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

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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.

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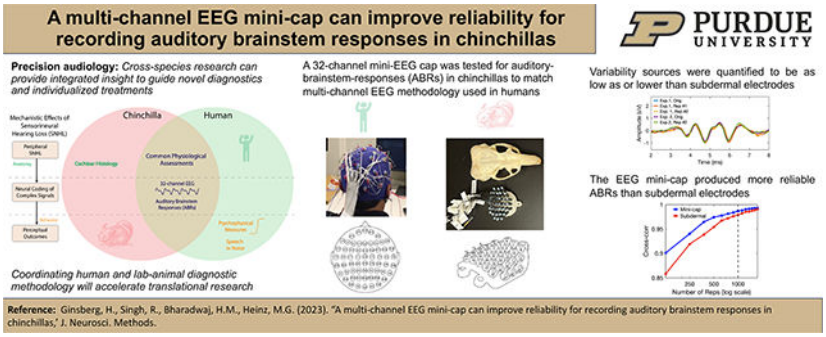
Conclusions: These results suggest the EEG mini-cap can improve translational studies of peripheral auditory evoked responses. Future work will evaluate the potential of the mini-cap to improve the reliability of more centrally evoked (e.g., cortical) EEG responses.

Graphical Abstract



Keywords

auditory neuroscience; sensorineural hearing loss; electroencephalography; EEG cap; auditory evoked potential

1. Introduction

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

In research, ABRs provide further insight into the diagnosis and localization of pathologies affecting the ascending auditory pathway that may be hidden in audiograms (Picton et al., 1973). For instance, ABR wave characteristics of amplitudes and latencies have been proposed as biomarkers for cochlear synaptopathy (CS), a covert and insidious condition

defined as the loss of synapses between sensory inner hair cells (IHC) and auditory nerve fibers (ANF). Since inner hair cells remain intact and not all synapses are lost, the clinical audiogram is often not affected (which has led this condition to be associated with “hidden hearing loss”); and suprathreshold ABR waveforms provide an alternative assay as they are altered by CS. A temporary increase in threshold after a noise exposure (i.e., a temporary threshold shift) has been correlated with CS, even in cases with no permanent threshold shift (PTS) (Hickox et al., 2017). Furthermore, it has been shown in animals after temporary threshold shift (TTS) noise exposures or with aging, wave-I amplitudes can be permanently reduced (Kujawa and Liberman, 2009) and after PTS noise exposures ABR thresholds are elevated and wave-1 latencies are reduced (Henry et al., 2011). In humans, where wave-I amplitudes are difficult to measure but wave-V is more prominent, 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 (e.g., 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.

2. Methods

2.1. Animals

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

2.2. Data collection

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

Acoustic bursts at a frequency of 4 kHz, consisting of a 5-millisecond duration burst with 0.5 millisecond linear onset and offset ramps (20 repetitions/second), were presented into the right ear of the animal. ABRs were recorded for input levels from 0 to 80 dB SPL in 10 dB increments, termed an ABR waterfall. After visually estimating the lowest level at which an ABR response was identified, one to two additional runs at 5 dB increments near the threshold were recorded. ABRs were simultaneously recorded with subdermal needle electrodes (Ambu Neuroline 74512–150/24, Ballerup, Denmark) and with the EEG mini-cap consisting of 32 active electrodes and a DRL and CMS lead (32 Channel EEG Mini-Cap, DA-AR-EMCL32, Cortech Solutions, Wilmington, NC, USA). All channels were connected

to the Biosemi ActiveTwo AD-box (Biosemi Active II system, Amsterdam, Netherlands) which further amplified and digitized the signal. Subdermal needle electrodes were inserted at the dorsal midline between the bullae (vertex), underneath the pinna and adjacent to the posterior bulla (mastoid), and at the bridge of the nose (ground) (Henry et al., 2011). Since the Biosemi ActiveTwo AD-box allowed for recording of up to eight external channels, the three needle electrodes were recorded simultaneously with the mini-cap using the same Biosemi ActiveTwo AD-box. Two 10mm in-ear gold-foiled tiptrodes (ER3–26A combined with ER3–28S) were additionally placed into the ear canal of the ipsilateral and contralateral ear which were recorded as external channels. The ipsilateral tiptrode acted as the reference channel during post-processing of the mini-cap EEG signals. The ground subdermal needle electrode placed at the bridge of the nose acted as the reference channel for the subdermal EEG signals.

2.2.1. EEG mini-cap for ABR data collection—Each of the thirty-two active Ag/AgCl electrodes with 10mm spacing between electrodes (Fig. 1A) recorded a continuous EEG signal that was visually analyzed using Actiview software (Biosemi, Amsterdam, Netherlands). The signal was digitally sampled at 16.384 kHz. Each electrode contained an inner tube with a silver wire and conductive saline-diluted EEG paste that could be moved perpendicularly through an external fixed sliding tube to ensure adequate scalp contact.

The animal's scalp was shaved using an electric razor and then further cleaned using Nair Hair removal lotion and isopropyl alcohol. A saline soaked cloth was placed on the exposed scalp until the mini-cap was placed. To place the mini-cap on the animal's head, the tympanic bullae (Fig. 1B) were located anatomically. A customized device was designed to tightly secure the mini-cap to the animal's head using firm elastic bands. The final experimental recording setup (Fig. 1C) included the mini-cap, three subdermal needle electrodes, and two tiptrodes.

Stimulus triggers corresponding to each instance of ipsilateral tone burst stimulus were sent to the Actiview software from the TDT stimulus-generation hardware. The continuous time EEG signals were aligned according to the stimulus onset and epoched in post-processing to generate the auditory evoked potential. The raw data from the mini-cap was saved as the non-epoched, non-filtered continuous time signal, along with the trigger times. For each ABR, 1000 repetitions (500 positive polarity, 500 negative) were collected and averaged.

2.3. Data analysis

Data processing was performed using the mne-python toolbox (Gramfort et al., 2013) combined with custom Matlab and Python software. First, the continuous EEG signal was referenced to an external channel. The mini-cap EEG signal was referenced to the ipsilateral ear-canal tiptrode whereas the mastoid and vertex subdermal needle electrodes were referenced to the ground subdermal electrode. Afterwards, the continuous mini-cap and subdermal needle EEG signals were band-pass filtered (300 Hz to 3000 Hz), and a 60 Hz notch filter was applied to remove any remnants of line noise. These low and high-pass filter values have been shown to produce ABR waveforms that predict auditory-nerve-fiber thresholds and frequency selectivity in hearing impaired chinchillas (Henry et al., 2011).

The continuous signal was then divided into epochs according to stimulus onset triggers. The subdermal ABR response was generated by subtracting the vertex non-inverting and reference inverting signals and averaging across all repetitions.

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

2.3.1. Thresholds and amplitudes—

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

2.4. Experimental Design

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

The study was designed (Table 1) to quantify the contributions of sources of 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.

3. Results

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

Several improvements were performed after the initial feasibility study to obtain reliable ABRs from the mini-cap. Ensuring adequate scalp contact was critical to obtaining a clean EEG signal. A customized 3D printed device attached to the stereotax ear-bar holders to hold the rubber band in place (see arrows in Fig. 1C) was created to ensure the mini-cap had adequate scalp contact. Other methodological improvements were crucial, including removing all fur from the scalp to ensure clean contact between electrodes and scalp and inserting a gel into each electrode channel that contained EEG electrode paste diluted with

saline. After implementing these improvements, clean ABRs were recorded using the mini-cap. During data collection, it was critical that the reference channel was not noisy (e.g., ipsilateral tiptrode) for post processing. The Biosemi software allowed for visualization of each EEG channel, so one crucial step in the procedure was establishing a clean reference channel during data collection to reduce additional noise when each channel was referenced after-the-fact. For example, in cases where the tiptrode needed to be inserted several times to generate a quality seal, the gold-foil partially rubbed off and added noise to the reference channel signal. Since all channels were averaged to establish a single ABR response, identifying and removing any noisy channels during data analysis was critical. As described in the PREP pipeline (Bigdely-Shamlo et al., 2015), a z-score deviation criterion was used to automatically detect amplitude differences across channels and remove any channels with a z-score greater than 2. To confirm feasibility after performing these improvements, ABRs were collected from one animal on two days, separated by one week. The feasibility of the mini-cap to produce ABR thresholds across multiple days was verified.

3.2. Mini-cap improves the reliability of ABR waveforms over subdermal electrodes

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

Cross-correlation coefficients at each intensity level for each source of variability were summarized (Fig. 2B). As level increases, the response becomes 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 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 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 five sources of variability appear minimal and do not affect the ability of the mini-cap to produce reliable, repeatable, and reproducible ABRs.

Next, a similar temporal analysis was performed on subdermal ABRs collected concurrently with the mini-cap ABRs. Since the subdermal needles were 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 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 ($F(1)=1.04$, $p=0.310$), 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 reliably produced ABRs of comparable morphology within a short duration of time (e.g., within a single experiment). Besides X-Time comparisons, 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 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 ($F(10)=143.97$, $p<0.0001$), but no interaction between the two main factors ($F(10)=0.69$, $p=0.738$). 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.

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

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). 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 they have been suggested as an assay of cochlear synaptopathy (Kujawa and Liberman, 2009). In comparison to the cross-correlation coefficient based on a temporal comparison of each ABR waveform, the ICC is a common statistical metric to assess test-retest reliability that reflects both the degree of correlation and the agreement between measurements (Koo and Li, 2016). Here, the ICC was used to quantify certain ABR biomarkers commonly used in the hearing science field. ICCs were also computed for the subdermal waveforms and compared directly to the corresponding mini-cap ICC. Since the subdermal needles were not replaced during an experiment, the X-Time source of variability could be directly compared between the two methodologies whereas X-Removal, X-Experimenter, and X-Cap were not empirically equivalent. X-Day variability could also be directly compared since the experimental design between the two methodologies was the same (i.e., two experiments conducted one week apart). An ICC value less than 0.40 was considered poor, between or equal to 0.4 and 0.60 was considered fair, between or equal to 0.6 and 0.75 was considered good, and greater than or equal to 0.75 was considered excellent (based on Rentzsch et al., 2008).

The mini-cap ICCs for both threshold and wave-1 amplitude portray excellent reliability for each of the four sources of variability during a single experiment, excluding threshold comparisons across multiple days. The subdermal ICC that represents a short duration of time (e.g., X-Time) appears consistently lower, especially for thresholds, when directly compared to the corresponding mini-cap ICC, suggesting less favorable reliability than the mini-cap in their ability to produce reliable, repeatable, and reproducible ABRs within a

single experiment. Overall, these results indicate the mini-cap was the more reliable and less variable methodology for ABR data collection within a single experiment. The only source of variability that appears to have below excellent reliability for both mini-cap and subdermal methods (besides for mini-cap wave-1 amplitudes) is X-Day. For wave-1 amplitudes, the mini-cap shows excellent reliability (ICC = 0.89) across two days, whereas the subdermal approach shows below-moderate reliability (ICC = 0.30), suggesting the mini-cap has an improved capability to produce reliable biomarkers (e.g., wave amplitudes) across two days. Thresholds are determined for each experiment, but there is the possibility of slight changes in threshold when measured a week apart based on animal physiology, especially within the 5 dB range (which is audiologically insignificant as 5-dB is the typical step size in manual audiometric tracking by an audiologist). It was, in fact, confirmed that when directly analyzing thresholds from the first day to the second day, most of the thresholds were within 5 dB of one another (70% for mini-cap, 63% for subdermal). The mean difference between the thresholds across two days was 5.12 dB for the mini-cap and 6.22 dB for the subdermal needles. The ICC suggests fair and poor reliability across two days for mini-cap and subdermal needles respectively, but when solely considering the thresholds, it does appear that both methodologies are able to produce accurate ABR thresholds (within 5 dB) across experiments on different days. Moreover, a 5-dB difference using subdermal needles is accepted for animal studies, so the mini-cap results exhibiting a 5-dB difference across two days are acceptable. It is hypothesized that the placement of the subdermal needles can vary from each experiment, as only anatomical landmarks are utilized. However, the mini-cap placement is placed over the majority of the scalp covered by the 32 mini-cap electrodes, so it is less likely that placement of the mini-cap will affect thresholds or waveform amplitudes (as indicated by X-Removal ICC of 0.80).

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

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

A correlation analysis was performed to further quantify the number of repetitions necessary to obtain a highly correlated response to the averaged waveform across all repetitions, labeled the gold standard. The signal window of each of the twenty boot responses was cross correlated to the signal window of the gold standard response. Next, the twenty cross-correlation coefficients were averaged together to produce a single cross-correlation coefficient for each repetition count. A greater averaged cross-correlation coefficient indicates that the number of repetitions averaged in the boot response was adequate to produce a clear and concise ABR response. Overall, it was found that about 1000

repetitions are adequate for the mini-cap methodology to produce a highly correlated ABR response (Fig. 3G). In the mini-cap experiments performed for this research, 1000 repetitions were collected for each response. At 1000 repetitions, there is a plateauing, indicating additional repetitions after 1000 are of diminishing value. The correlation analysis results were compared for the mini-cap and subdermal methods (Fig. 3H). In comparison to the subdermal method, the mini-cap required fewer repetitions to produce an equivalently correlated response. At all repetition counts, the mini-cap correlation values were greater, suggesting that fewer repetitions are necessary in the mini-cap method to obtain a clear and reliable ABR response.

4. Discussion

4.1. Summary of mini-cap variability

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

4.2. Comparison to subdermal variability

Simultaneous data collection with the mini-cap and subdermal needle electrodes allowed for direct comparisons between the two methodologies. Two of the five identified potential sources of variability, X-Time and X-Day, were equivalent in methodology since the subdermal needles were not removed or replaced during a single experiment (i.e., day). For comparisons across a short period of time, both the mini-cap and subdermal method produced reliable ABRs when evaluating waveform morphology (Fig. 2C). However, both thresholds and wave-1 amplitudes were statistically more robust using the mini-cap

than the subdermal needles, especially when evaluating threshold ICCs (mini-cap ICC = 0.98, subdermal ICC = 0.6). In general, both ABR methods are capable of producing reliable ABRs when the apparatus (i.e., mini-cap or needles) are not altered during a single experiment ("X-Time"). When the subdermal needles were altered across a different experiment on a second day ("X-Day"), there was a convincing difference when looking at the subdermal method's capability to produce robust ABRs in comparison to the mini-cap (mini-cap ICC = 0.49, subdermal ICC = 0.04). Statistically, two waveforms collected using the mini-cap across two experiments were more consistent regarding waveform morphology than two waveforms collected using subdermal needle electrodes across two experiments (Fig. 2D). Additionally, both thresholds and wave-1 amplitudes were more robust across two days using the mini-cap (Table 2).

4.3. Mini-cap benefits and limitations

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

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

In this study, the mini-cap was used to collect an auditory evoked response originating from deep-seated sources in the brainstem. Another promising benefit of the mini-cap is that other more centrally evoked potentials can also be recorded. In these cortical responses (closer to electrodes), there is the likelihood that individual channels can provide unique responses, which would provide a means to complete source localization of an evoked potential. To explore this benefit of using the multi-channel mini-cap to identify between-channel differences, a cortical response (4-kHz amplitude-modulated SAM noise) was measured in an awake chinchilla. The reference channel for this cortical response was the average of all channels. The topological map of the mini-cap was placed onto a phantom scalp (Fig. 4A). First, topological mapping was performed using an ABR response collected using the mini-cap (Fig. 4B). As seen visually in the response, at the chosen time points,

the responses across all channels show similar magnitude responses. The ABR originates from deep-seated sources and, hence, between-channel differences are minimal. Averaging across the channels, however, does appear to reduce the noise in the final ABR response. Conversely, the cortical response shows significant between-channel differences, especially from the onset response (Fig. 4C). The mini-cap can show between-channel differences from EEG scalp recordings on a chinchilla when the response is more centrally evoked. For these auditory evoked potentials where the goal is to identify channel differences in magnitude across time, the mini-cap is especially useful.

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

4.4. Future directions

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

4.5. Animal to human translation

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights :

- A mini-EEG cap was tested for ABRs in chinchillas to match methodology used in humans
- Reliable multi-channel EEG data from chinchillas were obtained using the mini-cap
- Sources of variability quantified to be as low as or lower than subdermal electrodes
- Mini-cap produces more reliable ABRs regarding waveform morphology than subdermal
- Mini-cap ICCs for threshold and wave-1 amplitude showed excellent reliability

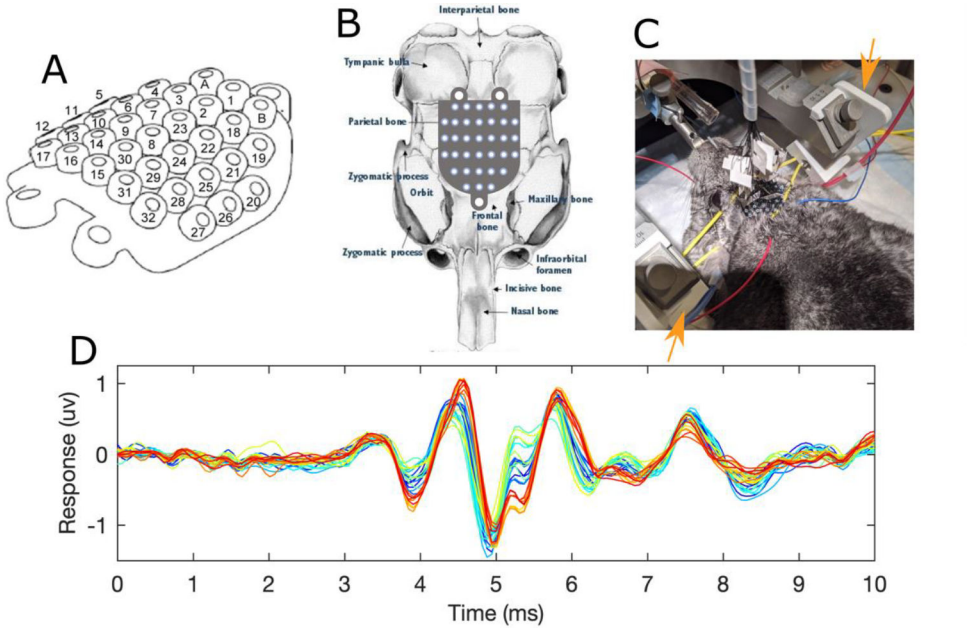

Figure 1. EEG mini-cap and associated experimental setup to record ABRs in chinchillas.

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

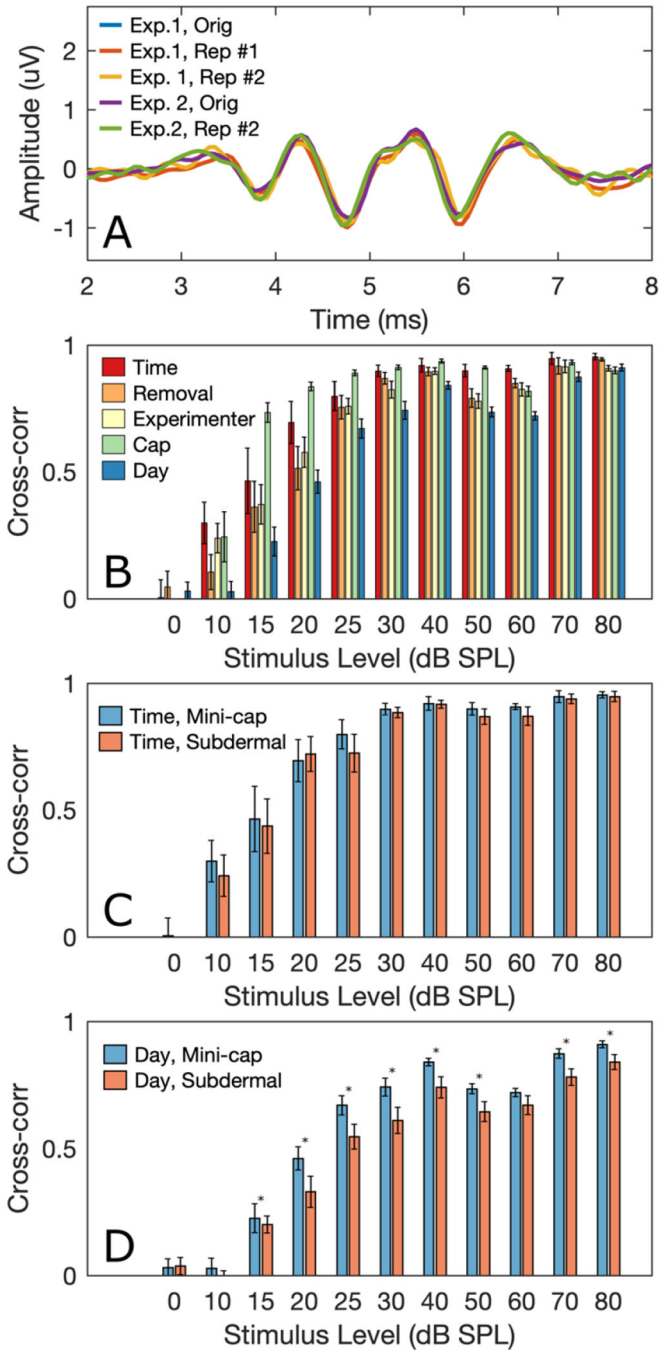

Figure 2. Comparisons of temporal waveforms across conditions.

(A) Five 40-dB SPL responses (signal window, 2–8ms) from each waterfall collected during a single experiment were compared to quantify the contribution of each source of variability. For example, an X-Time comparison would include comparing Experimenter #1, Original waveform with Experimenter #1, Replicate #1 waveform. (B) Summary of five sources of variability using the mini-cap to record ABRs in chinchillas. All comparisons for each sound level were categorized by the source of variability and cross-correlation coefficients were averaged together. As expected, the two sources of variability that notably affected

the cross-correlation coefficient were Experimenter and Day. Overall, across each source of variability, the mini-cap was able to produce reliable, repeatable, and reproducible ABRs. (C) Comparison of mini-cap and subdermal variability, X-Time when a second response was recorded shortly after the first ("Replicate #1"). Significant post-hoc t-tests are indicated with an asterisk (alpha = 0.05). The X-Time cross-correlation was statistically equivalent when using the mini-cap in comparison to the subdermal needle electrodes. Within a short period of time under the same conditions, the mini-cap and subdermal needles can produce reliable and repeatable ABRs. (D) Comparison of mini-cap and subdermal variability, X-Day when a second response was recorded on a different day than the first. Significant post-hoc t-tests are indicated with an asterisk (alpha = 0.05). The mini-cap produced statistically less variable ABRs across two days than the subdermal needles. When comparing ABR responses from two different experiments on separate days, the mini-cap showed greater reliability and reproducibility.

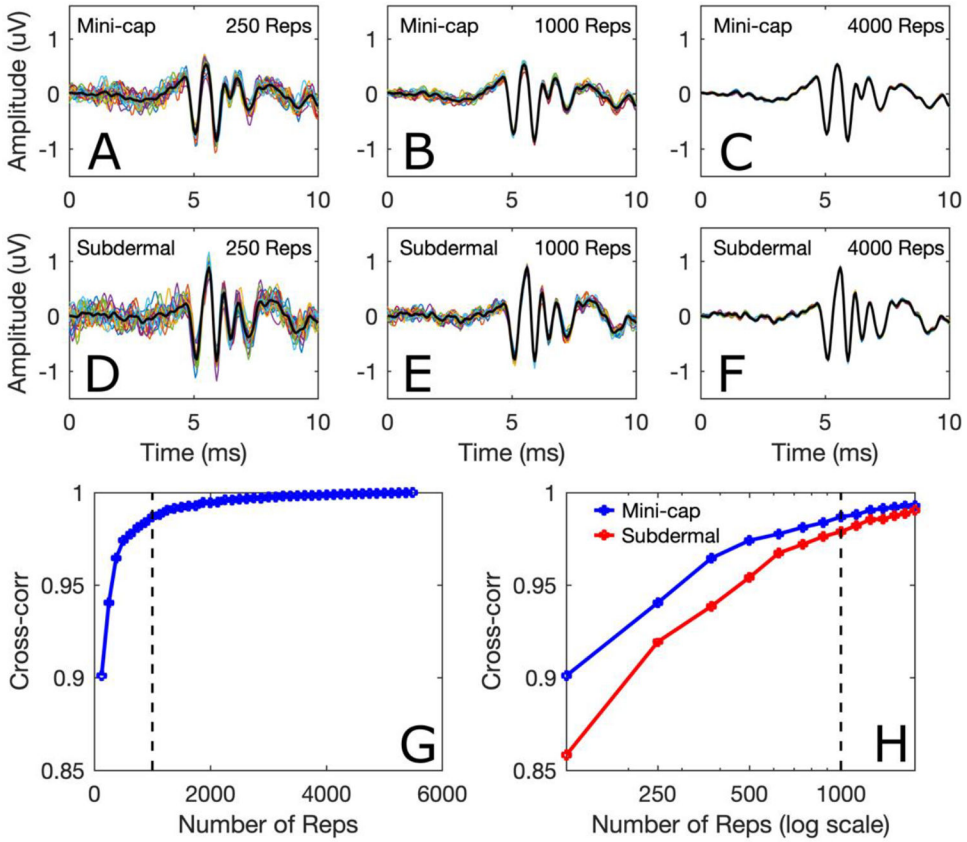

Figure 3. Bootstrapping analyses of mini-cap versus sub-dermal efficiency.

(A, B, C) Bootstrapping results using the mini-cap method. For each stimulus repetition number, twenty subsets of the total mini-cap repetitions were randomly chosen and averaged together to produce twenty total bootstrap responses. (D, E, F) Bootstrapping results using the subdermal needle method. (G) Bootstrapping analysis for mini-cap only, linear x-axis. The signal window (2–8ms) from each bootstrap response was cross correlated with the signal window (2–8ms) of the gold standard, the response averaged across all repetitions (N=5500). These 20 cross-correlation values were averaged together to produce a correlation value representing each repetition number. From this analysis, 1000 repetitions (see black dotted line) are seen to be acceptable to attain a highly correlated mini-cap response (waveform correlation coefficient > 0.98). Thus, in our typical experimental setup, we chose to record 1000 repetitions for each sound level. (H) Bootstrapping analysis for mini-cap and subdermal needles, on logarithmic x-axis. For all repetition values, the mini-cap correlations (i.e., reliability) are higher than for the subdermal results. Thus, these analyses show that in comparison to the subdermal method, the mini-cap requires fewer repetitions to produce an equitably correlated response.

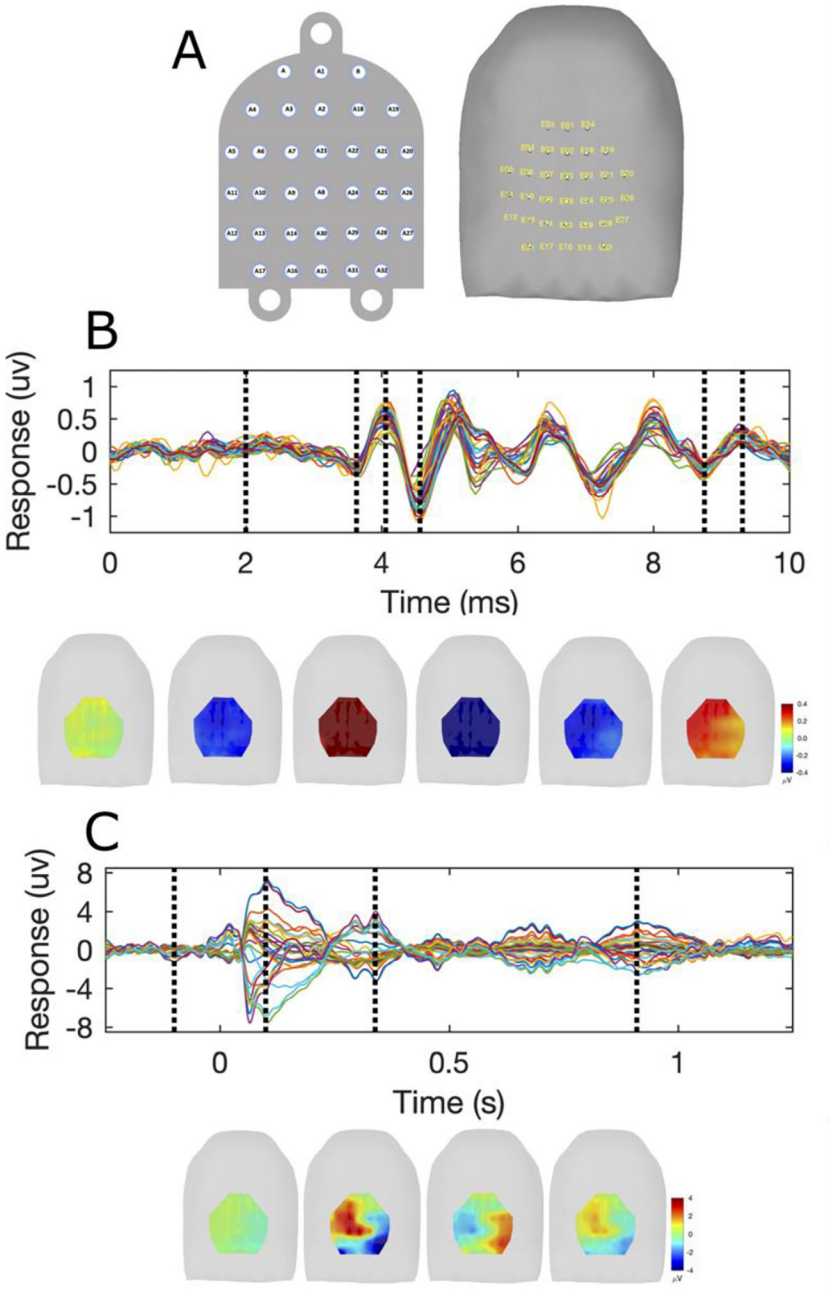

Figure 4. Topological mapping shows differences between brainstem and cortical responses.

(A) The 32-channel mini-cap layout (left) was placed onto a topological layout (right) to visualize the between-channel differences within a response. (B) Topological map of an ABR response at six different time points (labeled as T1-T6 with black dotted lines). Overall, all channels of the mini-cap appear to show similar magnitude responses at each of the six time points. Since the ABR originates from deep-seated sources within the brain, between-channels differences were minimal. (C) Topological map of a cortical response to 4-Hz amplitude modulated SAM noise in an awake chinchilla at four different time points

(labeled as T1-T4 with black dotted lines). From the onset response, cortical activation is observable, confirming the mini-cap can generate responses where between-channel differences are significant. Therefore, the neural source of the response determines whether between-channel differences are significant or not.

Table 1.

Experimental design to characterize five sources of variability in mini-cap ABRs.

Comparison Name	Measurement qualities tested	Variability Source	Consistent Variables	Comparison Examples	Possible Comparison per Experiment	Total Experimental Comparisons
X-Time	Reliability Repeatability	Short duration of time	Measurand Experimenter Instrument Day	O vs. R1 E.g. • D1-E1-C1-O vs. D1-E1-C1-R1 • D1-E2-C1-O vs. D1-E2-C1-R1	2	9
X-Removal	Reliability Repeatability	Replacement of the minicap	Measurand Experimenter Instrument Day	O vs. R2 R1 vs. R2 E.g. • D1-E1-C1-O vs. D1-E1-C1-R2 • D1-E1-C1-R1 vs. D1-E1-C1-R2	4	13
X-Experimenter	Reliability Reproducibility	Different Experimenter	Measurand Instrument Day	E1 vs. E2 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	9	24
X-Cap	Reproducibility	Different mini-cap9	Measurand Day Experimenter	C1 vs. C2 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	9	10
X-Day	Reliability Reproducibility	Longer duration of time (>= 1 day)	Measurand Experimenter Instrument	D1 vs. D2 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	18	29

[a]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.

Table 2.

Intraclass correlation coefficients (ICCs) for thresholds and suprathreshold wave-1 amplitudes for mini-cap and subdermal ABRs.

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)

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 ≤ ICC < 0.60: fair; 0.6 ≤ ICC < 0.75: good; ICC ≥ 0.75: excellent.