applications of saline.

357

358 **Sound presentation**

359 Auditory stimuli were delivered monaurally via a closed-field, calibrated system (modified DT770
360 drivers; Beyerdynamic Heilbronn, Germany) coupled to hollow ear bars. The speakers were
361 driven by a Tucker-Davis Technologies (TDT; Alachua, FL, USA) System 3 (RZ6, PA5 & HB7),
362 controlled by TDT Synapse and custom MATLAB 2021a (Mathworks; Natick, U.S.A) software.

363

364 **Neural recordings**

365 Multi-channel recording probes (NeuroNexus; Ann Arbor, MI, USA) were advanced
366 stereotaxically through the cerebellum towards the left CN using an MP-285 microdrive (Sutter
367 Instruments; Novato, CA, USA). Signals were amplified by a TDT PZ5 preamp connected to a TDT
368 RZ2 processor for filtering (0.3 – 5 kHz), and data was collected using Synapse software. Spikes
369 were detected on-line with threshold set at 4 standard deviations from mean background noise,
370 then spike-sorted post-hoc using a PCA approach. Electrodes were positioned so that shanks
371 spanned the DCN, SCC and AVCN. In 2 animals, electrode tracts were marked to confirm SCC
372 positioning. Units were characterized by responses to tone-burst and BBN signals, also used to
373 create receptive fields, PSTHs and RLFs (Ghoshal & Kim, 1997; Palmer, 1987; Stabler et al., 1996;
374 Winter & Palmer, 1995), and divided into 4 categories; PL (bushy cells), Ch (T-stellates), SC (small
375 cells) and B (buildups). Poorly driven units were discarded.

376

377 **Rate-level function and intensity coding analyses**

378

379 Receptive field stimuli consisted of randomized tonebursts (50 ms duration, 200 ms ISI, 0.1 octave
380 spacing, 5 dB steps, 15 repeats, randomized). From these, threshold and BF were obtained from
381 the lowest sound level to produce firing >2 standard deviations above spontaneous firing rates.
382 RLFs at BF were then used for further analyses. Total inhibitory area of the receptive field was
383 calculated as the number of squares of the receptive field where firing rate was <2 standard
384 deviations below spontaneous firing rates.

385
386 For intensity coding, PSTH-based stimulus classification was performed using methods described
387 in Foffani & Moxon (2004). Briefly, spike responses (serialized PSTH; 1 ms bins concatenated
388 across units) of the neural population generated in each trial (50 trials total) were compared to
389 the “template,” or average PSTH, of a given stimulus intensity. Euclidean distances between each
390 trial and the templates (19 total: 0 to 90 dB with 5 dB steps) were computed and the template
391 with the shortest distance was then assigned as “classified intensity.” Classification accuracy was
392 defined as the percentage of trials that were classified within <10 dB of the actual intensity, or
393 “hits.” Coefficient of variation for classification result across all 50 trials was calculated as
394 $\text{std}(x^2)/\text{mean}(x^2)$, where x counts the discretized step away from the “hit.”

395

396 **Drug delivery**

397 Local drug delivery of the muscarinic acetylcholine receptor antagonist, atropine was achieved
398 using a NeuroNexus D16 puffer-probe. The probe was connected to a 100 μl syringe containing
399 an 80 μM solution. The syringe was fixed to a digital syringe driver (UMP3 microsyringe injector
400 with Micro4 controller; World Precision Instruments; Sarasota, FL). This system allows
401 simultaneous drug delivery and neural recordings as described and validated in previous studies
402 (Rohatgi et al., 2009; Stefanescu & Shore, 2015, 2017). After assessing baseline CN neuronal
403 activity, 2 μl of solution was delivered at a rate of 100 nl/min, as in previous studies (Rohatgi et
404 al., 2009; Stefanescu & Shore, 2015, 2017).

405

406 **Round window recordings**

407 Cochlear compound action potential (CAP) recordings were performed using an insulated silver-
408 wire ball inserted through the bulla to rest on the cochlear round window. The trailing wire was
409 sealed to the skull, with a small hole left open for fluid wicking. CAPs were recorded in response
410 to 10 ms tones with rise/fall times of 2 ms. Amplitudes were calculated as P_1-N_1 of the mean
411 CAP waveform (200 repeats).

412

413 **Electrical stimulation**

414 MOC neurons in the VNTB were electrically stimulated with biphasic current pulses (100 ms
415 duration, 0.1 ms pulse width, 100 Hz, 500-1000 μA ; 5/sec) applied through concentric bipolar
416 electrodes (CBCEF75; FHC; Bowdoin, ME). CAPs were recorded to verify MOC neuron location (16
417 kHz, 70 dB SPL tone). CAP amplitudes were compared at baseline and 5 ms after the cessation of
418 electrical stimulation. A CAP-amplitude reduction of >30% confirmed accurate MOC locations in
419 the VNTB for stimulation. Current levels were titrated to ensure maximal MOC activation with no
420 elicited muscular contractions.

421

422 **Imaging**

423 In a separate guinea pig, a tract tracing experiment was performed to confirm VNTB projections
424 to and from SCC. FluroEmerald (10%; 0.5 μ l, Thermo Fisher Scientific, Waltham, MA, USA) was
425 pressure-injected (0.1 μ l min⁻¹) into the contralateral VNTB (1 mm anterior to the interaural line,
426 1.5 mm lateral to midline, 12 mm from the dural surface). The animal was anaesthetised with
427 ketamine-xylazine mixture in the same manner as described for electrophysiology experiments
428 but allowed to recover and maintained in the vivarium for 5 days before transcardial perfusion
429 (100 mL 1 \times PBS, 400 mL 4% paraformaldehyde) after euthanasia. The brain was extracted, post-
430 fixed for 2 h, immersed in 30% sucrose solution for 2 days, frozen and cryosectioned at 50 μ m in
431 the coronal plane (Leica, CM3050S). Coverslipped brain sections were examined under confocal
432 epifluorescence (PMT; Leica, SP5-x).

433

434 **Experimental design and statistical analysis**

435 SC sound evoked responses were compared to other CN cell types. Changes in these responses
436 during MOC modulation were also analysed. Statistical tests, including Kruskal-Wallis test,
437 ANCOVA, and two-way ANOVA were used to compare between cell-types or conditions (α =
438 0.05). Post hoc analyses were performed using Tukey's multiple comparison test.

439

440 **REFERENCES**

- 441 Arnott, R. H., Wallace, M. N., Shackleton, T. M., & Palmer, A. R. (2004). Onset Neurones in the
442 Anteroventral Cochlear Nucleus Project to the Dorsal Cochlear Nucleus. *Journal of the*
443 *Association for Research in Otolaryngology*, 5(2), 153–170.
- 444 Baashar, A., Robertson, D., Yates, N. J., & Mulders, W. H. A. M. (2019). Targets of Olivocochlear
445 Collaterals in Cochlear Nucleus of Rat and Guinea Pig. *Journal of Comparative Neurology*,
446 527(March), 2273–2290.
- 447 Benson, T. E., Berglund, A. M., & Brown, M. C. (1996). Synaptic input to cochlear nucleus
448 dendrites that receive medial olivocochlear synapses. *Journal of Comparative Neurology*,
449 365(1), 27–41.
- 450 Benson, T. E., & Brown, M. C. (1990). Synapses formed by olivocochlear axon branches in the
451 mouse cochlear nucleus. *The Journal of Comparative Neurology*, 295(1), 52–70.
- 452 Berryman, J. C. (1976). Guinea-pig Vocalizations: Their Structure, Causation and Function.
453 *Zeitschrift Für Tierpsychologie*, 41(1), 80–106. [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0310.1976.tb00471.x)
454 [0310.1976.tb00471.x](https://doi.org/10.1111/j.1439-0310.1976.tb00471.x)
- 455 Bharadwaj, H. M., Verhulst, S., Shaheen, L., Liberman, M. C., Shinn-Cunningham, B. G., Peelle, J.
456 E., Léger, A. C., & Elisabeth Gockel, H. (2014). Cochlear neuropathy and the coding of
457 supra-threshold sound. *Frontiers in Systems Neuroscience*, 8(26).
- 458 Boero, L. E., Castagna, V. C., Di Guilmi, M. N., Goutman, J. D., Elgoyhen, A. B., & Gómez-Casati,
459 M. E. (2018). Enhancement of the Medial Olivocochlear System Prevents Hidden Hearing
460 Loss. *Journal of Neuroscience*, 38(34), 7440–7451.
- 461 Cant, N. B. (1993). The Synaptic Organization of the Ventral Cochlear Nucleus of the Cat: The
462 Peripheral Cap of Small Cells. In *The Mammalian Cochlear Nuclei* (pp. 91–105). Springer
463 US.
- 464 Coomes, D. L., & Schofield, B. R. (2004). Projections from the auditory cortex to the superior
465 olivary complex in guinea pigs. *European Journal of Neuroscience*, 19(8), 2188–2200.
466 <https://doi.org/10.1111/j.0953-816X.2004.03317.x>
- 467 Darrow, K. N., Benson, T. E., & Brown, M. C. (2012). Planar multipolar cells in the cochlear
468 nucleus project to medial olivocochlear neurons in mouse. *The Journal of Comparative*
469 *Neurology*, 520(7), 1365–1375.
- 470 de Boer, J., Thornton, A. R. D., & Krumbholz, K. (2012). What is the role of the medial
471 olivocochlear system in speech-in-noise processing? *Journal of Neurophysiology*, 107(5),
472 1301–1312.
- 473 De Venecia, R. K., Liberman, M. C., Guinan, J. J., & Brown, M. C. (2005). Medial olivocochlear
474 reflex interneurons are located in the posteroventral cochlear nucleus: A kainic acid lesion
475 study in guinea pigs. *Journal of Comparative Neurology*, 487(4), 345–360.
- 476 Dolan, D. F., & Nuttall, A. L. (1988). Masked cochlear whole-nerve response intensity functions
477 altered by electrical stimulation of the crossed olivocochlear bundle. *J. Acoust. Soc. Am*,
478 83(3), 1081–1086.
- 479 Foffani, G., & Moxon, K. A. (2004). PSTH-based classification of sensory stimuli using ensembles
480 of single neurons. *Journal of Neuroscience Methods*, 135(1–2), 107–120.
481 <https://doi.org/10.1016/j.jneumeth.2003.12.011>
- 482 Fujino, K., & Oertel, D. (2001). Cholinergic modulation of stellate cells in the mammalian ventral

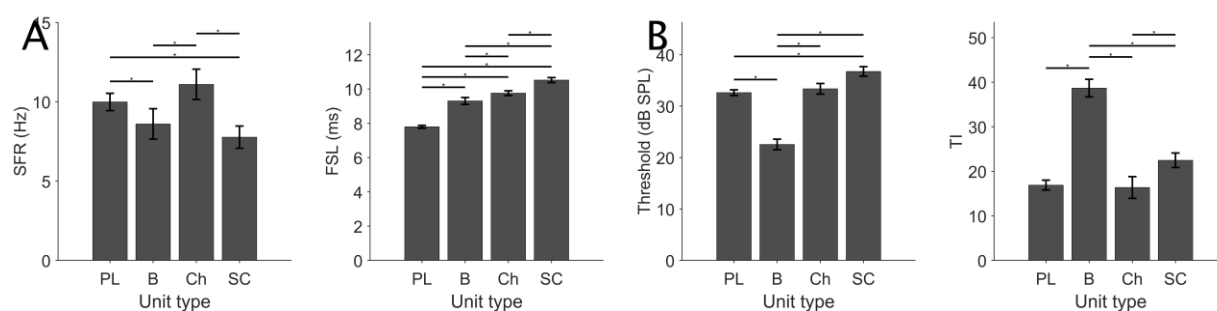
- 483 cochlear nucleus. *Journal of Neuroscience*, 21(18), 7372–7383.
- 484 Furman, A. C., Kujawa, S. G., & Liberman, M. C. (2013). Noise-induced cochlear neuropathy is
485 selective for fibers with low spontaneous rates. *Journal of Neurophysiology*, 110(3), 577–
486 586.
- 487 Ghoshal, S., & Kim, D. O. (1996). Marginal shell of the anteroventral cochlear nucleus: intensity
488 coding in single units of the unanesthetized, decerebrate cat. *Neuroscience Letters*, 205,
489 71–74.
- 490 Ghoshal, S., & Kim, D. O. (1997). Marginal Shell of the Anteroventral Cochlear Nucleus: Single-
491 Unit Response Properties in the Unanesthetized Decerebrate Cat. *J Neurophysiol*, 77(4),
492 2083–2097.
- 493 Goldberg, J. M., & Brown, P. B. (1969). Response of Binaural Neurons of Dog Superior Olivary
494 Complex to Dichotic Tonal Stimuli: Some Physiological Mechanisms of Sound Localization.
495 *American Physiological Society*, 32(4), 613–636.
- 496 Heeringa, A. N., Zhang, L., Ashida, G., Beutelmann, R., Steenken, F., & Köppl, C. (2020).
497 Temporal Coding of Single Auditory Nerve Fibers Is Not Degraded in Aging Gerbils. *The*
498 *Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 40(2), 343–
499 354.
- 500 Kawase, T., & Liberman, M. C. (1993). Antimasking Effects of the Olivocochlear Reflex. I.
501 Enhancement of Compound Action Potentials to Masked Tones. *Journal of Physiology*,
502 70(6).
- 503 Knudson, I. M., Shera, C. A., & Melcher, J. R. (2014). Increased contralateral suppression of
504 otoacoustic emissions indicates a hyperresponsive medial olivocochlear system in humans
505 with tinnitus and hyperacusis. *Journal of Neurophysiology*, 112(12), 3197–3208.
- 506 Kujawa, S. G., Glattke, T. J., Fallon, M., & Bobbin, R. P. (1993). Contralateral sound suppresses
507 distortion product otoacoustic emissions through cholinergic mechanisms. *Hearing*
508 *Research*, 68(1), 97–106. [https://doi.org/10.1016/0378-5955\(93\)90068-C](https://doi.org/10.1016/0378-5955(93)90068-C)
- 509 Lee, D. J., De Venecia, R. K., Guinan, J. J., & Brown, M. C. (2006). Central auditory pathways
510 mediating the rat middle ear muscle reflexes. *Anatomical Record - Part A Discoveries in*
511 *Molecular, Cellular, and Evolutionary Biology*, 288(4), 358–369.
512 <https://doi.org/10.1002/ar.a.20296>
- 513 Liberman, M. C. (1978). Auditory-nerve response from cats raised in a low-noise chamber. *The*
514 *Journal of the Acoustical Society of America*, 63, 442.
- 515 Liberman, M. C. (1991). Central projections of auditory-nerve fibers of differing spontaneous
516 rate. I. Anteroventral cochlear nucleus. *Journal of Comparative Neurology*, 313(2), 240–
517 258.
- 518 Malmierca, M. S., Le Beau, F. E. N., & Rees, A. (1996). The topographical organization of
519 descending projections from the central nucleus of the inferior colliculus in guinea pig.
520 *Hearing Research*, 93(1–2), 167–180.
- 521 Mellott, J. G., Motts, S. D., & Schofield, B. R. (2011). Multiple origins of cholinergic innervation
522 of the cochlear nucleus. *Neuroscience*, 180, 138–147.
- 523 Moore, J. K., & Osen, K. K. (1979). The cochlear nuclei in man. *American Journal of Anatomy*,
524 154(3), 393–417.
- 525 Mulders, W. H. A. M., Harvey, A. R., & Robertson, D. (2007). Electrically Evoked Responses in
526 Onset Chopper Neurons in Guinea Pig Cochlear Nucleus. *Journal of Neurophysiology*, 97(5),

- 527 3288–3297.
- 528 Mulders, W. H. A. M., Paolini, A. G., Needham, K., & Robertson, D. (2003). Olivocochlear
529 collaterals evoke excitatory effects in onset neurones of the rat cochlear nucleus. *Hearing*
530 *Research*, 176(1–2), 113–121.
- 531 Mulders, W. H. A. M., & Robertson, D. (2000). Evidence for direct cortical innervation of medial
532 olivocochlear neurones in rats. *Hearing Research*, 144(1–2), 65–72.
533 [https://doi.org/10.1016/S0378-5955\(00\)00046-0](https://doi.org/10.1016/S0378-5955(00)00046-0)
- 534 Mulders, W. H. A. M., Winter, I. M., & Robertson, D. (2002). Dual action of olivocochlear
535 collaterals in the guinea pig cochlear nucleus. *Hearing Research*, 174(1–2), 264–280.
536 [https://doi.org/10.1016/S0378-5955\(02\)00701-3](https://doi.org/10.1016/S0378-5955(02)00701-3)
- 537 Ngodup, T., Romero, G. E., & Trussell, L. O. (2020). Identification of an inhibitory neuron
538 subtype, the I-stellate cell of the cochlear nucleus. *ELife*, 9, 1–40.
539 <https://doi.org/10.7554/eLife.54350>
- 540 Oertel, D., Wu, S. H., Garb, M. W., & Dizack, C. (1990). Morphology and physiology of cells in
541 slice preparations of the posteroventral cochlear nucleus of mice. *Journal of Comparative*
542 *Neurology*, 295(1), 136–154.
- 543 Palmer, A. R. (1987). Physiology of the cochlear nerve and cochlear nucleus. *Br Med Bull*, 43(4),
544 838–855.
- 545 Pichora-Fuller, M. K. (1997). Language comprehension in older listeners. *Journal of Speech-*
546 *Language Pathology and Audiology*, 21(2), 125–142. [https://psycnet.apa.org/record/1997-](https://psycnet.apa.org/record/1997-05664-003)
547 [05664-003](https://psycnet.apa.org/record/1997-05664-003)
- 548 Plack, C. J., Barker, D., & Prendergast, G. (2014). Perceptual Consequences of “Hidden” Hearing
549 Loss. *Trends in Hearing*, 18, 1–11.
- 550 Robertson, D., & Mulders, W. H. A. M. (2000). Distribution and possible functional roles of some
551 neuroactive peptides in the mammalian superior olivary complex. *Microscopy Research*
552 *and Technique*, 51(4), 307–317.
- 553 Rohatgi, P., Langhals, N. B., Kipke, D. R., & Patil, P. G. (2009). In vivo performance of a
554 microelectrode neural probe with integrated drug delivery. *Neurosurg Focus*, 27(1), 8.
- 555 Ryan, A. F., Keithley, E. M., Wang, Z. -X, & Schwartz, I. R. (1990). Collaterals from lateral and
556 medial olivocochlear efferent neurons innervate different regions of the cochlear nucleus
557 and adjacent brainstem. *Journal of Comparative Neurology*, 300(4), 572–582.
- 558 Ryugo, D. K. (2008). Projections of low spontaneous rate, high threshold auditory nerve fibers
559 to the small cell cap of the cochlear nucleus in cats. *Neuroscience*, 154(1), 114–126.
- 560 Sayles, M., Füllgrabe, C., & Winter, I. M. (2013). Neurometric amplitude-modulation detection
561 threshold in the guinea-pig ventral cochlear nucleus. *The Journal of Physiology*
562 *Neuroscience C*, 591(13), 3401–3419.
- 563 Schofield, B. R., & Cant, N. B. (1999). Descending auditory pathways: Projections from the
564 inferior colliculus contact superior olivary cells that project bilaterally to the cochlear
565 nuclei. *Journal of Comparative Neurology*, 409(2), 210–223.
- 566 Schofield, B. R., Mellott, J. G., & Motts, S. D. (2014). Subcollicular projections to the auditory
567 thalamus and collateral projections to the inferior colliculus. *Frontiers in Neuroanatomy*,
568 8(70), 1–16.
- 569 Schofield, B. R., Motts, S. D., Mellott, J. G., & Foster, N. L. (2014). Projections from the dorsal
570 and ventral cochlear nuclei to the medial geniculate body. *Frontiers in Neuroanatomy*,

- 571 8(10), 1–12.
- 572 Shannon, R. V, Zeng, F., Kamath, V., Wygonski, J., & Ekelid, M. (1995). Speech Recognition with
573 Primarily Temporal Cues. *Science*, 270(5234), 303–304.
- 574 Stabler, S. E., Palmer, A. R., & Winter, I. M. (1996). Temporal and mean rate discharge patterns
575 of single units in the dorsal cochlear nucleus of the anesthetized guinea pig. *Journal of*
576 *Neurophysiology*, 76(3), 1667–1688.
- 577 Stefanescu, R. A., & Shore, S. E. (2015). NMDA receptors mediate stimulus-timing-dependent
578 plasticity and neural synchrony in the dorsal cochlear nucleus. *Frontiers in Neural Circuits*,
579 9(November), 1–14.
- 580 Stefanescu, R. A., & Shore, S. E. (2017). Muscarinic acetylcholine receptors control baseline
581 activity and hebbian stimulus timing-dependent plasticity in fusiform cells of the dorsal
582 cochlear nucleus. *Journal of Neurophysiology*, 117(3), 1229–1238.
- 583 Thompson, A. M., & Thompson, G. C. (1991). Posteroventral cochlear nucleus projections to
584 olivocochlear neurons. *The Journal of Comparative Neurology*, 303(2), 267–285.
- 585 Torres Cadenas, L., Matthew, X., Fischl, J., & Weisz, C. J. C. (2020). Synaptic Inhibition of Medial
586 Olivocochlear Efferent Neurons by Neurons of the Medial Nucleus of the Trapezoid Body.
587 *The Journal of Neuroscience*, 40(3), 509–525.
- 588 Viana, L. M., O, J. T., Burgess, B. J., Jones, D. D., ACP Oliveira, C., Santos, F., Merchant, S. N.,
589 Liberman, L. D., & Charles Liberman, M. (2015). Cochlear neuropathy in human
590 presbycusis: confocal analysis of hidden hearing loss in post-mortem tissue HHS Public
591 Access. *Hear Res*, 327, 78–88. <https://doi.org/10.1016/j.heares.2015.04.014>
- 592 Winslow, R. L., & Sachs, M. B. (1987). Effect of Electrical Stimulation of the Crossed
593 Olivocochlear Bundle on Auditory Nerve Response to Tones in Noise. *Journal of*
594 *Neurophysiology*, 57(4), 1002–1021.
- 595 Winter, I. M., & Palmer, A. R. (1991). Intensity coding in low-frequency auditory-nerve fibers of
596 the guinea pig. *The Journal of the Acoustical Society of America*, 90(4), 1958–1967.
- 597 Winter, I. M., & Palmer, A. R. (1995). Level dependence of cochlear nucleus onset unit
598 responses and facilitation by second tones or broadband noise. *Journal of*
599 *Neurophysiology*, 73(1), 141–159.
- 600 Ye, Y., Machado, D. G., & Kim, D. O. (2000). Projection of the marginal shell of the anteroventral
601 cochlear nucleus to olivocochlear neurons in the cat. *The Journal of Comparative*
602 *Neurology*, 420(1), 127–138.
- 603 Young, E. D., Robert, J. M., & Shofner, W. P. (1988). Regularity and latency of units in ventral
604 cochlear nucleus: implications for unit classification and generation of response properties.
605 *Journal of Neurophysiology*, 60(1), 1–29.

606

607



608

609 Fig S1: Small cells show lower SFRs and longer FSLs than other VCN units. A) Bar plot (mean \pm
610 SEM) of SFR for CN cell types (primary-like (PL), chopper (Ch), small cell (SC) and buildup. B) Bar
611 plot (mean \pm SEM) for FSL. C) Bar plot (mean \pm SEM) of thresholds for each CN cell type. D) Bar
612 plot (mean \pm SEM) of total inhibitory (TI) area of the receptive field. Comparisons between groups
613 were made using a Kruskal-Wallis test with Tukey's multiple comparison test * $p < 0.05$.

614