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TITLE:

A Method to Study Adaptation to Left-Right Reversed Audition

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SHORT ABSTRACT:
The present study proposes a protocol to investigate the adaptation to left-right reversed audition achieved only by wearable devices, using neuroimaging, which can be an effective tool for uncovering the adaptability of humans to a novel environment in the auditory domain.

LONG ABSTRACT:
An unusual sensory space is one of the effective tools to uncover the mechanism of adaptability of humans to a novel environment. Although most of the previous studies have used special spectacles with prisms to achieve unusual spaces in the visual domain, a methodology for studying the adaptation to unusual auditory spaces has yet to be fully established. This study proposes a new protocol to set-up, validate, and use a left-right reversed stereophonic system using only wearable devices, and to study the adaptation to left-right reversed audition with the help of neuroimaging. Although individual acoustic characteristics are not yet implemented, and slight spillover of unreversed sounds is relatively uncontrollable, the constructed apparatus shows high performance in a 360° sound source localization coupled with hearing characteristics with little delay. Moreover, it looks like a mobile music player and enables a participant to focus on daily life without arousing curiosity or drawing attention of other individuals. Since the effects of adaptation were successfully detected at the perceptual, behavioral, and neural levels, it is concluded that this protocol provides a promising methodology for studying adaptation to left-right reversed audition, and is an effective tool for uncovering the adaptability of humans to a novel environments in the auditory domain.

INTRODUCTION:

Adaptability to a novel environment is one of the fundamental functions for humans to live robustly in any situation. One effective tool for uncovering the mechanism of environmental adaptability in humans is an unusual sensory space that is artificially produced by apparatuses. In the majority of the previous studies dealing with this topic, special spectacles with prisms have been used to achieve left-right reversed vision[1–5] or up-down reversed vision[6, 7]. Furthermore, exposure to such vision from a few days to more than a month has revealed perceptual and behavioral adaptation[1–7] (*e.g.*, capability to ride a bicycle[2, 5, 7]). Moreover, periodic measurements of the brain activity using neuroimaging techniques, such as electroencephalography (EEG)[1], magnetoencephalography (MEG)[3], and functional magnetic resonance imaging (fMRI)[2, 4, 5, 7], have detected changes in the neural activity underlying the adaptation (*e.g.*, bilateral visual activation for unilateral visual stimulation[4, 5]). Although the participant's appearance becomes strange to some extent and great care is needed for the observer to maintain the participant's safety, reversed vision with prisms provides precise three-dimensional (3D) visual information without any delay in a wearable manner. Therefore, the methodology for uncovering the mechanism of environmental adaptability is relatively established in the visual domain.

In 1879, Thompson proposed a concept of pseudophone, "an instrument for investigating the laws of binaural audition by means of the illusions it produces in the acoustic perception of space"[8]. However, in contrast to the visual cases[1–7], few attempts have been made to study the adaptation to unusual auditory spaces, and no noticeable knowledge has been obtained to date. Despite a long history of developing virtual auditory displays[9, 10], wearable apparatuses for controlling 3D audition have rarely been developed. Hence, only a few reports examined the adaptation to left-right reversed audition. One traditional apparatus consists of a pair of curved trumpets that are crossed and inserted into a participant's ear canals in a contrariwise manner[11, 12]. In 1928, Young first reported the use of these crossed trumpets and wore them continuously for 3 days at most or a total of 85 h to test adaptation to left-right reversed audition. Willey *et al.*[12] retested the adaptation in three participants wearing the trumpets for 3, 7, and 8 days, respectively. The curved trumpets easily provided left-right reversed audition, but had an issue with the reliability of spatial accuracy, wearability, and strange appearance. A more advanced apparatus for the reversed audition is an electronic system in which left and right lines of head/earphones and microphones are reversely connected[13, 14]. Ohtsubo *et al.*[13] achieved auditory reversal using the first ever binaural headphone-microphones that were connected to a fixed amplifier and evaluated its performance. More recently, Hofman *et al.*[14] cross-linked complete-in-canal hearing aids and tested adaptation in two participants that wore the aids for 49 h in 3 days and 3 weeks, respectively. Although these studies have reported high performance of sound source localization in the front auditory field, the sound source localization in the backfield and a potential delay of electrical devices have never been evaluated. Especially in Hofman *et al.*'s study, the spatial performance of the hearing aids was guaranteed for the front 60° in the head-fixed condition and for the front 150° in the head-free condition, suggesting unknown omniazimuth performance. Moreover, the exposure period may be too short to detect phenomena related to the adaptation as compared with the longer cases of reversed vision[2, 4, 5]. None of these studies have measured brain activity using neuroimaging techniques. Therefore, the uncertainty in spatiotemporal accuracy, the short exposure periods, and the non-utilization of neuroimaging could be reasons for the small number of reports and the limited amount of knowledge on adaptation to left-right reversed audition.

Thanks to the recent advances in wearable acoustic technology, Aoyama and Kuriki[15] succeeded in constructing a left-right reversed 3D audition using only wearable devices that recently became available and achieved the omniazimuth system with high spatiotemporal accuracy. Moreover, approximately a 1-month exposure to reversed audition using the apparatus exhibited some representative results for MEG measurements. Based on this report, we describe, in this article, a detailed protocol to set-up, validate and use the system, and to test the adaptation to left-right reversed audition with the help of neuroimaging that is performed periodically without the system. This approach is effective for uncovering the adaptability of humans to a novel environment in the auditory domain.

PROTOCOL:

All methods described here have been approved by the Ethics Committee of Tokyo Denki University. For every participant, informed consent was obtained after the participant received a detailed explanation of the protocol.

1. Setup of the Left-Right Reversed Audition System

1.1. Setup of the Reversed Audition System without a Participant

1.1.1. Prepare a linear pulse-code-modulation (LPCM) recorder, binaural microphones, and binaural in-ear earphones.

1.1.1.1. First, connect the left and right lines of the microphones crossly to the LPCM recorder so that left-right reversed analogue sound signals are digitalized.

1.1.1.2. Second, connect the left and right lines of the earphones straight through to the recorder so that the reversed digitalized signals are immediately played.

1.1.1.3. Finally, put the bodies of the microphones and the earphones together for each ear with slight isolation by sound proofing materials, and cover the microphones with dedicated windscreens for suppressing the wind noise.

Note: In the case of employing the binaural earphone-microphones as binaural earphones, do not use the earphone parts in order to reduce the spillover of the sounds that go through the microphone parts.

1.1.2. Insert rechargeable batteries and a large-capacity high-speed memory card into the LPCM recorder and turn it on. Set the recording conditions properly in such a manner that the sound signals are recorded on the memory card as an LPCM format at a sampling rate of 96 kHz with a 24-bit depth.

1.1.3. Place the body of the system into a pocket-sized bag.

1.2. Setup of the Reversed Audition System with a Participant

1.2.1. Instruct a participant to insert the earphones of the reversed audition system tightly into the ear canals.

1.2.2. Disconnect the lines for the left and right microphones and connect the dominant-ear side of the microphone straight through to the recorder. Subsequently, instruct the participant to take off and put on the dominant-ear side of the system repetitively while adjusting the sound volume of the recorder to make the subjective loudness of direct (normal) and indirect (reversed) sounds equal (as close as possible). Check the loudness for the non-dominant ear as well, and connect all the lines of the system back again.

1.2.3. Place the system into the participant's pocket, fix the cords on the participant's clothes appropriately to prevent them from becoming entangled, and pick up unwanted noises.

2. Validation of the Left-Right Reversed Audition System

Note: Perform the following steps to validate the left-right reversed audition system, irrespective of experiments studying adaptation to left-right reversal.

2.1. Validation of the Sound Source Localization of the Reversed Audition System

2.1.1. Locate a digital angle protractor whose initial direction is defined as 0° at the center of an anechoic room, and assume a virtual circle centered at this point with a radius of 2 m. Along the virtual circle, mark 72 possible sound sources at every 5° from -180° to 175° in a clockwise manner, and set up plane-wave speakers at these points directed towards the center of the circle.

2.1.2. Set up a video camera near the center of the room to record the display of the digital protractor.

Note: Since the display of the protractor moves with the protractor's body, the field of view of the video should be large enough to cover all the possible areas. Moreover, the video camera should be carefully placed in order to not disturb the participant's sitting position and the sound presentation.

2.1.3 Prepare for two sessions of sound source localization: in the first session, the participant does not put on the reversed audition system. In the second session, the participant puts on the equipment, calibrates it, and checks the system (as explained in step 1.2) as quickly as possible.

2.1.4. Guide the participants to sit comfortably and blindfolded at the center of the circle facing a zero-degree sound source and wait for the experiment to start.

Note: Start validating the sound source localization here.

2.1.5. Conduct two sessions of sound source localization. In both sessions, have the participant use the protractor to indicate the perceived sound direction as precisely as possible without moving the head.

2.1.6. For each session, start video-recording the angle display of the protractor and present 1000-Hz sounds at 65-dB sound pressure level (SPL) from any of the sound sources using software stimulation.

Note: Here we use MATLAB with the Psychophysics Toolbox[16–18]. Although this toolbox is commonly used to present sounds, any reliable stimulation software can also be used. The sound at one location is randomly switched to the sound at another location every 10 s in such a way that each location is used once.

2.1.7. After each session, stop the video-recording and instruct the participants to take a break for sufficient amount of time.

Note: Finish validating the sound source localization here.

2.1.8. Read the trial-by-trial perceptual angles displayed on the protractor from the recorded video, and evaluate the spatial performance of the reversed audition system by comparing the perceptual angles in the normal and the reversed conditions against the physical angles defined by the direction of sound sources.

2.2. Validation of the Delay of the Reversed Audition System

2.2.1. Put the reversed audition system on a desk in a calm room with no participants.

2.2.2. Disconnect a line to the left microphone, and place a plane-wave speaker and the left earphone as close as possible to the right microphone.

Note: Start validating the delay of the system here.

2.2.3. Start recording direct (normal) sounds from the speaker and indirect (reversed) sounds from the left earphone simultaneously through the right microphone.

2.2.4. Present 1-ms click sounds from the speaker with a moderate inter-stimulus interval at 65-dB SPL using a psychophysics software toolbox.

2.2.5. After a sufficient number of trials, stop presenting and recording the sounds.

2.2.6. In order to confirm the symmetrical configuration of the system, repeat the same steps above using the right earphone and the left microphone.

Note: Finish validating the delay of the system here.

2.2.7. Read the recorded sound data using software (*e.g.*, MATLAB) and evaluate the difference between the onset timings of the direct (normal) sounds and indirect (reversed) sounds, which corresponds to a potential delay caused by the time spent passing through the electrical path in the system.

3. Studying the Adaptation to Left-Right Reversed Audition

3.1. Procedure of the Exposure to Reversed Audition

3.1.1. Remind the participants repeatedly of their right to quit the exposure at any time.

Note: Stop the exposure as soon as possible if the participant reports sickness or if an observer notices any sign that the participant wants to quit the exposure for any reason.

3.1.2. Prepare a sufficient number of spare rechargeable batteries and large-capacity high-speed memory cards to allow the participant to replace them at anytime.

Note: Start the exposure to the reversed audition here.

3.1.3. Instruct the participant to wear, calibrate, and check the reversed audition system by themselves daily, as explained in step 1.2. Perform the same procedure each time the participant wears the system after each interruption.

3.1.4. Instruct the participant to perform daily-life activities while wearing the system continuously for approximately a month, except while sleeping, bathing, neuroimaging, and other emergency times. In these cases, ask participants to remove the system and immediately insert earplugs into their ears to prevent recovery of adaptation.

Note: Although it is ideal for the participant to wear the system all day and night, it is strongly recommended that the system not be worn while sleeping and bathing in order to prevent unexpected loud noises and electrical shocks, respectively.

3.1.5. Replace the batteries and memory cards routinely before battery exhaustion and memory overcapacity, respectively. Remove the system and replace it with earplugs during sleep and bath times, or perform the replacement quickly in a silent place without producing any sound.

3.1.6. When a participant needs to move around outside, drive the participant in a car, accompany the participant on the move, or ask them to use safe means of transportation for acts performed alone.

Note: Great care should be taken by the researcher in order to not endanger the participant's safety during the exposure period, especially when the participant goes outside. Prohibit the participant from performing any dangerous behaviors.

3.1.7. In order to facilitate adaptation, instruct the participant to experience situations involving high auditory input, such as walking in a shopping mall or a campus, having a conversation with more than two persons, and playing 3D video games, for as long as possible.

3.1.8. Instruct the participant to keep a diary or provide a subjective report to an observer as frequently as possible about perceptual and behavioral changes, experienced events, and anything that the participant notices.

3.1.9. After the target exposure period, instruct the participant to take off the reversed audition system.

Note: Terminate the exposure to the reversed audition here. It is also important to follow up about the perceptual and behavioral changes in order to examine the recovery process from the adaption to left-right reversed audition.

3.2. Neuroimaging During the Exposure to Reversed Audition

3.2.1. Instruct the participant to train on a task that will be used during the neuroimaging experiments as sufficiently as possible.

3.2.1.1. For example, train the participant to perform a selective reaction time task in two conditions, compatible and incompatible[15]. The compatible condition consists of responding immediately to the right-ear sound with the right index finger and to the left-ear sound with the left index finger. The incompatible condition consists of responding immediately to the right-ear sound with the left index finger and to the left-ear sound with the right index finger.

3.2.1.2. Use 1000-Hz sounds at 65-dB SPL for 0.1 s with an inter-stimulus interval of 2.5 – 3.5 s, which appears pseudorandomly on either ear side, using a psychophysics software toolbox.

Note: Start a series of neuroimaging experiments here.

3.2.2. Before the exposure to reversed audition, conduct a neuroimaging experiment under the trained task.

3.2.2.1. For example, record either MEG or EEG responses, as well as the left and right finger responses under the selective reaction time task[15]. The task consists of two compatible and two incompatible blocks that are alternatively arranged with an inter-block interval of at least 30 s, and with sounds appearing 80 times for each block through the inserted earphones with plastic ear tubes.

3.2.2.2. For the MEG/EEG recording, set the sampling rate at 1 kHz and the analog recording passband at 0.03 – 200 Hz.

Note: Although a 122-channel MEG system was used in Aoyama and Kuriki[15], a multi-channel EEG system is also suitable for this protocol.

3.2.3. During approximately a 1-month exposure to reversed audition, conduct neuroimaging experiments under the trained task every week without the reversed audition system in exactly the same way as in the pre-exposure experiment (step 3.2.1).

Note: The system is removed immediately before and put on immediately after each experiment.

3.2.4. One week after the exposure, conduct a neuroimaging experiment under the trained task in exactly the same way as the pre-exposure experiment (step 3.2.1).

Note: Finish a series of neuroimaging experiments here.

3.2.5. Analyze the collected data before, during, and after the exposure to left-right reversed audition.

3.2.5.1. For example, after rejecting the epochs contaminated with eye-related artifacts, removing the offset in the pre-stimulus interval, and setting the low-pass filtering at 40 Hz, average the MEG/EEG data from 100 ms before to 500 ms after the sound onset for the stimulus-response compatible and incompatible conditions[15].

3.2.5.2. Using an MNE software package[19, 20], estimate the sources of the brain activity with dynamic statistical parametric maps (dSPMs) overlaid on cortical surface images.

3.2.5.3. Additionally, quantify the intensities of brain activity with minimum-norm estimates (MNEs) for each time point of the averaged data.

3.2.5.4. Furthermore, calculate the auditory-motor functional connectivity from single-trial zero-mean MEG/EEG data from 90 to 500 ms after the sound onset for each condition (*e.g.,* MATLAB with the Multivariate Granger Causality Toolbox)[21].

3.2.5.5. For the behavioral data, calculate the mean reaction times for the stimulus-response compatible and incompatible conditions.

REPRESENTATIVE RESULTS:
The representative results shown here are based on Aoyama and Kuriki[15]. The present protocol achieved left-right reversed audition with high spatiotemporal accuracy. **Figure 1** shows the sound source localization in directions over 360° before and immediately after putting on the left-right reversed audition system (**Figure 1A**), in six participants, as indicated by the cosine similarity. As shown in **Figure 1B**, the perceptual angles in the normal condition were quite well

correlated with the physical angles (positive correlation, adjusted R^2 = 0.99). The perceptual angles in the reversed condition were also well correlated with the physical angles (negative correlation, adjusted R^2 = 0.96; see also **Figure 4** in Aoyama and Kuriki[15]), although there existed a slight perceptual bias toward the counterclockwise rotation, especially for sounds coming from the right-front and the left-back directions. Notably, the perceptual angles in the reversed condition were more correlated with the oppositely arranged perceptual angles in the normal condition (adjusted R^2 = 0.98) than the physical angles, as shown in **Figure 1C**. Furthermore, a potential delay of the system was estimated to be a constant 2 ms. The present protocol also achieved a natural wearing appearance, like listening to music with a mobile music player, thereby avoiding any stress of being noticed by other individuals.

[Place **Figure 1** here]

The present protocol revealed perceptual changes to the reversed audition from a relatively early stage during the approximately 1-month exposure. Although a feeling of strangeness was reported just after the exposure, it began to decrease within a week of the exposure and continued to drop further over time. Mirror-image sounds were gradually perceived as normal, which also occurred with visual information and movements. One week after the end of the exposure period, all changes returned to the pre-exposure level. The present protocol detected not only perceptual but also behavioral and neural changes underlying the adaptation. **Figure 2** shows changes in behavioral and neural responses during the selective reaction time task over the exposure time in a representative participant. As shown in **Figure 2A**, the mean reaction times for response-incompatible sounds were overall longer than those for response-compatible sounds from the pre-exposure period to the third week, but became slightly shorter in the fourth week. This relative inversion followed the transient elongation of the mean reaction times irrespective of compatibility in the second week. After the exposure, all mean reaction times returned to the initial level. The MNE intensities of the left and right N1m components exhibited similar trends to the mean reaction times, as shown in **Figure 2B**, although the compatible-incompatible relationship was inversed. The N1m components are distinct auditory evoked fields observed at about 90 ms after sound onset, and their source was confirmed to be located in the bilateral superior temporal planes using dSPMs. Overall, the intensities in the stimulus-response compatible conditions were higher than those in the incompatible conditions from the pre-exposure period to the third week, but were slightly lower in the fourth week. This relative inversion followed the transient enhancement of the intensities irrespective of compatibility and laterality in the second week. After the exposure, they returned to the initial levels.

[Place **Figure 2** here]

Furthermore, the present protocol revealed changes in the functional connectivity across the left and right auditory and motor areas during the selective reaction time task in two participants, as shown in **Figure 3**. The functional connectivity was tested with the Granger causality test at a threshold of $p < 0.05$. Initially, these auditory-motor areas communicated with each other irrespective of stimulus and response. However, after exposure to the reversed audition, the auditory-motor connectivity became unstable. Notably, in the second week, the auditory-motor connectivity was disrupted drastically, especially in the right motor-to-auditory feedback and left-to-right motor communication. Immediately after that, the connectivity recovered at the level of the first week, and returned to the initial level after the exposure.

[Place **Figure 3** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Sound source localization in 360° directions, before and immediately after putting on the left-right reversed audition system, in six participants. (A) The constructed left-right reversed audition system. **(B)** Cosine similarity between perceptual angles and sign-regulated physical angles in the normal (blue) and reversed (red) conditions plotted against (unregulated) physical angles, respectively. While the physical angles are directly used for the cosine similarity in the normal condition, the signs of physical angles are inverted in the reversed condition. **(C)** Cosine similarity between perceptual angles in the reversed condition and oppositely arranged perceptual angles in the normal condition plotted against physical angles (purple). This figure has been modified from Aoyama and Kuriki[15].

Figure 2: Behavioral and neural responses during the selective reaction time task in a representative participant. (A) Mean reaction times for stimulus-response compatible and incompatible conditions. **(B)** Left and right auditory N1m intensities for stimulus-response compatible and incompatible conditions, as evaluated by minimum-norm estimates. Yellow zones indicate a period exposed to left-right reversed audition. This figure has been modified from Aoyama and Kuriki[15].

Figure 3: Auditory-motor functional connectivity as tested by Granger causality tests during the selective reaction time task in two participants. Red, yellow, and no arrow(s) indicate the number of participants who showed significance at a threshold of $p < 0.05$ ($N = 2$, 1, and 0, respectively). LM and RM denote left and right motor areas, respectively, and LA and RA denote left and right auditory areas, respectively. This figure has been modified from Aoyama and Kuriki[15].

DISCUSSION:

The proposed protocol aimed to establish a methodology for studying adaptation to left-right reversed audition as an effective tool for uncovering the adaptability of humans to a novel auditory environment. As evidenced by the representative results, the constructed apparatus achieved left-right reversed audition with high spatiotemporal accuracy. Although the previous apparatuses for reversed audition[11–14] were mostly reliable in the front auditory field, this protocol provides high performance in a 360-degree sound source localization coupled with hearing characteristics. Moreover, a potential delay of 2 ms lost through the electrical path in the system, which has never been evaluated in other electronic apparatuses[13, 14], is considered to be negligible due to the human temporal auditory acuity[22]. Unlike the traditional apparatus of curved trumpets[11, 12] with a strange appearance and uncomfortable fit, the reversed audition system used in the present protocol looks like a mobile music player and enables a participant to focus on daily life without arousing curiosity or drawing attention of other individuals. At this point, it is even superior to the apparatuses for reversed vision using prisms[1–7]. Indeed, as evidenced by the representative results, around 1 month of wearing the apparatus achieved adaptation to left-right reversed audition at the perceptual, behavioral, and neural levels. As in previous protocols[11–14], it was quite challenging to perform experiments with many participants, due to the long research period and difficulties in participant recruitment. However, individual results provided reliable, rich and valuable information about auditory adaptation (for details, see Aoyama and Kuriki[15]). Therefore, the present protocol is much better suited for facilitating the adaptation to reversed audition than any other previous protocols that have failed to noticeably advance knowledge about the adaptation[11–14].

As a basic premise, the highest priority in the proposed protocol should be the participant's safety, health, and will during the exposure to the reversed audition. In order to preserve these, an observer must take great care and communicate with the participant as much as possible, especially during and immediately after the exposure period. If any of the conditions are unsatisfactory, an observer must stop the exposure immediately. Apart from that, one of the most critical steps of the protocol is to instruct the participant to experience situations involving high auditory input for as long as possible. Unlike visual cases where the retinal input has fine spatial resolution[23, 24], exposure to reversed audition is less effective due to low auditory spatial resolution[25, 26]. In addition, non-environmental auditory events rarely occur in daily life, unless a person is subjected to high auditory inputs. Moreover, it is not enough for sounds to be directional and lateralized, but the sounds should also be accompanied by other sensory information or movement to facilitate the adaptation. Without this step, lower, or even no adaptive effect, is expected. Another critical step is to instruct the participant to train on a task as sufficiently as possible before the first neuroimaging experiment so that task performance converges at a certain level. This is necessary for a precise evaluation of the adaptive effect on behavioral and neural responses, because it is quite difficult to dissociate between the adaptive and the task learning effects over time. Preliminary reduction of the task learning effect thus promotes further analysis of the adaptation.

The present protocol can be flexibly modified, depending on the availability of experimental equipment and the purpose of study. For example, to validate the sound source localization of the reversed audition system, it is acceptable to employ another established method for sound source localization, instead of the digital angle protractor, and a sufficiently calm soundproof room, instead of an anechoic room. To study the adaptation to left-right reversed audition, the exposure period can be either shortened or prolonged and the frequency of neuroimaging can be either lower or higher, according to the situation. For further study, it is recommended to perform neuroimaging more frequently after the exposure period to investigate the recovery process after the adaptation. If neuroimaging is unavailable, it is possible to replace neuroimaging experiments by behavioral experiments. In this protocol, there is a possibility that a participant will request temporary suspension of the exposure due to inevitable reasons. Unless the participant agrees to insert earplugs into the ears during the suspended period, the exposure should be terminated due to unknown recovery effects on readaptation; a new experiment should be started with another participant. Another possible issue is that a balance of subjective loudness between left and right sounds becomes uncertain due to physical contact with the system or for other reasons. In that case, it is recommended for the participant to confirm, with the eyes closed, if the sounds emanating from the front are only localized at the front before readjusting the volume.

Even though the present apparatus showed high performance in 360° sound source localization, the results indicated a slight perceptual bias toward the counterclockwise rotation, especially for sounds coming from the right-front and the left-back directions. Assuming that the earphones are properly inserted into the participant's ear canals, two possibilities are considered for the asymmetrical distortion of the localization: individual acoustic characteristics and spillover of unreversed sounds. Acoustic characteristics are typically modeled as head-related transfer functions (HRTFs)[27], and common HRTFs are used for any participant in the current version of the apparatus without specific optimization. Thus, there is room to improve the apparatus by implementing individual HRTFs for each ear and participant. In contrast, slight spillover of unreversed sounds is relatively uncontrollable. Although separation of microphone and earphone parts of the system reduces the spillover and usual sounds are unlikely to generate perceptible bone conduction[28], it is technically difficult to prevent the spillover completely in a wearable way. Moreover, during the exposure, it is almost impossible to control bone-conducted self-produced voices; thus, there is nothing to do but to assume a symmetric distribution for them. Therefore, it is considered that the implementation of individual HRTFs is the priority to improve the apparatus and achieve more effective adaptation.

To our knowledge, this is the first successful protocol established for studying the long-term adaptation to precise left-right reversed audition with neuroimaging. In addition, this protocol has a great potential for extensive applicability in both auditory and multisensory research. For example, the system incorporating a microcomputer could be set up to induce different alterations in auditory space, such as an overall rightward shift or a compression of auditory space toward the center. Since spatial information is concordantly processed across sensory modalities, altered auditory space could be a strong tool to reveal mechanisms of multisensory spatial recalibration in a way similar to Zwiers *et al.*[29], who reported the effects of wearing prism lenses with spatially compressed vision on sound source localization. Nowadays, it is becoming increasingly popular to use currently available techniques in a multimodal manner, such as the simultaneous use of EEG and fMRI[30], and a delayed combined use of transcranial brain stimulation and EEG/MEG[31]. While the simultaneous use of two neuroimaging techniques compensates for their weaknesses reciprocally, the delayed combined use of neurostimulation and neuroimaging techniques reveals brain functions related to after-effects caused by the neurostimulation using the neuroimaging. Notably, an experimental scheme of the present protocol can be regarded as an expanded version of the latter case. Similar to the neurostimulation techniques, continuous wearing of a wearable apparatus with unusual sensory space causes after-effects of adaptation. These effects can be then measured by a neuroimaging technique. Therefore, the delayed combined use of a wearable apparatus and a neuroimaging technique reveals brain functions related to adaptation (as briefly pointed out in Aoyama and Kuriki[15]). From a general point of view, this scheme can provide new insights into neuroimaging studies with a variety of adaptive effects. In conclusion, the present protocol, under this scheme, provides a promising methodology for studying left-right reversed audition as a tool to uncover the adaptability of humans to a novel environment in the auditory domain.

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DISCLOSURES:

The author has nothing to disclose.

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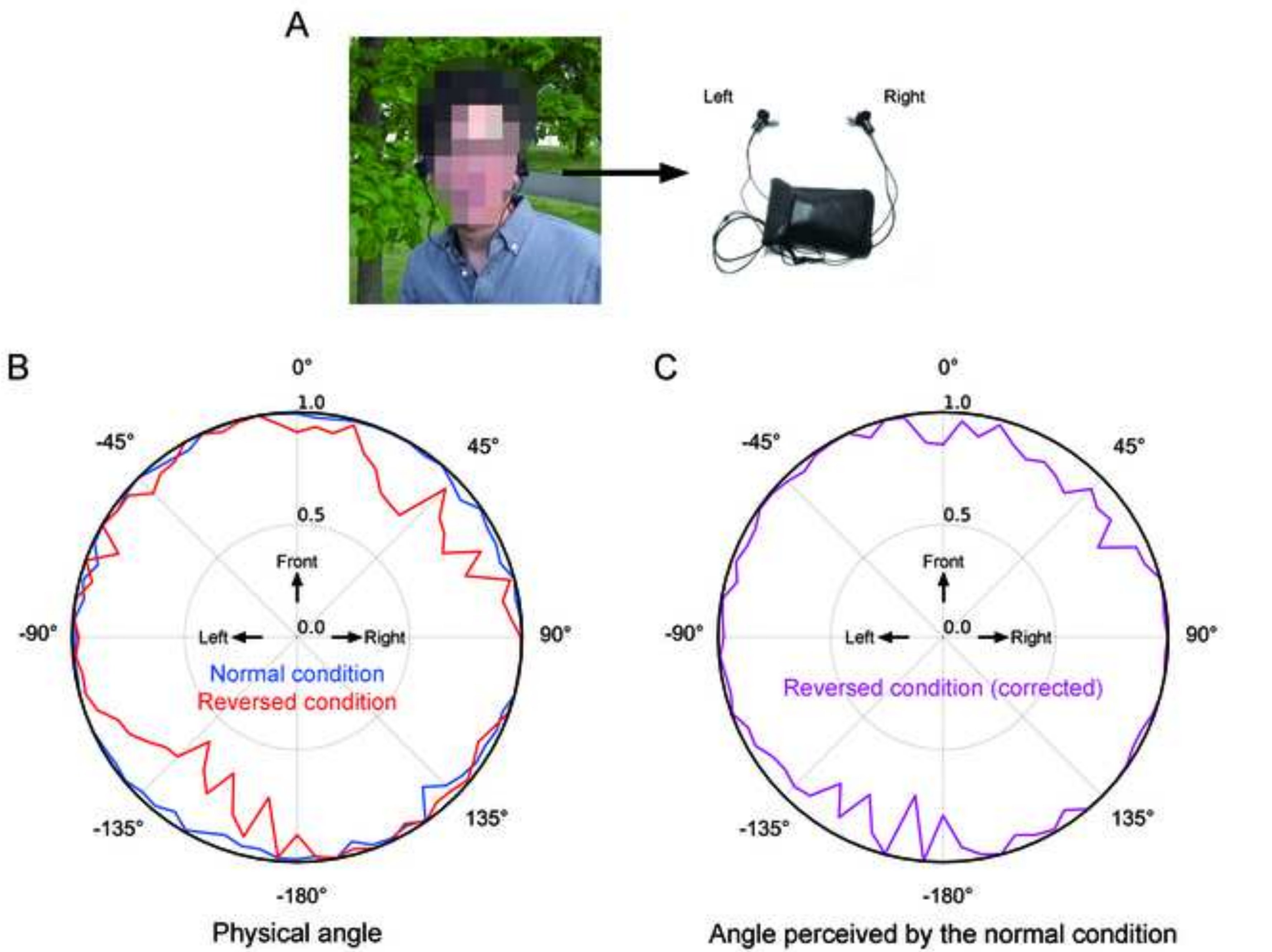

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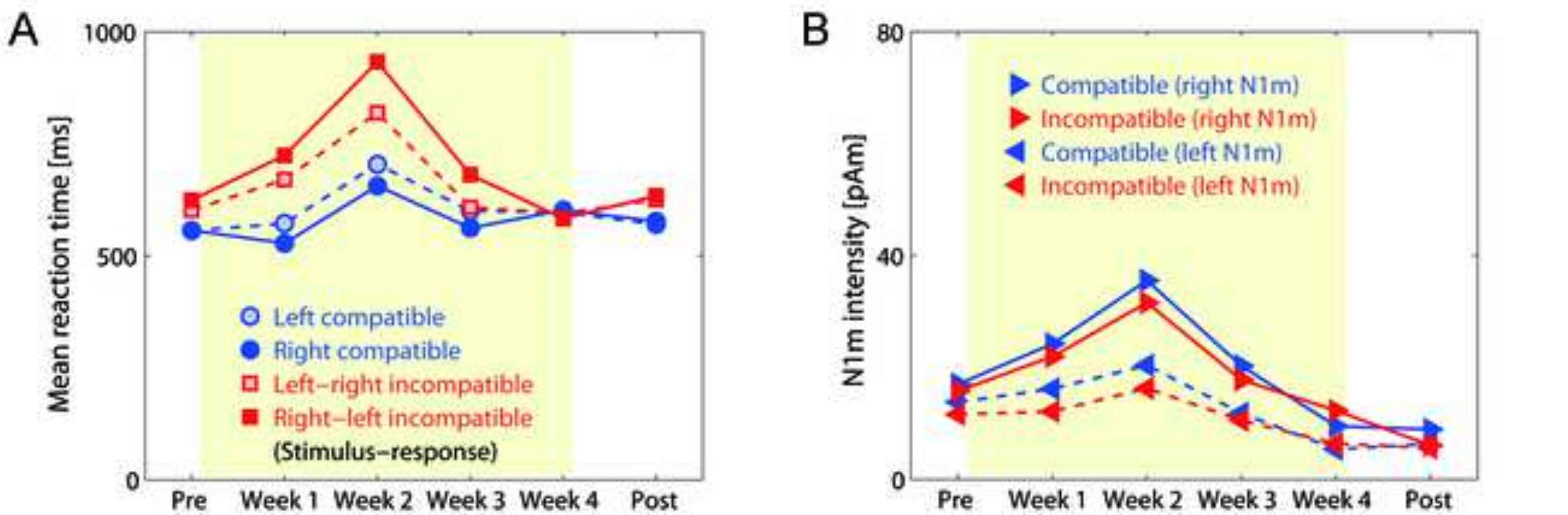

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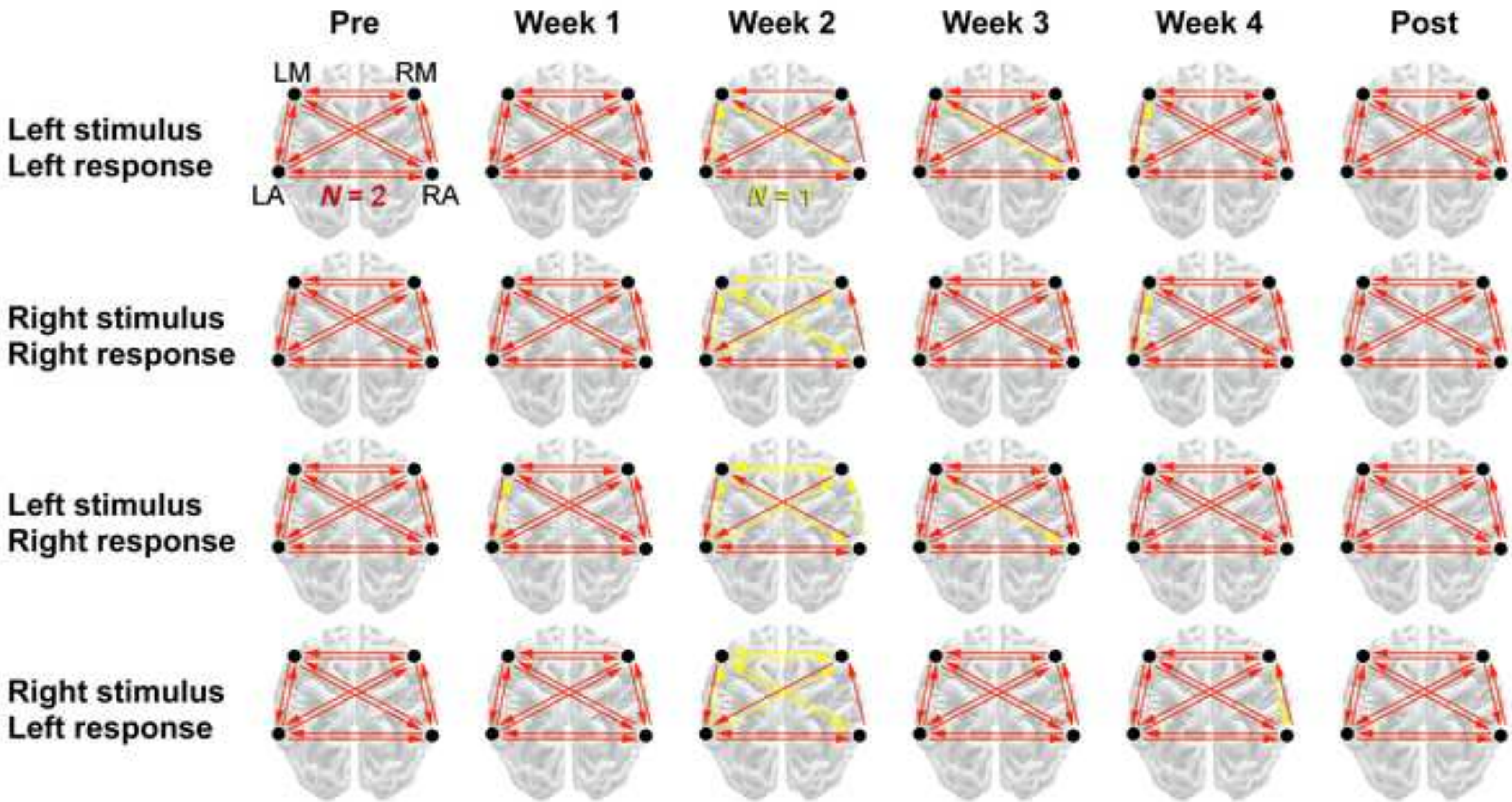

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Dear Dr. Alisha DSouza,

First of all, I am sorry that my resubmission has become so late, and thank you very much for your supportive and constructive letter, in which you and the other reviewers commented on my manuscript “A Method to Study Adaptation to Left-Right Reversed Audition” submitted to *JoVE*.

I am very grateful to you for the chance to revise this manuscript and thank all the reviewers for their positive and constructive comments and valuable suggestions, which I believe have contributed considerably to the improvement of the manuscript.

The revised version of the manuscript is enclosed, and comments on each of the points can be found in this letter. The original comments are highlighted in *italic*. I am convinced that this resubmission properly addresses the suggestions and hope that the revised manuscript is now appropriate to be published in *JoVE*.

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Responses to the JoVE Scientific Review Editor

Comment 1

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

I have now thoroughly proofread the manuscript and it has been checked by a native English speaker.

Comment 2

1.2.2: "Concurrently, adjust the sound volume of the recorder based on the point of subjective equality so that the two perceived loudness become as similar as possible." Unclear what is done here, or what we would show. May be better to unhighlight.

1.2.3: Unclear what we would film here.

Though the previous description about these steps were difficult to film, the steps cannot be skipped to perform the proper experiment. Therefore, I have combined these two steps together, moved the description about confirmation of a balance of subjective loudness to the third paragraph of DISCUSSION, and changed the description so that it can be filmed easily.

Comment 3

3.2.1: What is the task? Please describe it here.

3.2.2: The task will need to be describe and highlighted to film it, so the Note following this step will have to be a highlighted step, described in the imperative tense as a set of instructions. Please provide specific settings for experiments.

3.2.5: Similar idea as above. The current step does not include information on how to perform the action.

Ideally instructional details should be provided as steps rather than in notes.

Now I have moved the detailed description about the task, the neuroimaging, and the analysis from notes to steps, and combined them with the original steps so that they are shown as examples of the original steps. For the video recording, I have added the description about EEG to the examples in PROTOCOL and included the multi-channel EEG system into the table of materials, in case the MEG system is stopped.

Comment 4

Protocol Highlight: After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your *protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE's instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.*

I have checked all the descriptions in PROTOCOL to follow the instructions, and only highlighted "1. Setup of the Left-Right Reversed Audition System" and "3. Studying the Adaptation to Left-Right Reversed Audition". Now the length of the highlighted part is less than 2.5 pages.

Comment 5

The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.

I have now addressed these points.

Comment 6

Results: Mention sample sizes.

Now I have specified the number of participants not only in FIGURE LEGENDS but also in the body text.

Comment 7

Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

I have included a paragraph about "modifications and troubleshooting" by moving some of the notes in PROTOCOL and adding description, and clarified the role of each paragraph in DISCUSSION. The first paragraph describes "significance with respect to existing methods", the second "critical steps within the protocol", the third "modifications and troubleshooting of the method", the fourth "limitations of the technique", and the fifth "future applications or directions of the method".

Comment 8

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"M11-M, Philips" and "HM-200, JVC" were not used in this study but in Ohtsubo et al. and Hofman et al., respectively. Since the information can be obtained in these articles, I have removed them.

Comment 9

Please define all abbreviations at first use.

I have changed the phrase from "using the MVGC Multivariate Granger Causality Matlab Toolbox" to "using MATLAB with the Multivariate Granger Causality Toolbox".

Comment 10

Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

I have carefully checked all the units appearing in the manuscript.

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Responses to Reviewer #1

Major comment 1

The details of the apparatus are not described very well in this paper.

Now I have clarified description about the apparatus in PROTOCOL and included a figure showing the apparatus into Figure 1.

Major comment 2

As declared by the author, all these results are essentially the same as those previously published in another journal, although the visuals of the graphs have been modified.

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Minor comment 1

Figure 1 B: it may be better to replace the index of the circulate axis (located under the circle) to read, "Angle perceived by the normal condition"

Thank you for your suggestion. I have corrected this.

Responses to Reviewer #2

Minor comment 1

Some parts of the text are still highlighted in yellow. Not sure if it was forgotten by the author or a mistake of the JOVE system.

Yellow highlighted text indicates protocol text that will be featured in the video and used to guide scriptwriters in preparing a video script.

Minor comment 2

Sections need to have standardized formatting(e.g. "2.1" is bold while "2.2" is not bold)

I have now addressed this point.

Responses to Reviewer #3

Major comment 1

The Introduction mentions studies by Ohtsubo et al. and Hofman et al. which describe similar apparatuses. It is, however, not clear what is the difference between their approach and the current protocol? For example, the apparatus described by Hofman et al. seems to be a wearable device as well. Why does the author think that the current setup is superior (given that the other studies could have measured delays and performance in rear space which seem to be the main points of criticism)?

The binaural headphone-microphones used in Ohtsubo et al., 1980 had to be connected to a fixed amplifier, and thus, the current apparatus is superior to them in wearability. As for Hofman et al., 2002, the spatial performance of the hearing aids was guaranteed for the front 60 degrees in the head-fixed condition and the front 150 degrees in the head-free condition. Thanks to the recent advances in wearable acoustic technology, the present protocol achieved high omniazimuth performance, where a participant localized perceived sound source without moving the head. Therefore, the omniazimuth performance of the apparatus by Hofman et al., 2002 is quite unknown as compared with the current apparatus. Moreover, the exposure period in the previous studies may be short to detect phenomena related to the adaptation, as compared with the longer cases of reversed vision, and none of these studies have measured brain activity using neuroimaging techniques. It is thus considered that not only the high spatiotemporal accuracy of the apparatus but also the know-how of the long exposure period and the utilization of neuroimaging are the new points of the current protocol.

Here, I would like to express my appreciation for Minor comments 4 and 5. The current protocol has a great potential for extensive applicability in both auditory and multisensory research as you mentioned.

Accordingly, I have added the detailed description about Ohtsubo et al., 1980, Hofman et al., 2002, and the current apparatus in the second and final paragraphs of INTRODUCTION. In addition, I have described that a participant performed the sound localization task without moving the head in PROTOCOL. Moreover, I have included the description about the potential of the system in the final paragraph of DISCUSSION.

Major comment 2

It seems that adaptation effects after wearing the device for a month(!) are surprisingly weak and I wonder how reliable they are. For example, performance in the compatible/incompatible RT task (Fig. 2) appears identical at Pre, Week 3 and Post. Thus, I wonder how reliable is the relative reversion effect at Week 4? With the small number of participants that can be tested, it seems possible that this effect might not be replicated and is just noise. In this case, the main effect of wearing the device would be an initial slowing of RTs for both compatible/incompatible conditions that then returns to normal. Also, why are participants more confused by the device *after 2 weeks (i.e., slower RTs) than immediately after putting on the device (week 1)? And how much time was between taking off the device and posttest?*

I agree that the number of participants shown in REPRESENTATIVE RESULTS is not enough to draw general conclusions based on statistics. However, like the previous studies dealing with long-term exposure to reversed audition (Young, 1928; Willey et al., 1937; Ohtsubo et al., 1980; Hofman et al., 2002), it is quite general to perform this kind of experiment with a small number of participants because of the long research period and the difficult participant recruitment. Within each participant, individual results provide rich and valuable information about the adaptation.

In the present case, two participants showed similar trends in the reaction times, the N1m intensities, and auditory-motor connectivity patterns (note that the reversed audition system was taken off during the MEG recording). Importantly, the trends in the reaction times and the N1m intensities were not distinct but correlated with each other in two points: (1) absolute values irrespective of response compatibility (i.e., their tendency to be maximum in the second week, with overall disruption of the auditory-motor connectivity), and (2) relative values depending on the stimulus-response compatibility (i.e., their tendency to be inverted in the fourth week). For the first point, it is considered that these phenomena do not reflect perceptual confusion related to stimulus-response compatibility but indicate implicit auditory-motor optimization processes caused by continuous exposure to the reversed audition for about 2 weeks and that efficient processing becomes possible again as the optimization proceeds (according to the unpublished results, perceptual confusion, which was maximum at the beginning of the exposure, is reflected in oscillatory activity associated with error propagation). For the second point, it is considered that the 4-week exposure to the reversed audition causes plastic modulation of early auditory processing along with the auditory-motor optimization. Though the reaction times appear identical at Pre, Week 3 and Post in the previous version of Figure 2A, the detailed reaction times at Week 3 were different from the others (please see the new version of Figure 2A). Moreover, the Post experiment was conducted one week after taking off the reversed audition system due to the limited opportunity to use the MEG system, and thus, it is natural that the reaction times at Pre appear identical to those at Post after fully recovering from the adaptation. Consequently, the abovementioned correlations with the reasonable explanation supports the reliability of the individual results.

In fact, the scope of JoVE is the methodology and not the novel data or the broad scientific interpretation. Especially in REPRESENTATIVE RESULTS, it is officially permitted to present the results previously published in another journal, and the discussion are focused on the methods and the protocol. As to this manuscript, the original article is Aoyama and Kuriki, 2017, and all of the findings and the interpretation have been already reviewed. Therefore, I have not included the novel data or the interpretation in the manuscript, but instead modified it as follows: (1) I have clarified the reason why the small number of participants was employed, and guided the readers to see Aoyama and Kuriki, 2017 for details in the first paragraph of DISCUSSION, (2) I have specified in PROTOCOL that the reversed audition system was removed immediately before and put on immediately after each neuroimaging experiment during the exposure period, (3) I have updated Figure 2A to show the detailed reaction times, and (4) I have specified the timing of the post-exposure experiment in PROTOCOL and added the description about the recommendation to perform neuroimaging more frequently after the exposure to investigate recovering process from the adaptation for further study in the third paragraph of DISCUSSION.

Major comment 3

Does adaptation to the device have any effects on sound localization, that is, does sound localization performance change over the course of adaptation? To what extent? I assume that the results shown in Fig. 1 represent a measurement immediately after putting on the device, i.e., without any possible adaptation?

Figure 1 shows the sound source localization in 360-degree directions before and immediately after putting on the system in six participants. This was evaluated irrespective of the experiments studying adaptation to reversed audition.

Though the effects of the adaptation on sound source localization were not reported in Aoyama and Kuriki, 2017, the effects were tested over exposure time in the front auditory field in the unpublished study dealing with the ventriloquist effect. According to the study, no adaptation effects were found for the localization from the beginning to the fourth week, while the localization got sensitive to visual information. Though I agree that these findings are scientifically valuable, the current purpose of the sound source localization is to validate the constructed reversed audition system. Moreover, the scope of JoVE is not the novel data or the broad scientific interpretation, and the manuscript of the unpublished study is under preparation.

Therefore, in the revised manuscript, I have included a note about the sound source localization (and the delay evaluation) in PROTOCOL, and clarified the details of the experimental conditions in PROTOCOL, the first paragraph of REPRESENTATIVE RESULTS, and FIGURE LEGENDS (Figure 1).

Minor comment 1

Validation of the Left-Right Reversed Audition System, l. 148 ff.: The described procedure requires the use of a digital angle protractor and the availability of an anechoic room. Note that sound localization could be measured by a number of methods different than a digital angle protractor. Also, I wonder if an anechoic room (which might not be available at every institution) is really necessary to merely show that the device is properly working and reversing left/right auditory space?

To validate the sound source localization of the reversed audition system, it is acceptable to employ another established method for sound source localization, instead of the digital angle protractor, and a sufficiently calm soundproof room, instead of an anechoic room. I have included the description about them in the third paragraph of DISCUSSION.

Minor comment 2

Procedure of the Exposure to Reversed Audition, l. 231 ff.: It is not clear if the participants are supposed to use the device on their own during daily life (which I assume is the case given the suggested adaptation period of one month), because *paragraph 3.1.6 mentions that "When a participant needs to move outside, drive the participant in a car…". This seems to imply that the participant cannot move outside on their own without an experimenter present? Please clarify.*

I have clarified the explanation about the procedure in PROTOCOL.

Minor comment 3

Neuroimaging: if my understanding is correct, the device has to be removed before neuroimaging testing sessions. Thus, it is not specifically designed for use with neuroimaging, although the manuscript gives the impression that it is.

To avoid giving the impression that the system is specifically designed for use with neuroimaging, I have clarified the explanation about it in the final paragraph of INTRODUCTION and changed the description in SHORT ABSTRACT, LONG ABSTRACT, and the final paragraph of DISCUSSION.

Minor comment 4

Altered sensory space is a very useful tool to study multisensory recalibration processes. For example, Zwiers et al. studied the effect of wearing prism lenses with compressed spatial vision on auditory localization. There should be some discussion of the potential usefulness of the device for multisensory research.

Thank you for the precious information and suggestion. I have now included the description about the potential usefulness of the system for multisensory research with a citation of Zwiers et al. in the final paragraph of DISCUSSION.

Minor comment 5

Could the device be set up to induce different alterations in auditory space (e.g., an overall rightward shift or a compression of auditory space toward the center)? If yes, this should be mentioned in the manuscript, because it would substantially widen applicability of the device.

Thank you for your suggestion. As you pointed out, it is possible to induce different alterations in auditory space with the system incorporating a microcomputer. Therefore, I have included the description about it in the final paragraph of DISCUSSION.

General remark 1

Various parts of the manuscript are highlighted in yellow, but what is the meaning of the highlight?

Yellow highlighted text indicates protocol text that will be featured in the video and used to guide scriptwriters in preparing a video script.

General remark 2

The whole manuscript needs a thorough language editing.

I have now thoroughly proofread the manuscript and it has been checked by a native English speaker.

General remark 3

A figure showing the apparatus would be helpful.

Now I have included a figure showing the apparatus into Figure 1.

CERTIFICATE OF ENGLISH EDITING

This document certifies that the paper listed below has been edited to ensure that the language is clear and free of errors. The edit was performed by professional editors at Editage, a division of Cactus Communications.The intent of the author's message was not altered in any way during the editing process. The quality of the edit has been guaranteed, with the assumption that our suggested changes have been accepted and have not been further altered without the knowledge of our editors.

Title Of The Paper

A Method to Study Adaptation to Left-Right Reversed Audition

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