

19 A multi-channel EEG mini-cap can improve reliability
20 for recording auditory brainstem responses in
21 chinchillas

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24 **Abstract**

Background: Disabling hearing loss affects nearly 466 million people worldwide (World Health Organization). The auditory brainstem response (ABR) is the most common non-invasive clinical measure of evoked potentials, e.g., as an objective measure for universal newborn hearing screening. In research, the ABR is widely used for estimating hearing thresholds and cochlear synaptopathy in animal models of hearing loss. The ABR contains multiple waves representing neural activity across different peripheral auditory pathway stages, which arise within the first 10 milliseconds after stimulus onset. Multi-channel (e.g., 32 or higher) caps provide robust measures for a wide variety of EEG applications for the study of human hearing. However, translational studies using preclinical animal models typically rely on only a few subdermal electrodes.

New Method: We evaluated the feasibility of a 32-channel rodent EEG mini-cap for improving the reliability of ABR measures in chinchillas, a common model of human hearing.

Results: After confirming initial feasibility, a systematic experimental design tested five potential sources of variability inherent to the mini-cap methodology. We found each source of variance minimally affected mini-cap ABR waveform morphology, thresholds, and wave-1 amplitudes.

Comparison with Existing Method: The mini-cap methodology was statistically more robust and less variable than the conventional subdermal-needle methodology, most notably when analyzing ABR thresholds. Additionally, fewer repetitions were required to produce a robust ABR response when using the mini-cap.

Conclusions: These results suggest the EEG mini-cap can improve translational studies of peripheral auditory evoked responses. Future work will evaluate the potential of the mini-cap to improve the reliability of more centrally evoked (e.g., cortical) EEG responses.

25 *Keywords:* auditory neuroscience, sensorineural hearing loss,
26 electroencephalography, EEG cap, auditory evoked potential

27 **1. Introduction**

28 Ninety percent of the 466 million people worldwide afflicted with hearing
29 loss are diagnosed with sensorineural hearing loss (SNHL). SNHL presents
30 anatomically as damage to the inner ear (e.g., cochlear hair cells) and/or to the
31 neural pathways connecting the inner ear and the brain. The auditory brain-
32 stem response (ABR) is an established clinical diagnostic tool widely used in the
33 identification of SNHL, a potentially disabling condition as it affects everyday
34 communication. SNHL is debilitating as it is difficult to alleviate completely,
35 especially in common listening situations with background noise, such as restaur-
36 ants. The ABR provides a method to non-invasively measure the neural activity
37 across the peripheral auditory pathway (auditory nerve to midbrain), with each
38 wave roughly corresponding to a particular location along the pathway. Wave-1,
39 for example, originates primarily in the proximal part of the auditory nerve. A
40 short-duration sound stimulus (e.g., click or tone burst) is presented into the
41 ear, and within 10 milliseconds an auditory evoked potential consisting of about
42 five waves is produced and can be recorded from scalp electrodes (Eggermont,
43 2017). In the audiology clinic, ABRs are commonly employed to estimate hear-
44 ing thresholds when behavioral audiometry is not sufficient or possible, such as
45 during intraoperative monitoring and newborn hearing screening or for retro-
46 cochlear pathology screenings (Abadi et al., 2016). Active scalp electrodes in a
47 1-channel or 2-channel configuration provide ABRs with low-noise due to their
48 very low output impedance and good signal-to-noise (Hill, 2018).

49 In research, ABRs provide further insight into the diagnosis and localization
50 of pathologies affecting the ascending auditory pathway that may be hidden in
51 audiograms (Picton et al., 1973). For instance, ABR wave characteristics of
52 amplitudes and latencies have been proposed as biomarkers for cochlear synap-
53 topathy (CS), a covert and insidious condition defined as the loss of synapses
54 between sensory inner hair cells (IHC) and auditory nerve fibers (ANF). Since
55 inner hair cells remain intact and not all synapses are lost, the clinical audiogram
56 is often not affected (which has led this condition to be associated with “hid-
57 den hearing loss”); and suprathreshold ABR waveforms provide an alternative
58 assay as they are altered by CS. A temporary increase in threshold after a noise
59 exposure (i.e., a temporary threshold shift) has been correlated with CS, even
60 in cases with no permanent threshold shift (PTS) (Hickox et al., 2017). Fur-
61 thermore, it has been shown in animals after temporary threshold shift (TTS)
62 noise exposures or with aging, wave-I amplitudes can be permanently reduced
63 (Kujawa and Liberman, 2009), and after PTS noise exposures ABR thresholds
64 are elevated and wave-1 latencies are reduced (Henry et al., 2011). In humans,
65 where wave-I amplitudes are difficult to measure but wave-V is more prominent,
66 wave-V latency for signals in noise has been suggested as a more useful assay

of CS (Bramhall, 2021; Mehraei et al., 2016), evidenced definitively with age (Wu et al., 2019), but actively debated (Bramhall et al., 2019). While much has been learned from both animal and human studies, they have largely been done independently, thus making rigorous translation difficult.

Improved experimental methods for comparing ABRs from animals and humans will help to resolve the best use of ABRs in diagnosing both hidden and overt hearing losses. Animal models, like the chinchilla in our research, provide an opportunity to study anatomical pathologies directly, but to succeed at human translation, it is critical to pursue analogous methodologies whenever possible. In humans, a high-density, multi-channel electroencephalogram (EEG) electrode cap system is often used for ABR data collection (Bharadwaj et al., 2019; Mehraei et al., 2016). EEG has been shown to provide notable benefits for data collection in humans, including excellent temporal precision, affordability, ease of maintenance, and efficient multi-channel signal processing to reduce the number of required stimulus repetitions (Bharadwaj et al., 2019; Bharadwaj and Shinn-Cunningham, 2014; Kam et al., 2019; Ledwidge et al., 2018). In animal models, previous research has suggested that high-quality EEG recordings in small animals (e.g., mice, rats) are not possible due to the signal generated by the underlying cortex being too weak and the potential for a high level of noise due to electrocardiogram artifacts (Lundt et al., 2019). ABRs are thus most often recorded using subdermal needle electrodes in small animals. In chinchillas, three subdermal needle electrodes are typically used: the vertex electrode (non-inverting) placed between the bullae at the dorsal midline, the reference electrode (inverting) placed posterior to the right pinna, and the ground electrode placed at the bridge of the nose (Henry et al., 2011). Consistent placement of the inverting reference electrode underneath the posterior bullae is often difficult due to limited visibility, and this can introduce variability between responses. Proper electrode placement is often confirmed based on observing expected waveform morphology, with electrodes replaced when deemed necessary.

To align our methodology between our animal and human studies (Bharadwaj et al., 2022), we implemented and evaluated a 32-channel small animal EEG mini-cap system for ABR data collection in chinchillas. This recent technological advancement was first applied to simultaneously record EEG and fMRI in Wistar Rats, and allows for a non-invasive approach to record EEG scalp recordings in small animals (Sumiyoshi et al., 2011). Prior to this advancement, only invasive approaches to EEG recordings (e.g., epicranial) had been examined in small animals (Mégevand et al., 2008). By aligning our chinchilla and human ABR data collection methods, we will enhance our capability to effectively translate our research advancements for subcortical and cortical responses in the chinchilla animal model using the same methodology to clinical advancements for humans. After confirming initial feasibility of the mini-cap, the reliability, repeatability, and reproducibility of auditory evoked potentials measured in chinchillas using the mini-cap was quantified. Thereupon, the variability inherent to the mini-cap method was characterized and shown to be reduced compared to the conventional subdermal needle method.

113 **2. Methods**

114 *2.1. Animals*

115 Four young adult male chinchillas weighing 400 to 700 g were used in these
116 experiments. Adequate cochlear function was confirmed by measuring distortion-
117 product otoacoustic emissions (DPOAEs) at the start of each experiment (Bharad-
118 waj et al., 2022). Anesthesia was induced with a xylazine injection (2-3 mg/kg
119 subcutaneous) followed by a ketamine injection (30-40 mg/kg subcutaneous).
120 Then, the animal was placed in a stereotaxic device on a closed-loop regulated
121 heating pad set at 37 degrees Celsius with a rectal probe used to monitor body
122 temperature (50-7220F, Harvard Apparatus, Holliston, MA, USA), eye ointment
123 was applied to keep the eyes lubricated, and a pulse oximeter was attached to
124 the animal's hind paw to monitor oxygen and heart rate. After the experiment,
125 atipamezole (0.4-0.5 mg/kg intraperitoneal) was administered to reverse the
126 sedative effects of xylazine. Lactated Ringers solution (6cc subcutaneous) was
127 provided at the start and end of the experiment to stimulate post-anesthesia
128 recovery. All procedures were approved by the Purdue Animal Care and Use
129 Committee (PACUC Protocol No: 1111000123).

130 *2.2. Data collection*

131 Each experiment was conducted in an electrically shielded, double-walled
132 sound-attenuating chamber (Industrial Acoustics Company, Bronx, NY, USA).
133 The acoustic stimuli were created using custom Matlab software that controlled
134 Tucker-Davis-Technology (TDT) System 3 hardware (RP2 Real-Time Proces-
135 sor, Alachua, FL, USA). Calibration of the stimulus level was performed at
136 the start of each experiment when a probe microphone (ER 10B+ Low Noise
137 Mic System, Etymotic Research, Elk Grove Village, IL, USA) and two trans-
138 ducers (ER-2 earphones, Etymotic Research, Elk Grove Village, IL, USA) were
139 inserted into the animal's ipsilateral ear canal. After calibration, DPOAEs were
140 collected.

141 Acoustic bursts at a frequency of 4 kHz, consisting of a 5-millisecond du-
142 ration burst with 0.5 millisecond linear onset and offset ramps (20 repeti-
143 tions/second), were presented into the right ear of the animal. ABRs were
144 recorded for input levels from 0 to 80 dB SPL in 10 dB increments, termed
145 an ABR waterfall. After visually estimating the lowest level at which an ABR
146 response was identified, one to two additional runs at 5 dB increments near the
147 threshold were recorded.

148 ABRs were simultaneously recorded with subdermal needle electrodes (Ambu
149 Neuroline 74512-150/24, Ballerup, Denmark) and with the EEG mini-cap con-
150 sisting of 32 active electrodes and a DRL and CMS lead (32 Channel EEG
151 Mini-Cap, DA-AR-EMCL32, Cortech Solutions, Wilmington, NC, USA). All
152 channels were connected to the Biosemi ActiveTwo AD-box (Biosemi Active
153 II system, Amsterdam, Netherlands) which further amplified and digitized the
154 signal. Subdermal needle electrodes were inserted at the dorsal midline between
155 the bullae (vertex), underneath the pinna and adjacent to the posterior bulla
156 (mastoid), and at the bridge of the nose (ground) (Henry et al., 2011). Since

157 the Biosemi ActiveTwo AD-box allowed for recording of up to eight external
158 channels, the three needle electrodes were recorded simultaneously with the
159 mini-cap using the same Biosemi ActiveTwo AD-box. Two 10mm in-ear gold-
160 foiled tiptrodes (ER3-26A combined with ER3-28S) were additionally placed
161 into the ear canal of the ipsilateral and contralateral ear which were recorded
162 as external channels. The ipsilateral tiptrode acted as the reference channel
163 during post processing of the mini-cap EEG signals. The ground subdermal
164 needle electrode placed at the bridge of the nose acted as the reference channel
165 for the subdermal EEG signals.

166 *2.2.1. EEG mini-cap for ABR data collection*

167 Each of the thirty-two active Ag/AgCl electrodes with 10mm spacing be-
168 tween electrodes (Fig. 1A) recorded a continuous EEG signal that was visually
169 analyzed using Actiview software (Biosemi, Amsterdam, Netherlands). The sig-
170 nals were digitally sampled at 16.384 kHz. Each electrode contained an inner tube
171 with a silver wire and conductive saline-diluted EEG paste that could be moved
172 perpendicularly through an external fixed sliding tube to ensure adequate scalp
173 contact.

174 The animal's scalp was shaved using an electric razor and then further
175 cleaned using Nair Hair removal lotion and isopropyl alcohol. A saline soaked
176 cloth was placed on the exposed scalp until the mini-cap was placed. To place
177 the mini-cap on the animal's head, the tympanic bullae (Fig. 1B) were located
178 anatomically. A customized device was designed to tightly secure the mini-cap
179 to the animal's head using firm elastic bands. The final experimental record-
180 ing setup (Fig. 1C) included the mini-cap, three subdermal needle electrodes,
181 and two tiptrodes. Stimulus triggers corresponding to each instance of ipsi-
182 lateral tone burst stimulus were sent to the Actiview software from the TDT
183 stimulus-generation hardware. The continuous time EEG signals were aligned
184 according to the stimulus onset and epoched in post processing to generate the
185 auditory evoked potential. The raw data from the mini-cap was saved as the
186 non-epoched, non-filtered continuous time signal, along with the trigger times.
187 For each ABR, 1000 repetitions (500 positive polarity, 500 negative) were col-
188 lected and averaged.

189 *2.3. Data analysis*

190 Data processing was performed using the mne-python toolbox (Gramfort
191 et al., 2013) combined with custom Matlab and Python software. First, the con-
192 tinuous EEG signal was referenced to an external channel. The mini-cap EEG
193 signal was referenced to the ipsilateral ear-canal tiptrode whereas the mastoid
194 and vertex subdermal needle electrodes were referenced to the ground subder-
195 mal electrode. Afterwards, the continuous mini-cap and subdermal needle EEG
196 signals were band-pass filtered (300 Hz to 3000 Hz), and a 60 Hz notch filter
197 was applied to remove any remnants of line noise. These low and high-pass filter
198 values have been shown to produce ABR waveforms that predict auditory-nerve-
199 fiber thresholds and frequency selectivity in hearing impaired chinchillas (Henry

200 et al., 2011). The continuous signal was then divided into epochs according to
201 stimulus onset triggers. The subdermal ABR response was generated by sub-
202 tracting the vertex non-inverting and reference inverting signals and averaging
203 across all repetitions.

204 Before averaging across epochs to generate the averaged mini-cap ABR re-
205 sponse, noisy EEG channels were identified and removed. Noisy channels were
206 identified using a deviation criterion, a metric used to detect differences in am-
207 plitude across channels as described in the PREP pipeline (Bigdely-Shamlo
208 et al., 2015). There were typically four to five noisy channels removed due to
209 anatomical differences between chinchilla and Wistar rat. Remaining channels
210 (Fig. 1D) were averaged together to obtain the processed mini-cap ABR.

211
212 <<*Insert Figure 1 here*>>

213 2.3.1. Thresholds and amplitudes

214 Thresholds and wave-1 amplitudes, both commonly used metrics for CS,
215 were quantified for each recorded mini-cap and subdermal ABR. Threshold,
216 often used as a measure of hearing sensitivity, is defined as the lowest sound
217 intensity level at which an ABR waveform can be detected. Thresholds were esti-
218 mated using a cross-correlation method (Henry et al., 2011), where a suprathresh-
219 old waveform is used as a template to evaluate the presence of response at lower
220 SPLs. In the ABR waveform, wave-1 originates in the proximal part of the au-
221 ditory nerve and can be reduced in animals after a TTS noise exposure. Wave-1
222 amplitudes were estimated using an automated procedure applying dynamic
223 time warping (Picton et al., 1988). These are both common approaches for
224 deriving summary statistics (thresholds and suprathreshold amplitudes) from
225 ABR waveforms (Bharadwaj et al., 2022; Henry et al., 2011).

226 2.4. Experimental Design

227 Quantifying both repeatability and reproducibility is crucial to ensure the
228 mini-cap can successively record ABRs within a single experiment and within
229 different experiments across more than one day. Reliability, referring to the
230 consistency of a measure, can be divided into three categories: (1) across-day
231 (i.e., test-retest reliability), (2) across items (i.e., internal reliability), and (3)
232 across experimenters (i.e., inter-experimenter reliability) (Price et al., 2015).
233 The same measurand (i.e., animal) and the same instrument (i.e., mini-cap)
234 are required for characterizing reliability. Repeatability describes the closeness
235 of agreement between successive measurements of the same measurand carried
236 out under the same conditions (Price et al., 2015). The same experimenter, the
237 same experiment (i.e., day), the same instrument, and the same measurand are
238 required for characterizing repeatability. Reproducibility describes the closeness
239 of agreement between measurements of the same measurand carried out under
240 changed conditions of measurement (of Standards and Technology, 2015).

241 The study was designed (Table 1) to quantify the contributions of sources of
242 variability affecting the mini-cap methodology and directly compare that to the

measurement variability affecting the subdermal methodology. At the start of each experiment, the first experimenter placed the mini-cap and the first (“original, or O”) ABR waterfall was collected. Next, a second ABR was collected shortly after the first ABR, called “replicate #1, or R1”, where the only source of variability introduced was a short duration of time (i.e., minutes). Finally, the first experimenter removed and replaced the mini-cap and a third ABR was collected, termed “replicate #2, or R2”, where the variability associated with the same experimenter removing and replacing the mini-cap was analyzed. A second experimenter repeated the same procedure and three additional ABR waterfalls were collected. To quantify the variability associated with using a different mini-cap, a second experimental design involved only one experimenter collecting three ABR waterfalls (O, R1, R2) with the first mini-cap (C1) and then the same three ABR waterfalls with a second identical-model mini-cap (C2). One week after the first experiment (D1, 1st day), a second experiment (D2, 2nd day) was conducted to study the variability associated with using the mini-cap across a longer duration of time (i.e., two separate experiments). For each experiment, ideally 6 waterfalls were collected, although the actual number of experimental conditions measured differed depending on experimental time constraints related to animal status under anesthesia. Subdermal ABRs were collected simultaneously as mini-cap ABRs were collected. However, the subdermal needles were not modified throughout the duration of the experiment, except if one accidentally became displaced. Therefore, only two sources of variability were equivalent between the mini-cap and subdermal needles: waveforms collected across a short duration of time (O, R1) and waveforms collected across a longer duration of time (D1, D2).

To quantify the contribution of each specific source of variability, direct comparisons were made between ABR waveforms from two conditions (see Table 1 and Supplemental Material). To quantify a single source of variability independently, only one source of variability was altered for each comparison and the other sources of variability remained constant. In total, five potential sources of variability that can affect the mini-cap measurements were identified and labeled as described in the experimental procedure: X-Time, X-Removal, X-Experimenter, X-Cap, and X-Day. Two metrics were used to quantify each source of variability. First, temporal comparisons between waveforms were quantified using the cross-correlation coefficient. Second, intraclass correlation coefficients (ICCs) were used to evaluate the summary statistics of thresholds and suprathreshold wave-1 amplitudes, two critical biomarkers in CS studies (Bharadwaj et al., 2022, 2019; Kujawa and Liberman, 2009). By evaluating each source of variability independently, we were able to characterize the reliability, repeatability, and reproducibility of the mini-cap to record ABRs in chinchillas.

<<*Insert Table 1 here*>>

286 **3. Results**

287 *3.1. Feasibility of mini-cap to record ABRs and practical adjustments to improve
288 reliability*

289 Several improvements were performed after the initial feasibility study to
290 obtain reliable ABRs from the mini-cap. Ensuring adequate scalp contact was
291 critical to obtaining a clean EEG signal. A customized 3D printed device at-
292 tached to the stereotax ear-bar holders to hold the rubber band in place (see
293 arrows in Fig. 1C) was created to ensure the mini-cap had adequate scalp con-
294 tact. Other methodological improvements were crucial, including removing all
295 fur from the scalp to ensure clean contact between electrodes and scalp and
296 inserting a gel into each electrode channel that contained EEG electrode paste
297 diluted with saline. After implementing these improvements, clean ABRs were
298 recorded using the mini-cap. During data collection, it was critical that the ref-
299 erence channel was not noisy (e.g., ipsilateral tiptrode) for post processing. The
300 Biosemi software allowed for visualization of each EEG channel, so one crucial
301 step in the procedure was establishing a clean reference channel during data
302 collection to reduce additional noise when each channel was referenced after-
303 the-fact. For example, in cases where the tiptrode needed to be inserted several
304 times to generate a quality seal, the gold-foil partially rubbed off and added noise
305 to the reference channel signal. Since all channels were averaged to establish a
306 single ABR response, identifying and removing any noisy channels during data
307 analysis was critical. As described in the PREP pipeline (Bigdely-Shamlo et al.,
308 2015), a z-score deviation criterion was used to automatically detect amplitude
309 differences across channels and remove any channels with a z-score greater than
310 2. To confirm feasibility after performing these improvements, ABRs were col-
311 lected from one animal on two days, separated by one week. The feasibility of
312 the mini-cap to produce ABR thresholds across multiple days was verified.

313 *3.2. Mini-cap improves the reliability of ABR waveforms over subdermal elec-
314 trodes*

315 For each comparison (e.g., see Table 1), a temporal analysis of the two ABR
316 waveforms of the same intensity and frequency gave us insight into substantial
317 sources of variability in the mini-cap methodology. Cross-correlation coefficients
318 were calculated between the two waveforms, and the temporal characteristics of
319 the first waveform were compared to the second waveform. The ABR signals
320 were windowed from 2-8 milliseconds after stimulus onset because the waveform
321 morphology of interest (i.e., waves 1-5) occurred in this window. Next, the
322 cross-correlation coefficient between the two signal windows was calculated with
323 zero delay (i.e., lag), as both signals were collected under equivalent conditions
324 (Fig. 2A). By windowing from 2-8 milliseconds, the cross-correlation coefficient
325 only compared waveform morphology between the two signals and excluded
326 noise (i.e., before 2 milliseconds and after 8 milliseconds). The resulting cross-
327 correlation coefficients ranged from 0 to 1, with a coefficient of 1 indicating
328 the two ABR signals were identical in morphology and all sources of variability
329 represented in the comparison were negligible.

330 Cross-correlation coefficients at each intensity level for each source of variability were summarized (Fig. 2B). As level increases, the response becomes
331 more well-defined, and, thus, the cross-correlation coefficients increase. At intensity levels above threshold (i.e., on average, about 25-30 dB SPL), the averaged cross-correlation coefficients indicate that the waveform morphologies
332 between the two ABRs in each comparison were highly equivalent. This, in turn, reveals that each particular source of variability minimally affected the capability of the mini-cap to record reliable ABRs with similar morphology. It
333 was found that X-Experimenter and X-Day variability, on average, had the most significant impact on waveform morphology (e.g., 0.78 and 0.74 respectively at 50 dB SPL), whereas X-Time and X-Cap variability impacted waveform morphology the least (e.g., 0.90 and 0.91 respectively at 50 dB SPL). However, all
334 five sources of variability appear minimal and do not affect the ability of the
335 mini-cap to produce reliable, repeatable, and reproducible ABRs.
336

337 Next, a similar temporal analysis was performed on subdermal ABRs collected concurrently with the mini-cap ABRs. Since the subdermal needles were
338 not removed or replaced during an experiment, the cross-correlation coefficients for subdermal responses collected within a short period of time (“X-Time”) could be directly compared to the cross-correlation coefficients for mini-cap responses within a short period of time (Fig. 2C). For X-Time comparisons, we
339 found a statistically significant difference in mean cross-correlation coefficient by sound level ($F(10)=55.69$, $p<0.0001$) but not by methodology type ($F(1)=1.04$,
340 $p=0.310$), and no interaction between the two factors ($F(10)=0.11$, $p=1.0$). Since there was no statistical difference between the X-Time cross-correlation coefficients of the mini-cap compared to the subdermal needles, both methodologies
341 reliably produced ABRs of comparable morphology within a short duration of time (e.g., within a single experiment). Besides X-Time comparisons,
342 X-Day comparisons could also be correlated between the two methodologies. The X-Day cross-correlation coefficients from the mini-cap were compared to the subdermal needles across all levels (Fig. 2D). For X-Day comparisons, we
343 found a statistically significant difference in mean cross-correlation coefficient by both methodology type ($F(1)=23.24$, $p<0.0001$) and by sound level
344 ($F(10)=143.97$, $p<0.0001$), but no interaction between the two main factors
345 ($F(10)=0.69$, $p=0.738$). Across two experiments on two separate days, the mini-cap appears to produce more reliable and less variable ABRs, with respect to waveform morphology, than the subdermal method.

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367 <<*Insert Figure 2 here*>>

368 3.3. Mini-cap improves the reliability of common ABR summary metrics

369 Intraclass correlation coefficients (ICC) were calculated to statistically assess the mini-cap’s ability to provide reliable thresholds and averaged wave-1 amplitudes across each of the five potential sources of variability (see Table 2).
370 The suprathreshold wave-1 amplitudes (i.e., 60, 70, and 80 dB SPL) were averaged together to generate the values used in the ICC calculations, because
371 they have been suggested as an assay of cochlear synaptopathy (Kujawa and
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375 Liberman, 2009). In comparison to the cross-correlation coefficient based on a
376 temporal comparison of each ABR waveform, the ICC is a common statistical
377 metric to assess test-retest reliability that reflects both the degree of correlation
378 and the agreement between measurements (Koo and Li, 2016). Here, the ICC
379 was used to quantify certain ABR biomarkers commonly used in the hearing
380 science field. ICCs were also computed for the subdermal waveforms and com-
381 pared directly to the corresponding mini-cap ICC. Since the subdermal needles
382 were not replaced during an experiment, the X-Time source of variability could
383 be directly compared between the two methodologies whereas X-Removal, X-
384 Experimenter, and X-Cap were not empirically equivalent. X-Day variability
385 could also be directly compared since the experimental design between the two
386 methodologies was the same (i.e., two experiments conducted one week apart).
387 An ICC value less than 0.40 was considered poor, between or equal to 0.40 and
388 0.60 was considered fair, between or equal to 0.60 and 0.75 was considered good,
389 and greater than or equal to 0.75 was considered excellent (based on Rentzsch
390 et al. (2008)).

391 The mini-cap ICCs for both threshold and wave-1 amplitude portray excel-
392 lent reliability for each of the four sources of variability during a single exper-
393 iment, excluding threshold comparisons across multiple days. The subdermal
394 ICC that represents a short duration of time (e.g., X-Time) appears consistently
395 lower, especially for thresholds, when directly compared to the corresponding
396 mini-cap ICC, suggesting less favorable reliability than the mini-cap in their
397 ability to produce reliable, repeatable, and reproducible ABRs within a single
398 experiment. Overall, these results indicate the mini-cap was the more reliable
399 and less variable methodology for ABR data collection within a single experi-
400 ment. The only source of variability that appears to have below excellent reli-
401 ability for both mini-cap and subdermal methods (besides for mini-cap wave-1
402 amplitudes) is X-Day. For wave-1 amplitudes, the mini-cap shows excellent re-
403 liability (ICC = 0.89) across two days, whereas the subdermal approach shows
404 below-moderate reliability (ICC = 0.30), suggesting the mini-cap has an im-
405 proved capability to produce reliable biomarkers (e.g., wave amplitudes) across
406 two days. Thresholds are determined for each experiment, but there is the
407 possibility of slight changes in threshold when measured a week apart based
408 on animal physiology, especially within the 5 dB range (which is audiologically
409 insignificant as 5-dB is the typical step size in manual audiometric tracking by
410 an audiologist). It was, in fact, confirmed that when directly analyzing thresh-
411 olds from the first day to the second day, most of the thresholds were within
412 5 dB of one another (70% for mini-cap, 63% for subdermal). The mean differ-
413 ence between the thresholds across two days was 5.12 dB for the mini-cap and
414 6.22 dB for the subdermal needles. The ICC suggests fair and poor reliabil-
415 ity across two days for mini-cap and subdermal needles respectively, but when
416 solely considering the thresholds, it does appear that both methodologies are
417 able to produce accurate ABR thresholds (within 5 dB) across experiments on
418 different days. Moreover, a 5-dB difference using subdermal needles is accepted
419 for animal studies, so the mini-cap results exhibiting a 5-dB difference across
420 two days are acceptable. It is hypothesized that the placement of the subder-

421 mal needles can vary from each experiment, as only anatomical landmarks are
422 utilized. However, the mini-cap placement is placed over the majority of the
423 scalp covered by the 32 mini-cap electrodes, so it is less likely that placement
424 of the mini-cap will affect thresholds or waveform amplitudes (as indicated by
425 X-Removal ICC of 0.80).

426
427 <<*Insert Table 2 here*>>

428 3.4. *Mini-cap improves the efficiency of ABR data collection over subdermal*
429 *electrodes*

430 A bootstrapping analysis was completed to evaluate the number of repe-
431 titions necessary to attain reliable ABR responses for both the mini-cap and
432 subdermal methods. The number of repetitions used in the final averaged ABR
433 waveform was varied. For each repetition count, a randomized subset of repe-
434 titions was selected (without replacement) from a high-repetition data set and
435 averaged, constituting a single boot. Overall, twenty boots were calculated per
436 repetition count and the repetition count was varied starting at 100 repetitions
437 in increments of 125 repetitions. As the repetition count increased, the aver-
438 aged ABR waveform became less noisy and more pronounced. Three repetition
439 counts for each method are shown in Figure 3 (see subpanels A versus D, B
440 versus E, C versus F). Visually, it appears the mini-cap produces less noisy
441 waveforms at each repetition count.

442 A correlation analysis was performed to further quantify the number of repe-
443 titions necessary to obtain a highly correlated response to the averaged wave-
444 form across all repetitions, labeled the gold standard. The signal window of
445 each of the twenty boot responses was cross correlated to the signal window of
446 the gold standard response. Next, the twenty cross-correlation coefficients were
447 averaged together to produce a single cross-correlation coefficient for each repe-
448 tition count. A greater averaged cross-correlation coefficient indicates that the
449 number of repetitions averaged in the boot response was adequate to produce a
450 clear and concise ABR response. Overall, it was found that about 1000 repeti-
451 tions are adequate for the mini-cap methodology to produce a highly correlated
452 ABR response (Fig. 3G). In the mini-cap experiments performed for this re-
453 search, 1000 repetitions were collected for each response. At 1000 repetitions,
454 there is a plateauing, indicating additional repetitions after 1000 are of dimin-
455 ishing value. The correlation analysis results were compared for the mini-cap
456 and subdermal methods (Fig. 3H). In comparison to the subdermal method,
457 the mini-cap required fewer repetitions to produce an equivalently correlated
458 response. At all repetition counts, the mini-cap correlation values were greater,
459 suggesting that fewer repetitions are necessary in the mini-cap method to obtain
460 a clear and reliable ABR response.

461
462 <<*Insert Figure 3 here*>>

463 **4. Discussion**

464 *4.1. Summary of mini-cap variability*

465 Using the 32-channel mini-cap, high-quality EEG recordings of an evoked
466 potential were achievable in our chinchilla model, improving upon previous lim-
467 itations suggested by Lundt et al. (2019). The variability associated with using
468 the mini-cap to collect ABRs in chinchillas was quantified across five potential
469 sources of variability. Each source of variability was demonstrated to minimally
470 affect ABR waveform morphology, thresholds, and wave-1 amplitudes. Wave-
471 form morphology appeared consistent across measurement comparisons when
472 the ABR waveform itself was more pronounced at sound levels above threshold
473 (i.e., above 30 dB SPL) (Fig. 2B). The most impactful sources of variability to
474 waveform morphology were X-Experimenter and X-Day. Thresholds and wave-1
475 amplitudes, both critical ABR biomarkers (Kujawa and Liberman, 2009), were
476 generally consistent and reliable across all sources of variability for the mini-cap
477 (Table 2). X-Day variability most notably affected the reliability of thresholds
478 across two experiments. However, as mentioned, the differences in thresholds
479 across days were mainly within a 5-dB range of one another. Previous studies
480 (Bharadwaj and Shinn-Cunningham, 2014; Lu et al., 2022) have found benefits
481 from pooling data across channels from multichannel recordings. In this study,
482 time-averaging across all channels produced similar results (Ginsberg, 2020).
483 The signal component arising from subcortical sources is highly similar across
484 different mini-cap channels while the noise (e.g., from cortical activity) is more
485 variable, such that averaging across channels improves the signal-to-noise ratio.
486 These results suggest that the mini-cap can produce reliable, repeatable, and re-
487 producible ABRs when the replicate measurements are collected within a short
488 period of time, when the mini-cap is replaced, when a different experimenter
489 places the mini-cap, when a different mini-cap is used to collect data, or when
490 a second experiment is performed on a different day.

491 *4.2. Comparison to subdermal variability*

492 Simultaneous data collection with the mini-cap and subdermal needle elec-
493 trodes allowed for direct comparisons between the two methodologies. Two
494 of the five identified potential sources of variability, X-Time and X-Day, were
495 equivalent in methodology since the subdermal needles were not removed or re-
496 placed during a single experiment (i.e., day). For comparisons across a short pe-
497 riod of time, both the mini-cap and subdermal method produced reliable ABRs
498 when evaluating waveform morphology (Fig. 2C). However, both thresholds and
499 wave-1 amplitudes were statistically more robust using the mini-cap than the
500 subdermal needles, especially when evaluating threshold ICCs (mini-cap ICC
501 = 0.98, subdermal ICC = 0.6). In general, both ABR methods are capable of
502 producing reliable ABRs when the apparatus (i.e., mini-cap or needles) is not
503 altered during a single experiment (“X-Time”). When the subdermal needles
504 were altered across a different experiment on a second day (“X-Day”), there was
505 a convincing difference when looking at the subdermal method’s capability to

506 produce robust ABRs in comparison to the mini-cap (mini-cap ICC = 0.49, sub-
507 dermal ICC = 0.04). Statistically, two waveforms collected using the mini-cap
508 across two experiments were more consistent regarding waveform morphology
509 than two waveforms collected using subdermal needle electrodes across two ex-
510 periments (Fig. 2D). Additionally, both thresholds and wave-1 amplitudes were
511 more robust across two days using the mini-cap (Table 2).

512 *4.3. Mini-cap benefits and limitations*

513 There are methodological benefits of using the mini-cap for ABR data col-
514 lection. First, the mini-cap requires fewer repetitions to produce a robust ABR
515 response than the subdermal needles (Fig. 3H). Since the responses across
516 all mini-cap channels are roughly the same because the response itself origi-
517 nates from deep-seated sources of the brainstem (Fig. 4B), averaging across all
518 channels increases the signal-to noise ratio in the final response. As portrayed
519 through this study's results, the mini-cap produces less variable and more robust
520 ABRs in comparison to the subdermal methodology. Additionally, the poten-
521 tial sources of variability that could emerge when using the mini-cap have been
522 quantified and deemed to have a minimal impact on the final ABR response.
523 To compare, the subdermal needles have considerable variability introduced
524 through simple electrode placement.

525 There are three significant methodological benefits for using the 32-channel
526 mini-cap that drive its higher consistency and signal-to-noise ratio compared
527 to the subdermal 3-electrode approach. First, the mini-cap integrates active
528 electrodes which generally improve the signal-to-noise ratio compared to pas-
529 sive electrodes. Second, by averaging across up to thirty-two channels, within-
530 channel random noise (e.g., driven by cortical activity) is averaged out of the
531 final signal. Third, since the mini-cap covers the majority of the chinchilla's
532 scalp, there is less variability in the placement of the mini-cap than with the
533 subdermal needles, one of which is placed beneath the bulla in a difficult position
534 beneath the fur and skin.

535 In this study, the mini-cap was used to collect an auditory evoked response
536 originating from deep-seated sources in the brainstem. Another promising ben-
537 efit of the mini-cap is that other more centrally evoked potentials can also be
538 recorded. In these cortical responses (closer to electrodes), there is the likeli-
539 hood that individual channels can provide unique responses, which would pro-
540 vide a means to complete source localization of an evoked potential. To explore
541 this benefit of using the multi-channel mini-cap to identify between-channel
542 differences, a cortical response (4-kHz amplitude-modulated SAM noise) was
543 measured in an awake chinchilla. The reference channel for this cortical re-
544 sponse was the average of all channels. The topological map of the mini-cap
545 was placed onto a phantom scalp (Fig. 4A). First, topological mapping was
546 performed using an ABR response collected using the mini-cap (Fig. 4B). As
547 seen visually in the response, at the chosen time points, the responses across
548 all channels show similar magnitude responses. The ABR originates from deep-
549 seated sources and, hence, between-channel differences are minimal. Averaging
550 across the channels, however, does appear to reduce the noise in the final ABR

551 response. Conversely, the cortical response shows significant between-channel
552 differences, especially from the onset response (Fig. 4C). The mini-cap can show
553 between-channel differences from EEG scalp recordings on a chinchilla when the
554 response is more centrally evoked. For these auditory evoked potentials where
555 the goal is to identify channel differences in magnitude across time, the mini-cap
556 is especially useful.

557
558 <<*Insert Figure 4 here*>>
559

560 There are a few inherent limitations to using the mini-cap to record ABRs
561 in chinchillas, as was done in this study. First, during our experiments, we saw
562 a few consistently noisy channels (across both of our mini-caps) that had to
563 be removed during post processing using a selected noise criterion. Since this
564 mini-cap was designed based on the head anatomy of Wistar rats and not the
565 head anatomy of chinchillas, anatomical differences in the chinchilla likely led
566 to a few channels consistently not providing a good signal. That being said, it is
567 impressive that the majority of channels provided quality recording from chin-
568 chillas with no modifications to the rat mini-cap hardware. Obviously future
569 designs could be made specifically for the chinchilla (or other species), but the
570 generality of this min-cap to other rodent species is promising for broader use
571 without the need for expensive modifications for each species studied. Other
572 limitations relative to the standard subdermal approach include (1) increased
573 pre-experiment preparation time since the animal's head must be cleanly shaved
574 prior to collecting data, (2) an increased cost to purchase necessary equipment
575 (including mini-cap, ActiveTwo adapter cable, and Biosemi ActiveTwo ampli-
576 fier), and (3) additional post processing steps since all 32 channels provide raw
577 continuous-time signals that must be processed and combined; however, since
578 the data are in the same format as used in human studies (e.g., Bharadwaj
579 et al. (2022)) there are powerful open-source code packages that can be used
580 relatively easily for advanced post processing analyses (Gramfort et al., 2013).

581 *4.4. Future directions*

582 Future iterations of the mini-cap may include designing a mini-cap specific
583 to the chinchilla's anatomy. The mini-cap used in this research was designed
584 in accordance with the Wistar rat anatomy (Sumiyoshi et al., 2011). This
585 will become especially important when the mini-cap is used to measure cortical
586 responses as each channel will portray a unique response and, thus, ensuring the
587 mini-cap is designed according to the chinchilla's anatomy will help to facilitate
588 source localization. Since all channels showed equivalent ABR responses, it was
589 less critical that the mini-cap be designed according to the chinchilla anatomy
590 by Sumiyoshi et al. (2011). The first steps of designing a mini-cap specifically for
591 chinchillas would be to obtain an MRI of the chinchilla head and then develop
592 a chinchilla brain atlas (as was done in Wistar rats). Additional experiments
593 to measure cortical responses will be conducted. The feasibility of the mini-cap
594 to record cortical responses in awake chinchillas has been confirmed (Fig. 4C).

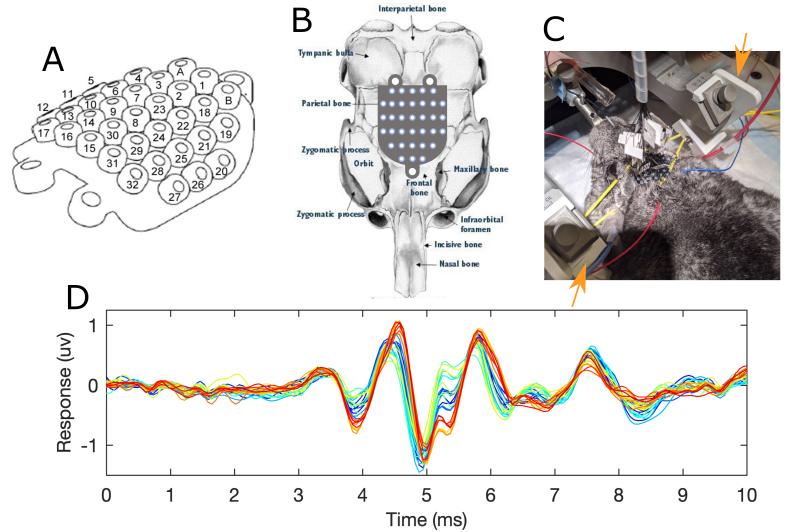
595 Further investigation into the cortical responses may focus on separating out
596 different anatomical sources within a single response.

597 *4.5. Animal to human translation*

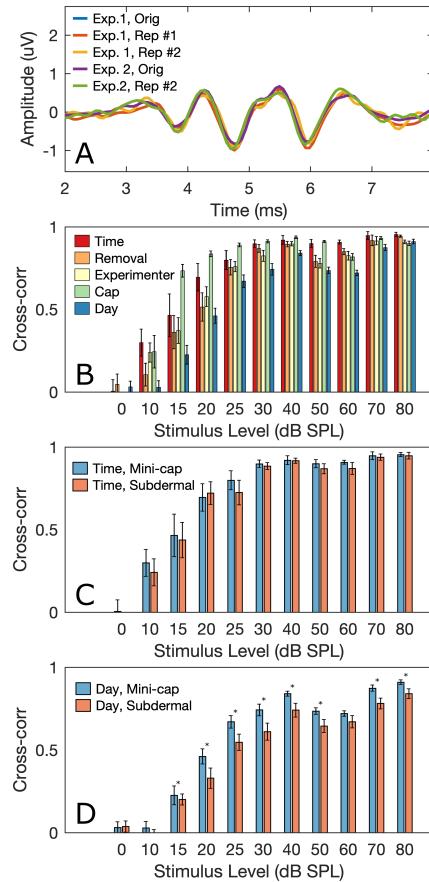
598 Cross-species translational studies have garnered unique insight into the
599 likely widespread prevalence of cochlear synaptopathy in human subjects with
600 audiometric normal hearing (Bharadwaj et al., 2022); however, the coordination
601 of methods across species was limited to peripheral measures (e.g., wideband
602 middle-ear muscle reflex). In the present study, the mini-cap allowed us to co-
603 ordinate neural measures and methodologies between chinchillas and humans.
604 Moving forward, the central effects of cochlear synaptopathy (e.g., central gain
605 effects as described in Schaette and McAlpine (2011)) can be further explored
606 using the mini-cap to record cortical responses in chinchillas. Now that the vari-
607 ability has been quantified and deemed minimal, the next step will be to collect
608 ABRs and cortical measures using the mini-cap before and after a temporary
609 threshold shift (TTS) noise exposure. The cochlear synaptopathy metrics can
610 then be quantified and directly compared to similarly measured metrics in hu-
611 mans. Fundamentally, we will continue to strive to develop new and improved
612 approaches to use our chinchilla data to advance human clinical diagnostics
613 and human clinical outcomes. This small animal EEG mini-cap is one major
614 advancement we have eagerly pursued to progress our alignment between chin-
615 chilla and human neural data collection to accelerate this valuable translation
616 between our significant findings in the chinchilla animal model and meaningful
617 clinical advancements for humans.

618 **Acknowledgements**

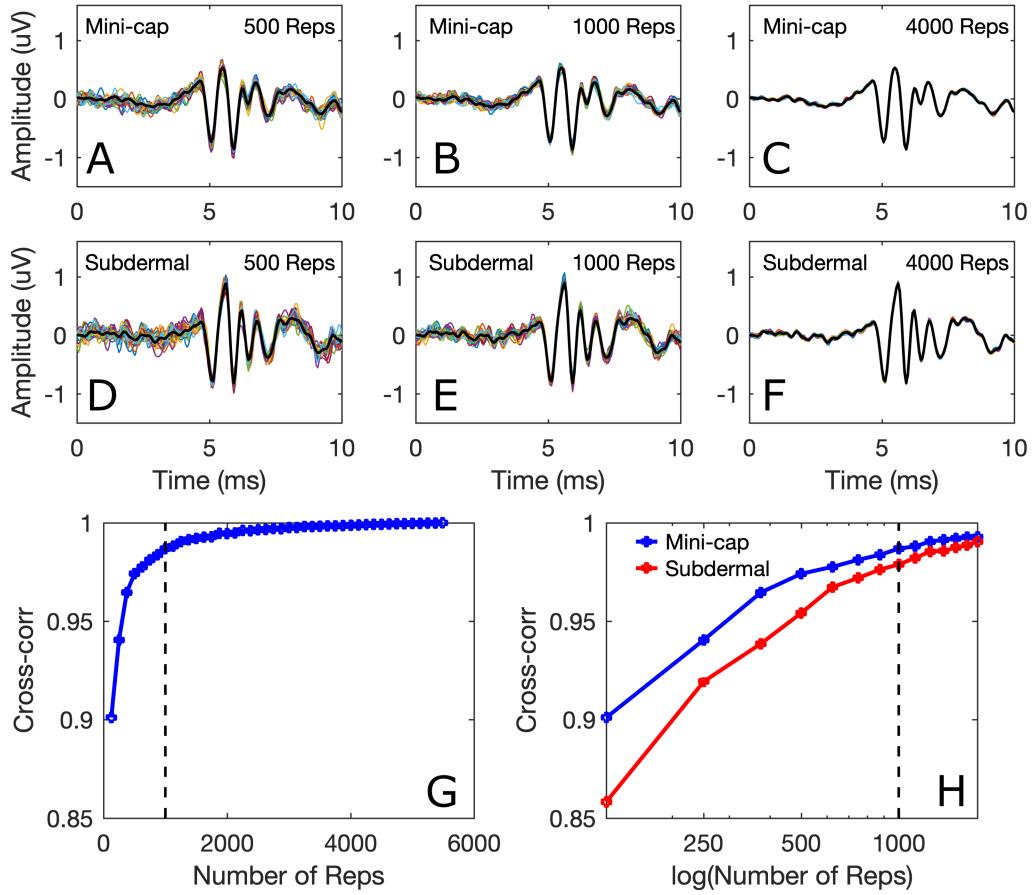
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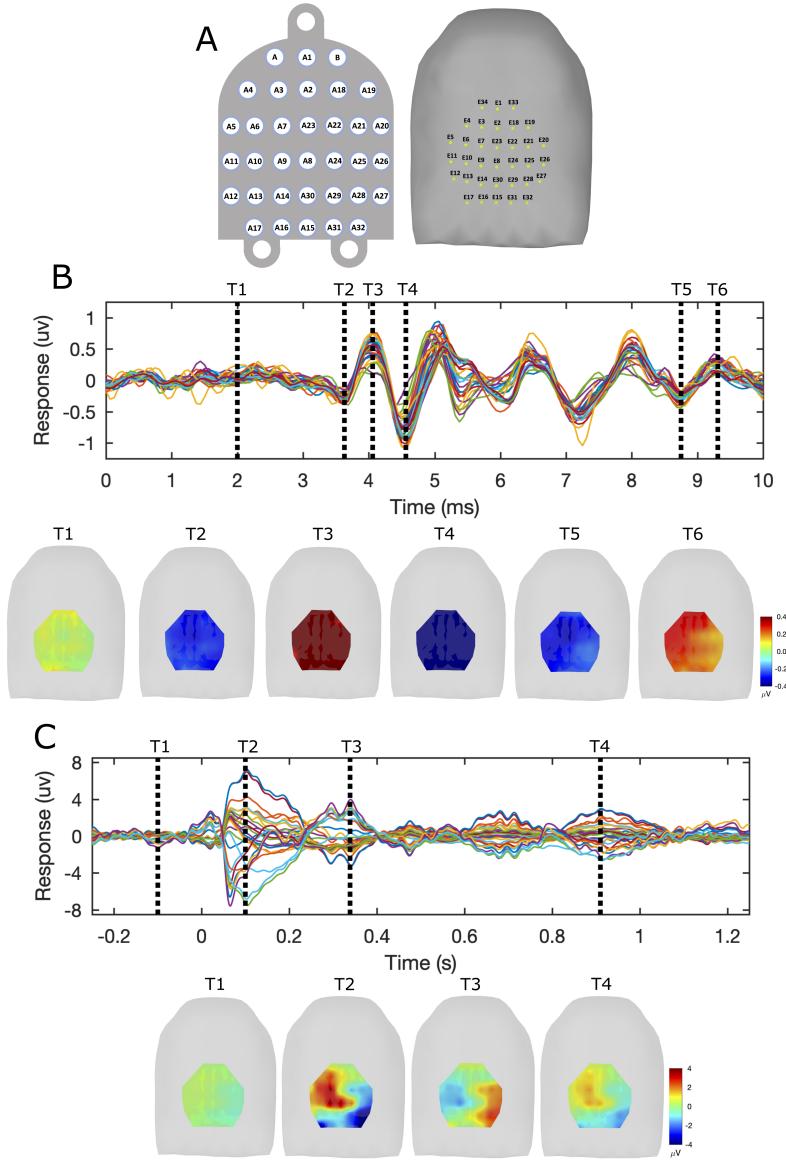
628 **Figure 1. EEG mini-cap and associated experimental setup to record**
 629 **ABRs in chinchillas.** (A) The small-animal high-density EEG mini-cap
 630 (Cortech Solutions) used in this research consists of 32 active electrodes. On the
 631 mini-cap: Electrode site A is the Common Mode Sense (CMS) active electrode
 632 and electrode site B is the Driven Right Leg (DRL) passive electrode; together
 633 these electrodes provide a feedback loop to drive the average potential of the
 634 subject close to the reference voltage. (B) The EEG mini-cap placed on the
 635 chinchilla skull, adapted from Brenner et al. (2005) with permission (Original
 636 illustration by Marc E. Goldyne, MD PhD). The two bullae were located on the
 637 chinchilla's scalp and the mini-cap was placed in front of the two bullae.
 638 (C) The final experimental setup of the EEG mini-cap for data collection. Two
 639 gold-foiled tiptrodes (not pictured) were placed into each ear canal. Three sub-
 640 dermal needle electrodes were placed to simultaneously record subdermal ABR
 641 responses: vertex electrode (red) placed at dorsal midline between bullae; mas-
 642 toid electrode (blue) placed underneath the pinna and adjacent to the posterior
 643 bulla (mastoid); ground electrode (green) placed on bridge of nose. The elastic
 644 yellow band attached to a customized 3D printed device (see arrows) tightly
 645 holds the mini-cap on the chinchilla's head. (D) An auditory brainstem re-
 646 sponse from a chinchilla recorded using the EEG mini-cap, with each colored
 647 line representing a different channel on the mini-cap that displays the averaged
 648 response across the 1000 stimulus repetitions recorded; noisy channels are not
 649 shown. Time zero indicates the stimulus onset.



650 **Figure 2. Comparisons of temporal waveforms across conditions.** (A)
651 Five 40-dB SPL responses (signal window, 2-8ms) from each waterfall collected
652 during a single experiment were compared to quantify the contribution of each
653 source of variability. For example, an X-Time comparison would include com-
654 paring Experimenter #1, Original waveform with Experimenter #1, Replicate
655 #1 waveform. (B) Summary of five sources of variability using the mini-cap to
656 record ABRs in chinchillas. All comparisons for each sound level were cate-
657 gorized by the source of variability and cross-correlation coefficients were averaged
658 together. As expected, the two sources of variability that notably affected the
659 cross-correlation coefficient were Experimenter and Day. Overall, across each
660 source of variability, the mini-cap was able to produce reliable, repeatable, and
661 reproducible ABRs. (C) Comparison of mini-cap and subdermal variability, X-
662 Time when a second response was recorded shortly after the first (“Replicate
663 #1”). Significant post-hoc t-tests are indicated with an asterisk ($\alpha = 0.05$).
664 The X-Time cross-correlation was statistically equivalent when using the mini-
665 cap in comparison to the subdermal needle electrodes. Within a short period of
666 time under the same conditions, the mini-cap and subdermal needles can pro-
667 duce reliable and repeatable ABRs. (D) Comparison of mini-cap and subdermal
668 variability, X-Day when a second response was recorded on a different day than
669 the first. Significant post-hoc t-tests are indicated with an asterisk ($\alpha = 0.05$).
670 The mini-cap produced statistically less variable ABRs across two days
671 than the subdermal needles. When comparing ABR responses from two differ-
672 ent experiments on separate days, the mini-cap showed greater reliability and
673 reproducibility.



674 **Figure 3. Bootstrapping analyses of mini-cap versus subdermal effi-**
 675 **ciency.** (A, B, C) Bootstrapping results using the mini-cap method. For each
 676 stimulus repetition number, twenty subsets of the total mini-cap repetitions
 677 were randomly chosen and averaged together to produce twenty total bootstrap
 678 responses. (D, E, F) Bootstrapping results using the subdermal needle method.
 679 (G) Bootstrapping analysis for mini-cap only, linear x-axis. The signal window
 680 (2-8ms) from each bootstrap response was cross correlated with the signal win-
 681 dow (2-8ms) of the gold standard, the response averaged across all repetitions
 682 ($N=5500$). These 20 cross-correlation values were averaged together to produce
 683 a correlation value representing each repetition number. From this analysis,
 684 1000 repetitions (see black dotted line) are seen to be acceptable to attain
 685 a highly correlated mini-cap response (waveform correlation coefficient >0.98).
 686 Thus, in our typical experimental setup, we chose to record 1000 repetitions for
 687 each sound level. (H) Bootstrapping analysis for mini-cap and subdermal nee-
 688 dles, on logarithmic x-axis. For all repetition values, the mini-cap correlations
 689 (i.e., reliability) are higher than for the subdermal results. Thus, these analyses
 690 show that in comparison to the subdermal method, the mini-cap requires fewer
 691 repetitions to produce an equitably correlated response.



692 **Figure 4. Topological mapping shows differences between brainstem
693 and cortical responses.** (A) The 32-channel mini-cap layout (left) was placed
694 onto a topological layout (right) to visualize the between-channel differences
695 within a response. (B) Topological map of an ABR response at six different
696 time points (labeled as T1-T6 with black dotted lines). Overall, all channels of
697 the mini-cap appear to show similar magnitude responses at each of the six time
698 points. Since the ABR originates from deep-seated sources within the brain,
699 between-channels differences were minimal. (C) Topological map of a cortical
700 response to 4-Hz amplitude modulated SAM noise in an awake chinchilla at four
701 different time points (labeled as T1-T4 with black dotted lines). From the onset
702 response, cortical activation is observable, confirming the mini-cap can gener-
20 ate responses where between-channel differences are significant. Therefore, the
703 neural source of the response determines whether between-channel differences
704 are significant or not.
705

Table 1: **Experimental design to characterize five sources of variability in mini-cap ABRs.** Abbreviations: E1/E2 - Experimenter; C1/C2 - Mini-cap; O/R1/R2 - Original/Replicate#; D1/D2 - Day; Total experimental comparisons are the actual number of comparisons used in this study based on the data collected from each experiment (one animal on one day). See Supplemental Material for the full table of all possible comparisons.

Comparison Name	Measurement qualities tested	Variability Source	Consistent Variables	Comparison Examples	Possible Comparisons per Experiment	Total Experimental Comparisons
X-Time	Reliability Repeatability	Short duration of time	Measurand Experimenter Instrument Day	E.g. D1-E1-C1-O vs. D1-E1-C1-R1 D1-E2-C1-O vs. D1-E2-C1-R1 O vs. R1	2	9
X-Removal	Reliability Repeatability	Replacement of the mini-cap	Measurand Experimenter Instrument Day	E.g. D1-E1-C1-O vs. D1-E1-C1-R2 D1-E1-C1-R1 vs. D1-E1-C1-R2 O vs. R2 R1 vs. R2	4	13
X-Experimenter	Reliability Reproducibility	Different experimenter	Measurand Instrument Day	E.g. D1-E1-C1-O vs. D1-E2-C1-O D1-E1-C1-O vs. D1-E2-C1-R1 D1-E1-C1-O vs. D1-E2-C1-R2 E1 vs. E2	24	24
X-Cap	Reproducibility	Different mini-cap	Measurand Instrument Day	E.g. D1-E1-C1-O vs. D1-E1-C2-O D1-E1-C1-O vs. D1-E1-C2-R1 D1-E1-C1-O vs. D1-E1-C2-R2 C1 vs. C2	9	9
X-Day	Reliability Reproducibility	Longer duration of time (>1 day)	Measurand Experimenter Instrument	E.g. D1-E1-C1-O vs. D2-E1-C1-O D1-E1-C1-O vs. D2-E1-C1-R1 D1-E1-C1-O vs. D2-E1-C1-R2 D1 vs. D2	18	29

Table 2: **Intraclass correlation coefficients (ICCs) for thresholds and suprathreshold wave-1 amplitudes for mini-cap and subdermal ABRs.** The mean ICCs for each source of variability for mini-cap and subdermal are shown along with the respective 95% confidence interval (depicted in parentheses: lower/upper). The subdermal needles were not replaced during an experiment, and, thus, subdermal ICCs were not able to be computed for X-Removal, X-Experimenter, and X-Cap, as indicated by N/A. ICC categories are as follows, based on Rentzsch et al. (2008): $ICC < 0.4$: poor; $0.4 \leq ICC < 0.60$: fair; $0.6 \leq ICC < 0.75$: good; $ICC \geq 0.75$: excellent.

Source of Variability	Threshold ICC		Wave-1 amplitude ICC	
	Mini-cap	Subdermal	Mini-cap	Subdermal
X-Time	0.98 (0.91/0.99)	0.60 (-0.1/0.89)	0.94 (0.75/0.99)	0.86 (0.36/0.97)
X-Removal	0.80 (0.46/0.93)	N/A	0.89 (0.69/0.97)	N/A
X-Experimenter	0.91 (0.74/0.96)	N/A	0.85 (0.68/0.93)	N/A
X-Cap	0.77 (0.32/0.94)	N/A	0.91 (0.67/0.98)	N/A
X-Day	0.49 (0.09/0.74)	0.04 (-0.32/0.40)	0.89 (0.78/0.95)	0.30 (-0.08/0.61)

⁷⁰⁶ **Appendix A. Supplemental Material**

⁷⁰⁷ All possible experimental comparisons are included in table attached.

Source of Variability		Possible Comparisons
X-Time		Experimenter #1-Cap #1-Original vs. Experimenter #1-Cap #1-Replicate #1 Experimenter #2-Cap #1-Original vs. Experimenter #2-Cap #1-Replicate #1 Experimenter #1-Cap #1-Original vs. Experimenter #1-Cap #1-Replicate #2 Experimenter #1-Cap #1-Replicate #1 vs. Experimenter #1-Cap #1-Replicate #2 Experimenter #2-Cap #1-Original vs. Experimenter #2-Cap #1-Replicate #2 Experimenter #2-Cap #1-Replicate #1 vs. Experimenter #1-Cap #1-Replicate #2
X-Removal		Experimenter #1-Cap #1-Original vs. Experimenter #2-Cap #1-Original Experimenter #1-Cap #1-Original vs. Experimenter #2-Cap #1-Replicate #2 Experimenter #1-Cap #1-Replicate #1 vs. Experimenter #2-Cap #1-Original Experimenter #1-Cap #1-Replicate #1 vs. Experimenter #2-Cap #1-Replicate #2 Experimenter #1-Cap #1-Replicate #1 vs. Experimenter #2-Cap #1-Replicate #1 Experimenter #1-Cap #1-Replicate #2 vs. Experimenter #2-Cap #1-Original Experimenter #1-Cap #1-Replicate #2 vs. Experimenter #2-Cap #1-Replicate #1 Experimenter #1-Cap #1-Replicate #2 vs. Experimenter #2-Cap #1-Replicate #2
X-Experimenter		Cap #1-Experimenter #1-Original vs. Cap #2-Experimenter #1-Original Cap #1-Experimenter #1-Original vs. Cap #2-Experimenter #1-Replicate #1 Cap #1-Experimenter #1-Original vs. Cap #2-Experimenter #1-Replicate #2 Cap #1-Experimenter #1-Replicate #1 vs. Cap #2-Experimenter #1-Original Cap #1-Experimenter #1-Replicate #1 vs. Cap #2-Experimenter #1-Replicate #2 Cap #1-Experimenter #1-Replicate #2 vs. Cap #2-Experimenter #1-Original Cap #1-Experimenter #1-Replicate #2 vs. Cap #2-Experimenter #1-Replicate #1 Cap #1-Experimenter #1-Replicate #2 vs. Cap #2-Experimenter #1-Replicate #2
X-Cap		Day #1-Experimenter #1-Cap #1-Original vs. Day #2-Experimenter #1-Cap #1-Original Day #1-Experimenter #1-Cap #1-Original vs. Day #2-Experimenter #1-Cap #1-Replicate #1 Day #1-Experimenter #1-Cap #1-Original vs. Day #2-Experimenter #1-Cap #1-Replicate #2 Day #1-Experimenter #1-Cap #1-Replicate #1 vs. Day #2-Experimenter #1-Cap #1-Original Day #1-Experimenter #1-Cap #1-Replicate #1 vs. Day #2-Experimenter #1-Cap #1-Replicate #2 Day #1-Experimenter #1-Cap #1-Replicate #2 vs. Day #2-Experimenter #1-Cap #1-Original Day #1-Experimenter #1-Cap #1-Replicate #2 vs. Day #2-Experimenter #1-Cap #1-Replicate #1 Day #1-Experimenter #1-Cap #1-Replicate #2 vs. Day #2-Experimenter #1-Cap #1-Replicate #2 Day #1-Experimenter #2-Cap #1-Original vs. Day #2-Experimenter #2-Cap #1-Original Day #1-Experimenter #2-Cap #1-Original vs. Day #2-Experimenter #2-Cap #1-Replicate #1 Day #1-Experimenter #2-Cap #1-Original vs. Day #2-Experimenter #2-Cap #1-Replicate #2 Day #1-Experimenter #2-Cap #1-Replicate #1 vs. Day #2-Experimenter #2-Cap #1-Original Day #1-Experimenter #2-Cap #1-Replicate #1 vs. Day #2-Experimenter #2-Cap #1-Replicate #2 Day #1-Experimenter #2-Cap #1-Replicate #2 vs. Day #2-Experimenter #2-Cap #1-Original Day #1-Experimenter #2-Cap #1-Replicate #2 vs. Day #2-Experimenter #2-Cap #1-Replicate #1 Day #1-Experimenter #2-Cap #1-Replicate #2 vs. Day #2-Experimenter #2-Cap #1-Replicate #2
X-Day		

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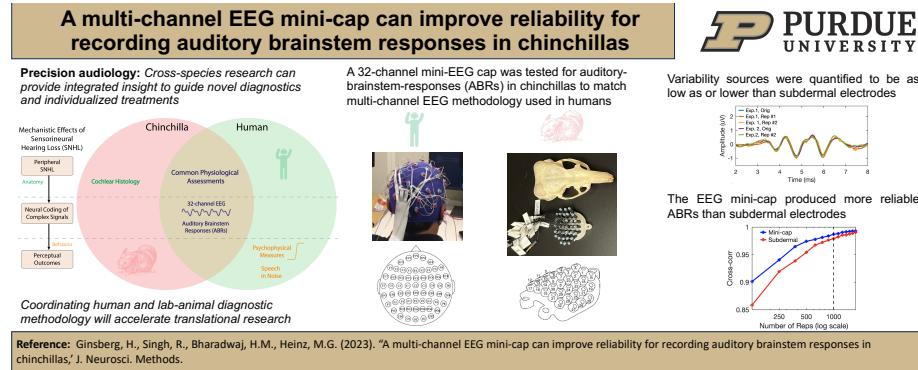
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1 Graphical Abstract

2 A multi-channel EEG mini-cap can improve reliability for recording
3 auditory brainstem responses in chinchillas

4 Hannah Ginsberg, Ravinderjit Singh, Hari M. Bharadwaj, Michael G. Heinz



5 Highlights

6 **A multi-channel EEG mini-cap can improve reliability for recording**
7 **auditory brainstem responses in chinchillas**

8 Hannah Ginsberg, Ravinderjit Singh, Hari M. Bharadwaj, Michael G. Heinz

9 • A mini-EEG cap was tested for ABRs in chinchillas to match methodology
10 used in humans

11 • Reliable multi-channel EEG data from chinchillas were obtained using the
12 mini-cap

13 • Sources of variability quantified to be as low as or lower than subdermal
14 electrodes

15 • Mini-cap produces more reliable ABRs regarding waveform morphology
16 than subdermal

17 • Mini-cap ICCs for threshold and wave-1 amplitude showed excellent reli-
18 ability