Neuroscience

Unraveling the impact of congenital deafness on individual brain organization

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eLife Assessment

This study presents **valuable** data on the increase in individual differences in functional connectivity with the auditory cortex in individuals with congenital/early-onset hearing loss compared to individuals with normal hearing. The evidence supporting the study's claims is **convincing**, although additional work using resting-state functional connectivity and further links to how the results align with the underlying biology could have further strengthened the study. The work will be of interest to neuroscientists working on brain plasticity and may have implications for the design of interventions and compensatory strategies.

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Abstract

Research on brain plasticity, particularly in the context of deafness, consistently emphasizes the reorganization of the auditory cortex. However, a critical question arises: to what extent do all individuals with deafness show the same level of reorganization? To address this question, we examined the individual differences in deafness functional connectivity (FC), specifically from the deprived auditory cortex. Our findings demonstrate a remarkable differentiation between individuals deriving from the absence of shared auditory experiences, resulting in heightened FC variability among deaf individuals, compared to more consistent FC in the hearing group. Notably, connectivity to language regions becomes more diverse across individuals in deafness. This does not stem from delayed language acquisition, as it is found in deaf native signers, who are exposed to rich natural language since birth. However, comparing FC diversity between deaf native signers and deaf delayed signers who were deprived of language in early development, we show that language experience also impacts individual differences, although to a more moderate extent. Overall, our research points out the intricate interplay between brain plasticity and individual differences, shedding light on the diverse ways reorganization manifests among individuals. It further joins findings in blindness, showing that individual differences are affected by

sensory experience. Finally, these findings highlight the importance of considering individual differences in personalized rehabilitation for hearing loss.

Introduction

Neural plasticity, a fundamental property of the brain, refers to its ability to adapt and reorganize in response to sensory input and environmental demands. Meaningful plasticity is found in response to extreme environmental scenarios, such as missing the typical input to an entire sensory channel. Extensive research into neural plasticity in congenital deafness has shown that deafness induces neural reorganization (e.g., Allen et al., 2013; Almeida et al., 2015, 2018; Amaral et al., 2016; Finney et al., 2003; Lomber et al., 2010; Ruttorf et al., 2023; Scott et al., 2014; for a review see Alencar et al., 2019; Lomber et al., 2020). For instance, the auditory cortex (AC) in deafness becomes highly responsive to visual stimuli, reflecting a compensatory adaptation to sensory loss (e.g., Codina et al., 2017; Hauthal et al., 2013; Simon et al., 2020). Importantly, the reorganization of the AC in deaf individuals also plays a role in language processing, responding to sign language, which uses the visual rather than the auditory modality (Nishimura et al., 1999; Trumpp & Kiefer, 2018). Although most findings in congenital deafness that suggest visual processing in the AC are caused by hearing loss, as opposed to using sign language (Cardin et al., 2013, 2016; Fine et al., 2005), sign language itself also affects cross-modal plasticity - for example in the processing of motion (Bavelier et al., 2001; Codina et al., 2017; McCullough et al., 2012). Therefore, both hearing loss and compensatory capacities are important factors when seeking to comprehend the plastic alterations in the AC in deafness.

Overall, hearing loss promotes cross-modal plasticity in the AC and beyond it; but do all individuals with deafness undergo the same *level* or even *type* of reorganization? Or can reorganization affect deaf people differently, shedding light on the nature of plasticity at the individual level? Recent evidence on blindness suggests that the variability between individuals may even be further increased due to sensory loss (Sen et al., 2022). In this study, we showed that people who were congenitally blind have significantly more individual differences in brain connectivity from their deprived visual cortex beyond what is found in sighted controls. This was especially true in areas where connectivity is reshaped by blindness (Sen et al., 2022). This suggests that plasticity may be more variable among people than previously thought. Further, it illustrates the role of postnatal experience, in driving individual differences in brain development. Is the expansion of individual differences due to plasticity a general principle of brain development? If so, we can expect to find increased individual differences in deafness as well.

Testing this question in deafness opens an additional question. Deafness is frequently accompanied by a secondary deprivation. Deaf children born to hearing parents who are raised without direct contact to other deaf adults often suffer from delayed language acquisition as they cannot perceive spoken or sign language in their environment (Hall, 2017; Mayberry et al., 2002; Mayberry & Eichen, 1991). This early-onset deprivation has unique effects on brain organization as well (Cheng et al., 2023; Lyness et al., 2013; Twomey et al., 2020; X. Wang et al., 2023). Therefore, testing individual differences in deafness allows testing a secondary question: If the absence of experience increases individual variability, would language acquisition delay cause additional variation in the link between the auditory and language systems? Or does only early-onset and long-lasting input loss cause such diversification? Last, individual variability in neural plasticity may also impact the restoration of hearing. In terms of auditory recovery, hearing aids and cochlear implantation are the main options in auditory rehabilitation. In congenital hearing loss, cochlear implants should be applied in younger rather than older children, as the efficacy of cochlear implants decreases if implemented later (Karltorp et al., 2020; Kral & Sharma, 2012; Lyness et al., 2013; Purcell et al., 2021; Sharma & Campbell, 2011). However, even then, the success of their application might be dependent on the level of the reorganization of the AC: an early work showed that in children prior to cochlear

implantation, the level of metabolism in their cortex, including the AC, predicted their speech perception outcomes (D. S. Lee et al., 2001), suggesting a challenge posed by reorganization to intact sensory restoration. In contrast, more recently, it was shown that recruitment of the broad AC (including language areas) for visual speech in deaf adults positively correlates to auditory speech perception following implantation (Anderson et al., 2017). Therefore, understanding the nuances of brain reorganization and specifically how it may vary among deaf individuals, may enable the implementation of more effective and individualized auditory rehabilitative interventions.

Therefore, the goal of the current study is to use brain connectivity to test if individual variability is modulated by sensory loss in deafness, and how it may be affected by delayed language acquisition. We examine whether the reorganization of the AC in congenital deafness results in connectivity that is particularly variable across individuals. We predict that higher variability will be observed in deafness, indicating a significant influence of postnatal sensory loss on brain organization across sensory systems. Alternatively, if increased individual variability is not observed for the deaf, this would challenge previous findings from the blind (Sen et al., 2022), arguing against the idea that sensory loss promotes individual variation in general, and suggesting instead that different sensory systems may promote more consistent or variable plasticity patterns. Last, testing the role of delayed language acquisition, we predict that deaf individuals with additional delayed language acquisition may show an additional increase in their individual connectivity differences, signifying that delayed language acquisition, as a form of short-term deprivation, can also affect brain variability across individuals.

Results

Does auditory cortex functional connectivity variability differ between congenitally deaf and hearing individuals?

We first investigated whether deafness causes changes to the individual differences in functional connectivity (FC) from the auditory cortex (AC). To achieve this, FC maps were assessed within each group, the deaf and hearing groups, for their voxel-wise variability across individuals. This was accomplished through the implementation of a whole-brain voxel-level test for homogeneity of variance (Brown Forsythe test, see Methods). We found that multiple regions showed significant inter-subject variability differences in FC between the deaf and hearing groups (**Figure 1A**; see also **Table S1** for the peaks of this effect). These included areas of the left temporal lobe (superior temporal gyrus – STG and middle temporal gyrus – MTG, including the auditory association cortex), the bilateral inferior frontal gyrus (IFG, including Broca's area), paracentral lobe, and a small part of the dorsal visual cortex. The clusters in the STG, MTG and IFG fall, to a great extent, within classically identified language regions (see white outline in **Figure 1A**; mapping language areas from Fedorenko et al. (2010)). This major effect of deafness on individual differences in FC was uniquely strong for the auditory cortex. Replicating this analysis with multiple control regions (all atlas cortex areas not involved in audition or language; Harvard-Oxford Atlas) showed that AC-FC had a much more substantial change in variability due to deafness (X^2 = 2303.18, p < .0001; **Figure S1**).

To determine which group has larger individual differences in these regions (**Figure 1B**), we computed the ratio of variability between the two groups (deaf/hearing) in the areas that showed a significant difference in variability (**Figure 1A**). The deaf show variability over twice as large as the hearing in most of the areas that show change to within-group variability - including the STG, MTG, and the IFG. The deaf group showed lower variability in only one cluster in the left early visual cortex. Thus, the findings from this analysis indicate that as in vision, in hearing individuals auditory experience appears to exert a general stabilizing influence on FC, whereas hearing loss leads to greater overall variability between individuals in the connectivity of the AC. A single

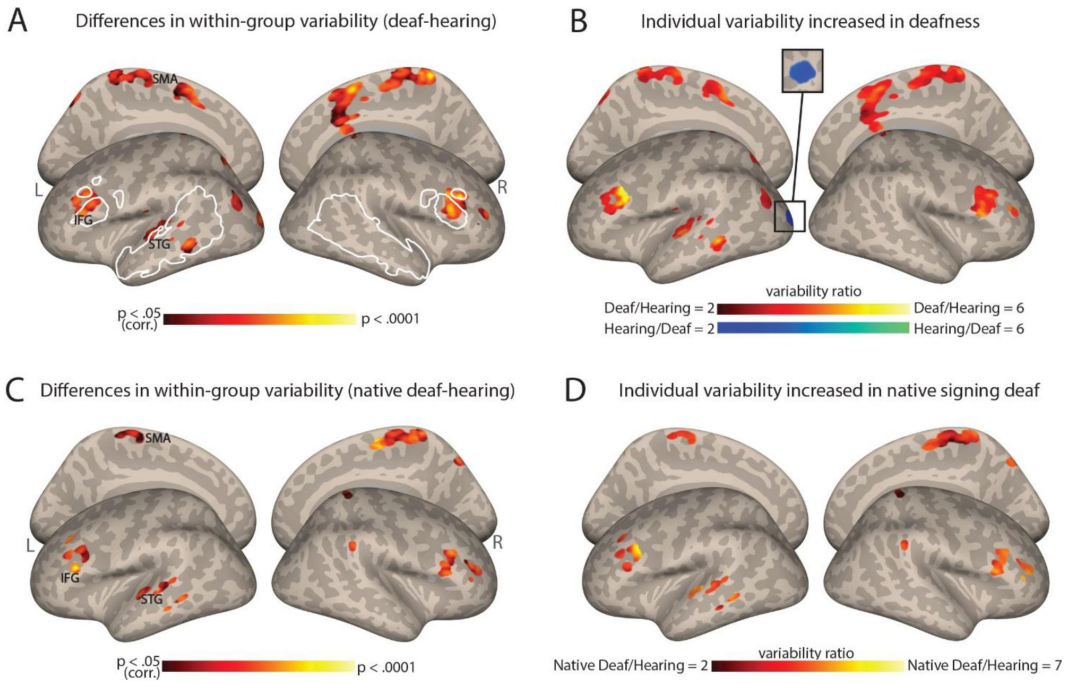

Figure 1.

Individual differences in functional connectivity (FC) from the auditory cortex (AC) increase in deafness.

A. Significant differences in the inter-individual variability of the AC-FC values between deaf and hearing groups ($p < .05$, cluster-corrected for multiple comparisons) are presented on inflated cortical hemispheres. These are found in the left STG (including the auditory association cortex), bilateral IFG (including part of Broca's area), paracentral lobule, along with the dorsal visual stream. **B.** The ratio of the within-group variability of AC-FC between the deaf and hearing groups is presented (within areas showing variability between the groups). Most areas showing a change in variability between the groups display larger individual differences in deafness, including the left auditory association cortex and Broca's area. **C.** Differences in native signing deaf subgroup and hearing group in their interindividual variability of the AC-FC values ($p < .05$, cluster-corrected for multiple comparisons) replicate the effect of the mixed deaf group (panel A). **D.** The ratio of the variability of auditory cortex FC between the native signing deaf and hearing (within areas showing variability difference between the groups). No area showed increased individual differences for the hearing group. Native-signing deaf participants have higher individual differences, despite having no delay in language acquisition.
Anatomical marks: SMA = Supplementary Motor Area; IFG = Inferior Frontal Gyrus; STG = Superior Temporal Gyrus. The regions outlined in white show some of the language-sensitive regions identified by Fedorenko et al. (2010), including the IFG, the anterior and the posterior temporal parcellations.

exception is that the deaf had more consistent connectivity between their early auditory and visual cortices. This suggests that as in vision loss (Sen et al., 2022), individual differences dramatically increase due to deafness.

Is the increased variability (mainly) explained by hearing loss?

Our sample of deaf individuals was rather homogenous in having severe to profound hearing loss from early life. However, it included a mix of native signers and adults who were deaf children to hearing parents, who were taught to sign later in life, and, in effect, experienced delayed language acquisition. Given that our sample of deaf individuals exhibited varying ages of language acquisition, it raises the question of whether the observed FC variability above is primarily attributable to delayed language acquisition or to hearing loss. To investigate this question, we tested if the increased variability would still be found when comparing native deaf signers to hearing individuals, all of whom had natural language experience (for sign or spoken language, respectively) from birth through their parents. Our results demonstrated a very similar pattern to the one described above, revealing increased variability in temporal, frontal, and medial regions (**Figure 1C**; see also **Table S1**). The FC variability is higher in the native-signing deaf individuals when compared to the hearing individuals (**Figure 1D**). Similar findings are seen when comparing the deaf delayed-language and hearing groups (e.g., for the IFG, **Figure S2**). This outcome suggests that deafness-related factors, even without delayed language acquisition, are sufficient to generate more diverse FC from the AC between individuals and that auditory experience, regardless of language exposure, exerts a broad stabilizing effect on FC.

Does AC variability increase especially for areas that reorganize in deafness?

To test if this change in individual differences stems from variable outcomes of deafness-related plasticity, we tested if areas that show reorganization in FC are especially susceptible to increased individual differences. We computed the change in FC from the AC between the hearing and deaf groups (**Figure 2A**). Consistent with prior research (e.g., Andin & Holmer, 2022; Ding et al., 2016), deaf individuals showed increased FC to the AC in frontal, temporal, and parietal regions, while for the hearing the connectivity was stronger to sensorimotor areas (**Figure 2B**). We then explored whether regions that had undergone functional reorganization due to deafness also exhibited high variability within the deaf group. We predicted that if plasticity due to deafness results in higher variability, than areas with overall FC change between the groups would also display heightened variability within the deaf group, leading to a correlation between the two spatial maps. We therefore conducted a correlation analysis between the spatial pattern of variability difference observed between the groups (**Figure 1A**) and the spatial pattern of the group effect in terms of AC-FC (**Figure 2A**). The Pearson's correlation coefficient between these two maps was modest but highly significant ($r = .24$, $p < .0001$ for both smoothed and unsmoothed permuted data; confirmed through a permutation test shuffling voxel location across 100,000 iterations; **Figure 2C**). This suggests a moderate link between variability and plasticity: not only is the AC-FC more variable in the deaf, but the variability seems to also be increased in areas that showed reorganization because of deafness. To test which of the regions that had undergone reorganization had particularly variable plasticity across individuals, we inspected the variability ratio between the deaf and hearing groups in the areas that had group-level changes to FC. We found that all the areas that showed changes to FC exhibited either greater variability within the deaf group (mainly in the parietal and right frontal cortex) or similar variability in both groups (**Figure 2D**). No region showed higher variability in the hearing. Together, this suggests that plasticity FC of the AC in deafness is overall linked to more variable outcomes across individuals.

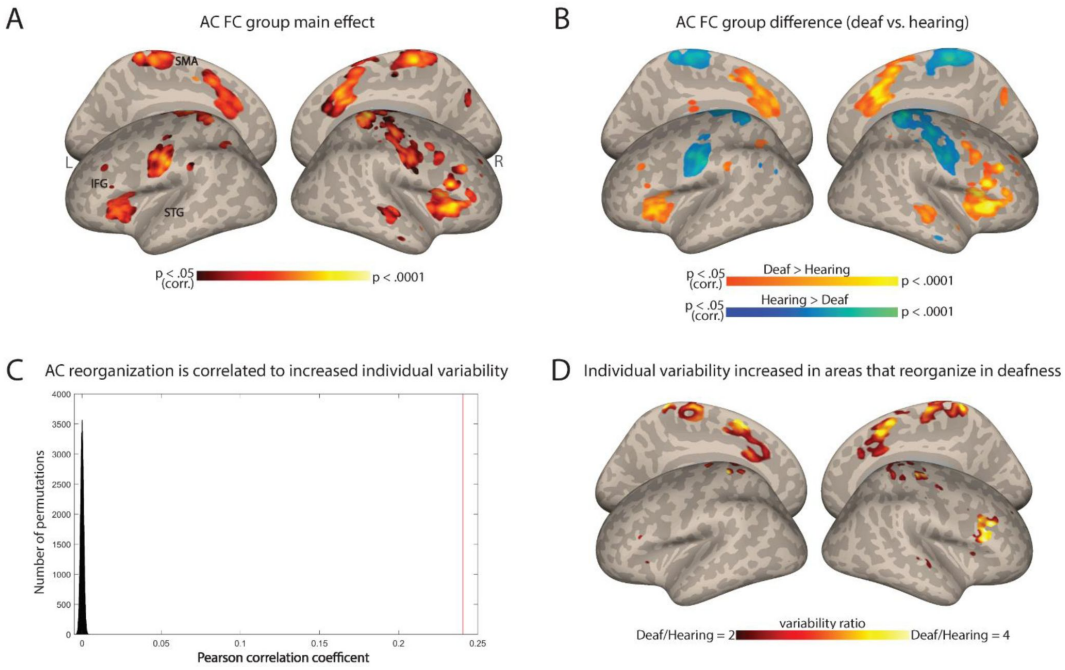

Figure 2.

Individual variability in deafness is related to brain plasticity.

A. ANOVA main effect showing which regions are reorganized in deafness (group difference between the deaf and hearing in AC-FC ($p < .05$, cluster-corrected for multiple comparisons). **B.** Direct comparison of AC-FC between deaf and hearing groups ($p < .05$, cluster-corrected for multiple comparisons) broadly replicated previous findings, showing broad reorganization in deafness. **C.** Correlation between regions that show increased individual differences (**Figure 1A**) with the regions that show reorganization in deafness (panel A) is shown as a red line ($r = .24$) compared with a spatial permutation test (distribution in black); the brain patterns of FC reorganization and that of increased individual differences are correlated, suggesting increased individual differences characterizes plasticity in deafness. **D.** The ratio of the intra-group variability of AC-FC between the deaf and hearing groups is shown within areas showing reorganization group-level changes to FC. No area showed increased individual differences for the hearing group. Among the areas showing a change in AC-FC in deafness, individual differences are overall increased (red-orange) or stable (uncolored). Anatomical marks: SMA = Supplementary Motor Area; IFG = Inferior Frontal Gyrus; STG = Superior Temporal Gyrus.

Does delayed language acquisition affect individual differences?

Finally, we aimed to investigate the independent impact of language exposure, and whether delayed language acquisition played an additional role in the heightened variability observed among deaf individuals. To address this, we replicated the FC variability analysis by comparing deaf native signers to deaf delayed signers, equating hearing loss. In contrast to the results above, which revealed extensive variability change across multiple brain regions, this analysis only identified significant differences between native and delayed deaf signers in four small clusters (**Figure 3A**; see also **Table S1**) in the left posterior Middle Frontal Gyrus (pMFG), close to the Precentral Gyrus (PreCG), the left posterior supramarginal gyrus (pSMG), left dorsal visual cortex (precuneus and cuneus), and the right anterior inferior frontal gyrus (aIFG). Interestingly, these regions did not all show a consistent effect in their direction, but instead increased variability was attributed to both sub-groups for different clusters. FC variability was increased for the deaf delayed signing individuals both in the left MFG and the right aIFG (**Figure 3B**). In contrast, the deaf native signing individuals showed higher variability in the pSMG and the dorsal stream (precuneus and cuneus) (**Figure 3B**). Interestingly, the two areas that had increased variability in delayed signers closely corresponded to language-related areas (e.g., pMFG and aIFG). These findings indicate that beyond the broader effects of deafness on individual differences in the FC of the early AC, delayed language acquisition can also affect individual differences, albeit to a lesser extent.

Could other individual factors explain individual differences in deafness?

Our results so far suggest that the early lack of hearing experience (i.e., congenital deafness) is the primary factor driving AC-FC variability. However, even congenital deafness is not completely homogenous, and other factors related to partial hearing experience could also contribute to this individual variability among the deaf. For instance, the degree of hearing loss and the use of hearing aids, which provide residual hearing (even if not sufficient for language comprehension in the case of our participants), might also influence individual differences. To test this, we computed the correlation between AC-FC and three factors related to hearing experience: the age when hearing aids were first used, the duration of hearing aid use, and the hearing threshold. At the whole-brain level, we observed that AC-FC to different brain regions, primarily in the occipital lobe, but also in posterior middle temporal gyrus (**Figure S3A**), are influenced by the age at which our deaf participants began using hearing aids, such that higher FC is correlated with older ages of onset. Interestingly, there was no correlation between the duration of hearing aid use and AC-FC with any brain region (no significant clusters, $p < .05$, cluster-corrected for multiple comparisons). Last, we tested the correlation between AC-FC and hearing threshold. This analysis was possible only on a subset of our sample ($N = 23$), with the remaining participants only able to report the level of hearing loss (i.e., 'profound') rather than specific hearing threshold values, and therefore should be interpreted cautiously. We found that AC-FC to the left fusiform gyrus is correlated with the hearing threshold of deaf participants (**Figure S3B**), indicating that more profound hearing loss is associated with stronger FC between the AC and the fusiform gyrus. Since these correlations did not implicate any of the areas we identified here as having higher AC-FC variability in the deaf (**Figure 1A, B**), we directly tested if the individual differences in AC-FC to these regions could be accounted for by these hearing-related parameters. We performed the same correlation analysis at the ROI level using individual clusters extracted from the map in **Figure 1A**. No significant correlations were found for any of the factors or ROIs (all $p > .05$ before correction for multiple comparisons, see **Figures S4**, **S5**, and **S6** for scatter plots of AC-FC and each individual factor). These findings suggest that while hearing experience factors such as

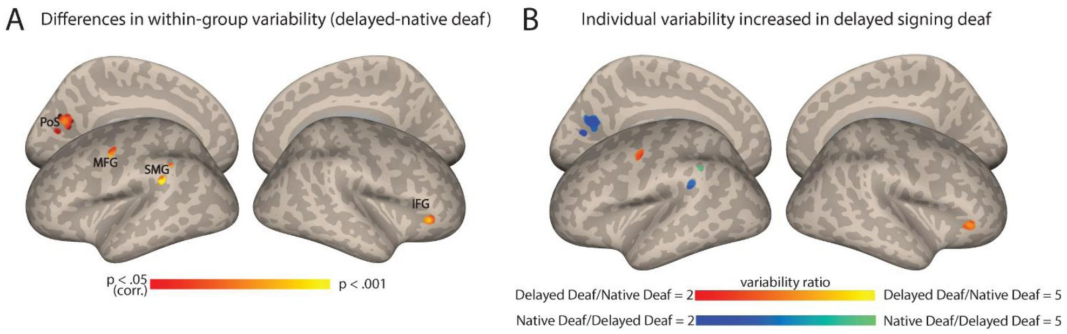

Figure 3.

Auditory cortex functional connectivity (AC-FC) variability is influenced by language exposure.

A. Differences in delayed and native signing deaf subgroups in their interindividual variability of the AC-FC values ($p < .05$, cluster-corrected for multiple comparisons) show changes to individual differences in four specific clusters. **B**. The ratio of the variability of AC-FC between the delayed signing and native signing deaf (within areas showing variability between the subgroups) shows that individual differences increase due to delayed language acquisition in the left MFG and right anterior IFG, but that early-onset sign language exposure stabilizes connectivity between the AC and the left SMGs and left dorsal medial visual cortex (cuneus and precuneus).
Anatomical marks: PoS = Parietooccipital Sulcus; IFG = Inferior Frontal Gyrus; SMG = Supramarginal Gyrus; MFG = Middle Frontal Gyrus

hearing threshold and age of hearing aid use can influence AC-FC, they do not account for the observed increased variability observed in our study, underscoring the role of congenital deafness in increasing AC-FC individual differences.

Discussion

This study demonstrates a link between neural plasticity and variability in the auditory cortex (AC) of deaf individuals. Our study has demonstrated that, in comparison to a hearing group, individuals in the deaf group display a greater degree of individual variability in their functional connectivity (FC) from the AC. These were not driven by differences in their hearing threshold or hearing aid use experience, but rather by congenital auditory loss. This shows consistency with previous findings of increased individual differences in blindness (Sen et al., 2022). Furthermore, there is a mild relationship between this heightened variability and the adaptive changes occurring within the deprived AC. Specifically, we found that overall, the spatial patterns of plasticity and increased individual differences are significantly correlated, and there is increased variability in many areas functionally connected to the AC that have undergone reorganization due to deafness. Further, some, although more modest, increased variability was found when comparing deaf individuals who had varying degrees of sign language acquisition, suggesting that language acquisition timing itself also plays an additional significant role in producing different AC functional connections. These findings suggest that although hearing loss in itself may be sufficient to increase individual differences, the variation of the FC patterns in the AC in response to deafness can increase further when considering a combination of auditory experience and timing of language acquisition. Together, these findings show how the interaction of auditory and language exposure may amplify the spectrum of FC diversity from the AC, allowing for a more comprehensive understanding of the complex factors shaping neural plasticity in response to deafness.

The auditory system, like the visual system, undergoes a critical phase during which its organization is fine-tuned by sensory experience (Knudsen, 2004). Given the fundamental role of auditory input for the AC organization, hearing loss leads to significant reorganization of this brain region. Importantly, while hearing loss induces cross-modal plasticity in the AC, we show that the extent of reorganization may vary among individuals with deafness. Recent evidence on blindness (Sen et al., 2022) suggests that the absence of visual experience increases individual differences in brain connectivity. Our study extends this inquiry to a different population experiencing a distinct type of sensory loss. Our findings demonstrate how hearing loss influences the neural connectivity profile of the deprived AC, introducing variability in the network outcomes. Much of the increased individual differences are found in areas that belong to the language system, including Broca's area (notably bilaterally) and the left STG and MTG (**Figure 1A, B**). This aligns with prior research demonstrating significant FC alterations between the AC and the language network in deaf infants and children (Shi et al., 2016; S. Wang et al., 2019). It also aligns with animal studies showing how deafness can impact top-down connectivity (Yusuf et al., 2021) and cortico-cortical interactions (Yusuf et al., 2017). Therefore, it appears that connectivity between the early AC and many language regions is stabilized or else affected by the use of audition for language in early life and becomes less consistent in its absence. Notably, while our findings mostly point to increased AC-FC variability within the deaf group, a single cluster in the left early visual cortex exhibits higher variability in the hearing group. This suggests a potential stabilizing impact of hearing loss on the interaction between the auditory and visual cortices, possibly due to the prevalent use of vision for adaptation. Overall, these results, coupled with those of Sen et al. (2022), highlight the impact of postnatal sensory experience in promoting consistency in brain organization, suggesting a general principle of brain development of the sensory systems.

The results of this study also provide evidence for the role of neural plasticity in generating diverse individual patterns of brain connectivity. Our finding that the exhibited heightened AC-FC variability by the deaf group corresponds spatially to regions that reorganize in deafness, even if only moderately (r = .24), suggests that the increased variability may be partly attributed to the reorganization and adaptation of neural circuits in response to hearing loss. Although not all areas that changed their mean FC to the AC showed increased variability in the deaf group, we found higher AC-FC variability in regions such as the inferior frontal cortex, and pre-supplementary motor area. These same brain regions exhibited functional reorganization in response to deafness, as illustrated in **Figure 2B**, consistent with prior resting-state fMRI research emphasizing functional changes following hearing loss (e.g., Andin & Holmer, 2022; Ding et al., 2016). Importantly, these changes in the mean connectivity of the AC likely reflect the change in its function in deafness. Studies have shown that the AC can be activated in deaf individuals when performing parallel visual tasks, indicating a shift in functional activation from auditory to visual processing (e.g., Almeida et al., 2015; Benetti et al., 2017, 2021; Bola et al., 2017; Bottari et al., 2014; Butler et al., 2017; Finney et al., 2001; Lomber et al., 2010; Meredith & Lomber, 2011; Petitto et al., 2016; Scott et al., 2014), albeit typically for the same type of functional computation (Cardin et al., 2020; Heimler et al., 2015; Lomber, 2017; Pascual-Leone & Hamilton, 2001). Our results suggest that such functional responses may vary across individuals in accordance with their variable levels of FC alterations. Interestingly, in addition to finding increased variability in connectivity for areas that increase their connectivity in deafness, we also found higher AC-FC variability in regions that show a decreased FC to the temporal lobe in deafness, specifically the somatosensory cortex (e.g., Andin & Holmer, 2022; Bonna et al., 2021; Ding et al., 2016). Here too, previous research has identified differences in somatosensory involvement between deaf and hearing individuals, which has been linked to sign language and visual processing (Bonna et al., 2021; Okada et al., 2016). Therefore, it appears that any type of plasticity in the connectivity of the AC, regardless of its direction (increased or decreased FC), may manifest variably across individuals.

What underlying mechanisms drive these changes, and what is their significance? Although the term "reorganization" is debated, and some concerns exist about the functionality of neural reorganization (Makin & Krakauer, 2023), functional recruitment of AC for non-auditory tasks is supported by evidence from animal studies demonstrating causal links (Lomber et al., 2010; Meredith & Lomber, 2011; for a review, Alencar et al., 2019; Lomber et al., 2020). Such functional changes appear to arise from a combination of unmasking and re-weighting of non-dominant inputs, rather than extensive anatomical alterations (Alencar et al., 2019; Kral & Sharma, 2012, 2023; Kupers et al., 2010; Pascual-Leone & Hamilton, 2001). Our findings of increased variability in FC from the AC in the deaf suggest more than mere unmasking, as this variability cannot easily be explained without assuming some degree of reorganization. Our study does not determine the origins of the individual differences observed, but we expect that they may arise from a blend of genetic and environmental factors. Auditory experiences seem to stabilize FC patterns, implying that experience-dependent pruning in the auditory system consolidates a consistent pattern of connectivity optimized for hearing. In its absence, AC connectivity may become more variable, depending on random and inherited individual differences in patterns of AC connectivity at birth. Further, diverse compensatory experiences of deaf individuals could enhance the variability of AC-FC. Our analysis showed that factors such as hearing threshold and the age at which hearing aids are first used can influence AC-FC. However, these factors do not fully account for the observed variability, suggesting that AC connectivity is susceptible to a complex interplay of environmental influences. Future studies involving deaf infants will be crucial to determining the balance in which AC-FC variability is driven by environmental influences and inherited connectivity patterns.

In addition to the effect of deafness itself, we have also demonstrated how a particular additional factor, namely language experience, may moderately affect variations in the FC of the AC. Although our data may be somewhat underpowered to fully explore this question, deaf

individuals who have had exposure to sign language from birth, for example, appear to exhibit more consistent connectivity between the AC and the left pMFG, as well as the right aIFG, compared to those who had experienced delayed language acquisition in early development (**Figure 3B**). Interestingly, the left pMFG/PreCG have been associated with sign language comprehension (Trettenbrein et al., 2021; Yang et al., 2024), and our results indicate that delayed language acquisition leads to higher FC variability in this area specifically. This provides some evidence that early experience with sign language consolidates this connectivity pattern. On the other hand, the aIFG, part of the inferior frontal cortex, is known to be involved in lexical comprehension and discourse semantics for sign language (Emmorey, 2021). The literature often highlights higher activation in the left hemisphere, typically accompanied by less extensive neural activity in the right hemisphere's homologous region. Therefore, it is unclear why the right aIFG shows higher FC variability in deaf delayed signers, as opposed to the left hemisphere; this may be due to variable reliance on areas capable of compensating for deficits in typical language systems (Martin et al., 2023; Newport et al., 2022; Tuckute et al., 2022). Further research is needed to elucidate the precise role of the right hemisphere's IFG in this context. In contrast, the AC connectivity to the supramarginal gyrus and the cuneus/precuneus is more consistent in people who experienced delayed language acquisition in addition to deafness. Although we only speculate why these areas show such interactions, these findings highlight the complex interplay between sensory experience, language acquisition, and neural plasticity in shaping the individual patterns of FC of the AC. However, these outcomes did not align with our initial hypothesis, which anticipated a more pronounced effect of increased individual differences in delayed signers, especially within the language network. This may be since the observed variations in brain connectivity from early AC are primarily attributed to hearing loss, rather than delayed language acquisition. In turn, this could be both due to the early AC function as primarily responsive to auditory stimulation as well as to the fairly early maturation of this region (Kral & O'Donoghue, 2010), which may make it more susceptible to hearing loss, rather than to delayed language acquisition itself. This conclusion is further reinforced by our analysis targeting the variability in deaf native signers: the findings showed similar patterns of increased individual variability for FC within this subgroup as compared to the hearing (**Figure 1C, D**) compared to the analysis involving both native and nonnative signers (**Figure 1A, B**). It would appear that auditory loss in itself, regardless of language experience, is a larger driver of increased individual differences. Along the same lines, we have also tested if increased individual differences may then be found in the connectivity from Broca's area in the case of delayed language acquisition, and did not find any significant effect. Though this may be due to insufficient power, this further emphasizes that the increase in individual differences in AC-FC, and possibly beyond it, during deafness are primarily attributed to hearing loss. An additional variable that may contribute to the relatively minor effect of language experience in our results is the relatively high language abilities within our cohort of delayed signers, which were comparable to those of the native signers. All deaf participants self-reported consistent levels of sign language proficiency, a factor that is typically affected following delayed language acquisition (Bogliotti et al., 2020; Caselli et al., 2021; Cheng & Mayberry, 2021; Tomaszewski et al., 2022). Furthermore, a subset of delayed deaf signers acquired sign language before the age of 6 (N = 6, see also **Table S2**), potentially rendering them less susceptible to the impact of language deprivation. To further elucidate these findings, future investigations should include a larger and more diverse sample, specifically in terms of sign language acquisition age, in order to comprehensively address this aspect.

Finally, hearing aids and cochlear implants represent the primary approaches in auditory rehabilitation, and individual differences could be considered with respect to these interventions. The effectiveness of these treatments, especially cochlear implantation, is intricately linked to the extent of reorganization within the AC (Feng et al., 2018; Heimler et al., 2014, 2015; Kral et al., 2019; D. S. Lee et al., 2001; H.-J. Lee et al., 2007). The ability to regain a lost sense (i.e., hearing) is likely influenced by the preservation of the auditory system, as cross-modal reorganization for a different function may hinder its capacity to process information from the original modality and computation. Although this link is nuanced, given that some portions of the

AC appear to reorganize for parallel functions to those they typically perform (Cardin et al., 2020; Heimler et al., 2015; Lomber, 2017; Pascual-Leone & Hamilton, 2001), reorganization appears to affect the ability to restore auditory function to AC. Although future research would need to establish a direct link between the individual brain connectivity patterns reported here and their functional utility, the diverse reorganization levels shown in this study hold potential clinical relevance for auditory rehabilitation. This is particularly true when considering the larger individual differences in how strongly the AC connects to the language system (**Figure 1B**), where a disconnect may form between the reorganized role in visual language and auditory feed-forward roles. Additionally, this study highlights the imperative of acknowledging and considering differences between hearing and deaf individuals, particularly when employing normative data in clinical contexts (e.g., neurosurgery). The recognition of variability in brain organization among diverse populations underscores the necessity for tailored approaches in clinical practices, ensuring more accurate and effective interventions for deaf individuals.

It is worth noting that we assessed individual differences based on FC during task performance and not at rest. Although it would be prudent for future research to explore this aspect, we expect that individual patterns of plasticity in the AC connectivity remain relatively consistent across different time periods and states. FC patterns of hearing individuals are primarily shaped by common system and stable individual features, and not by time, state, or task (Finn et al., 2015; Gratton et al., 2018; Tavor et al., 2016). While the task may impact FC variability, we have recently shown that individual FC patterns are stable across time and state even in the context of plasticity due to visual deprivation (Amaral et al., 2024). Therefore, we expect that in deafness as well there should not be meaningful differences between resting-state and task FC networks, in terms of FC individual differences.

In conclusion, this study demonstrates that the lack of auditory experience results in increased individual differences in brain organization. Notably, this increased variability is prominent in language areas and regions undergoing reorganization in response to deafness, highlighting the relationship between brain plasticity and individual differences. Furthermore, our findings indicate that this variability is not solely influenced by sensory loss due to deafness; deprivation from language during early life also plays a role in shaping this variability. Ultimately, these outcomes underscore the significance of postnatal experience in generating individual differences. Additionally, they support tailoring rehabilitation strategies to match the unique patterns of plasticity seen in individuals with sensory impairments, including those with deafness.

Methods

Participants

We recruited 39 congenitally or early deaf adults and 33 hearing college students, all native Mandarin Chinese speakers (15 males, mean age 21.97 ± 2.58 years, range: 18–28 years; see **Table 1** for the detailed characteristics of the participants). All of them possessed normal or corrected-to-normal vision, and their majority was right-handed (with the exception of three deaf individuals), as determined by the Edinburgh inventory (Oldfield, 1971). Prior to their involvement in the study, all participants provided written informed consent and received monetary compensation for their participation. The research protocol was reviewed and approved by the Human Subject Review Committee at Peking University, adhering to the principles outlined in the Declaration of Helsinki.

All participants with hearing impairment completed a background questionnaire, in which they provided information about their hearing loss conditions, history of language acquisition, and educational background (**Table S2**). Specifically, the etiology of hearing loss was collected in a

	Native deaf signers (N = 16)	Delayed deaf signers (N = 23)	Hearing non-signers (N = 33)
Age of Sign Language Acquisition	0 ± 0	6.91 ± 1.62	N/A
Age	28.50 ± 7.13	27.09 ± 5.87	21.97 ± 2.54
Years of Education	14.13 ± 2.31	15.09 ± 1.41	15.03 ± 1.93
Gender	11 M, 5 F	12 M, 11 F	15 M, 18 F

Table 1.

Participants' demographic information.

questionnaire, with the following 4 options: hereditary (selected by N = 15), maternal disease (N = 4), ototoxicity (N = 9), and other/unknown (N = 11 wrote “unknown”). Note that we are unable to confirm the self-reported etiology of hearing loss due to the lack of medical records and the lack of systematic medical examinations for hearing loss in China twenty to thirty years ago. All deaf participants indicated severe (N = 8) or profound (N = 31) deafness from birth, except for three participants who reported becoming deaf before the age of 3. Self-reported hearing thresholds ranged from 85 to 120 decibels (dB). Some of the participants used hearing aids during their lifetime, however, speech comprehension was reported as very poor, even when hearing aids were employed. At the time of testing, five deaf participants were using hearing aids frequently (either daily or 3-4 times per week); one reported to have used hearing aids, only but rarely (1-2 times per month); while others either had never used hearing aids or had used them for varying durations (with usage spanning from 0.5 to 20 years, see also **Table S2**). Only one deaf participant reported having received long-term oral training from teachers starting at age 2.

The deaf participants were divided into two distinct subgroups. The first subgroup, referred to as "native signers”, consisted of 16 individuals (11 males). These individuals were born to deaf parents and were exposed to Chinese Sign Language (CSL) shortly after birth. The second subgroup, known as "delayed signers" (nonnative signers) comprised 23 individuals (12 males). These participants were born into hearing families and began learning CSL after enrolling in special education schools, with the age of CSL initiation ranging from 4 to 10 years. The two deaf groups were carefully matched on various demographic variables, including gender, age, and years of education ($p > .15$). Additionally, in terms of language skills, both deaf groups were matched in terms of self-reported proficiency in CSL comprehension, production, and lipreading skills ($p > .34$).

The hearing group and the deaf group were matched based on gender and years of education ($p > .15$), but there was a significant age difference between these two groups ($p < .05$). Given this significant age difference, we used age as a nuisance variable in our FC analyses, and the differences in variability were assessed after statistically accounting for the age variable.

Image acquisition

Functional and structural MRI data were collected using a Siemens Prisma 3T Scanner with a 64-channel head-neck coil at the Center for MRI Research, Peking University. Functional data were acquired with a simultaneous multi-slice echoplanar imaging sequence supplied by Siemens (62 axial slices, repetition time [TR] = 2000 ms, echo time [TE] = 30 ms, multi-band factor = 2, flip angle [FA] = 90°, field of view [FOV] = 224 mm × 224 mm, matrix size = 112 × 112, slice thickness = 2 mm, gap = 0.2 mm, and voxel size = 2 mm × 2 mm × 2.2 mm). A high-resolution 3D T1-weighted anatomical scan was acquired using the magnetization-prepared rapid acquisition gradient echo sequence (192 sagittal slices, TR = 2530 ms, TE = 2.98 ms, inversion time = 1100 ms, FA = 7°, FOV = 224 mm × 256 mm, matrix size = 224 × 256, interpolated to 448 × 512, slice thickness = 1 mm, and voxel size = 0.5 mm × 0.5 mm × 1 mm).

Image preprocessing

We used SPM12 (Welcome Trust Centre for Neuroimaging, London, UK), run in Matlab R2018b (Mathworks, Inc., Sherborn, MA, USA), for processing and analysis of structural and functional data. For each participant, the first four volumes of each functional run were discarded for signal equilibrium. The remaining functional data were slice-time corrected to the first slice (middle slice in time) and corrected for head motion to the first volume of the first session using 7th degree b-spline interpolation. All participants had head motion less than 2mm/2°, except for one hearing participant that showed excessive head motion in 2 runs, which were excluded from analysis. Structural images were coregistered to the first functional images. Functional data were then

normalized to MNI anatomical space using a 12-parameter affine transformation model in DARTEL (Ashburner, 2007) and resampled to 2 mm^3 voxel size prior to applying a 6 mm FWHM Gaussian filter.

Stimuli and procedure

During the fMRI scanning, the participants performed a semantic task whose predictors were regressed out to focus on the underlying FC patterns. Design-regressed task data has been extensively used in the past to calculate FC (e.g., Amaral et al., 2021; Gratton et al., 2018; Norman-Haignere et al., 2012; Walbrin & Almeida, 2021), and it has been shown that it effectively leads to similar FC estimates as when using resting scans (Fair et al., 2007). Stimuli comprised a set of 90 written words. This set consisted of 40 concrete/object words and 50 abstract/nonobject words, the latter lacking explicit external referents. Participants were given instructions to visually examine each of these 90 target words, contemplate their meanings, and engage in an oddball one-back semantic judgment task (X. Wang et al., 2023).

Each participant completed a total of 10 runs of task fMRI scanning, with each run lasting for 360 seconds. One native signer completed only eight runs and subsequently withdrew from the study due to discomfort, so we analyzed 8 runs for this subject. In each run, there were 90 target word trials, each lasting for 2.5 s, as well as 14 catch trials, also lasting 2.5 s each. For more details about this experiment please see X. Wang et al. (2023). There was no difference in the activation for words across the brain ($p < .05$, cluster-corrected for multiple comparisons) between the deaf and hearing participants in this task. Hearing and deaf participants performed differently in terms of accuracy (acc_{deaf} = 74% vs. $acc_{hearing}$ = 89%, $t(70) = 5.6$, $p < .05$), but not in terms of reaction time (RT_{deaf} = 1083ms vs. $RT_{hearing}$ = 1147ms, $t(70) = 1.3$, $p = .2$). Despite the groups being matched for reaction time, to further control for task performance effects, both task accuracy and reaction time were included as nuisance variables in all analyses (deaf vs. hearing; delayed deaf vs. native deaf).

Functional connectivity analysis

Functional connectivity (FC) was computed using the CONN Toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). Time courses were extracted from the 10 runs after regressing out the task predictors, and potential confounding effects were estimated and removed separately for each voxel and for each participant and run. In addition, functional data were denoised using a standard denoising pipeline (Nieto-Castanon, 2020) including the regression of potential confounding effects characterized by white matter timeseries, CSF timeseries, motion parameters, session and task effects, and simultaneous bandpass frequency filtering of the BOLD timeseries (Hallquist et al., 2013) between 0.01 Hz and 0.1 Hz.

Seed region of interest

The seed region for the early auditory cortex (AC) was defined using the atlas provided by the CONN toolbox (Harvard-Oxford Atlas distributed with FSL, (Jenkinson et al., 2012). We extracted the Heschl's Gyrus parcellation (broadly corresponding to the location of the primary AC) for both hemispheres and used it as our seed region for the FC analysis.

Functional connectivity variability analysis

Seed-based connectivity maps for each subject were estimated characterizing the spatial pattern of FC with the seed area (bilateral Heschl's Gyrus). FC strength was represented by Fisher-transformed bivariate correlation coefficients from a weighted general linear model, modeling the association between their BOLD signal timeseries. To examine whether there were differences in the interindividual variability of FC values between the two groups, namely the deaf and hearing participants, we conducted the Brown–Forsythe test for equal variance (**Figure 1A**). The Brown–

Forsythe test (Brown & Forsythe, 1974) is a homogeneity of variance test like Levene's test, conventionally used to test for variability differences, but uses the median instead of the mean, safeguarding against false positives in cases of skewed data distribution (Olejnik & Algina, 1987). The regression of the age variable was implemented in the analyses comparing deaf vs. hearing (given the age difference between these groups), while the regression of task variables (i.e., accuracy and reaction times) was included in all analyses to account for task performance effects. The minimum significance level for all presented results was established at $p < .05$, cluster-corrected for multiple comparisons within the gray matter volume using the spatial extent method (a set-level statistical inference correction; (Forman et al., 1995; Friston et al., 1994). Correction was based on the Monte Carlo simulation approach, extended to 3D datasets using the threshold size plug-in for BrainVoyager QX (Brain Innovation, Maastricht, Netherlands). This analysis was replicated for additional control ROIs – including all ROIs from the Harvard-Oxford Atlas not related to audition or language, and the number of significant voxels was calculated per ROI to test if the change in variability in deafness is specific to the AC. To determine if the number of voxels in AC was significantly higher than in the other control ROIs, we conducted a chi-square test for goodness of fit (**Figure S1**).

To inspect the direction of the variability group effect, and determine which group had higher variance, we computed the ratio of variability between the groups (Variability Deaf/Variability Hearing, **Figure 1B**; (Sen et al., 2022)) for each voxel showing a significant Brown–Forsythe test effect ($p < .05$, corrected). We also conducted equivalent analyses on a subset of the deaf participants, with our investigation centering on the roots of the differences in individual variability, and whether they stem from hearing loss (deafness) or from late exposure to language. To test the role of hearing loss, we compared deaf individuals who are native signers to hearing participants (**Figure 1C**), both populations having access to full language (spoken and CSL, respectively) from birth. To test the role of delayed language acquisition, we compared native signing deaf individuals to deaf individuals who acquired sign language at a later stage (**Figure 3**).

In addition to the variability analysis, FC data was also analyzed to directly compare the connectivity between the groups, with a one-way ANOVA (**Figure 2A**). To inspect the direction of reorganization in AC-FC, we computed a *post hoc* t-test comparing FC between the groups (deaf vs. hearing, **Figure 2B**). To quantitatively examine the link between reorganization in deaf individuals and its impact on variability, we conducted a comparative analysis between the spatial pattern of FC variability (**Figure 1A**) and the spatial pattern of reorganization observed in the deaf (**Figure 2A**). This was done with the unthresholded maps to correlate the spatial pattern at large between these statistical effects. This was achieved by calculating the Pearson's correlation coefficient between these maps, specifically within the gray matter (**Figure 2C**). The significance level for the correlation was obtained using a permutation test (100,000 iterations), randomly shuffling voxels for each iteration and convolving each random map with a Gaussian kernel based on data smoothness estimation to account for spatial autocorrelation. To ensure the additional smoothing step does not introduce artificial correlations, we also calculated the significance level without applying Gaussian smoothing to the permuted maps. The resulting permutation distribution was then compared with the previously obtained Pearson's correlation coefficient. Finally, we also inspected the variability ratio within the areas that showed reorganization in deafness (**Figure 2D**).

Correlation with hearing experience variables

In order to inspect the effect of specific factors related to hearing experience (see also **Table S2**) on AC-FC variability, we calculated the correlation between the AC-FC of each voxel for deaf participants and: 1) hearing threshold, defined as the lowest dB across both ears (numeric value available for 23 participants); 2) hearing aid use start age, for 28 participants who reported having used hearing aids; 3) hearing aid use, for all deaf participants, except one who did not report the exact length of use for the hearing aids (N = 38). These correlations were computed at the whole-

brain level (**Figure S3**, using the same multiple-comparisons correction as previously mentioned). Further, they were computed at the ROI level (**Figures S4**, **S5** and **S6**) for all the clusters that showed a main effect of increased AC-FC variability in deafness (**Figure 1A**). None of these correlations were significant even before correction for multiple comparisons (all $p > .05$).

Data availability

Source data files have been provided for all the figures on OSF at the link *https://osf.io/yvkmf/*. Deidentified raw nifti files are not publicly available due to ethical constraints, however they are available from the corresponding authors upon reasonable request.

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Additional information

Author contributions

L.A., X.W., Y.B., and E.S.-A. designed research; X.W. and Y.B. performed research; L.A. analyzed data; L.A. wrote the original draft; all authors reviewed the manuscript.

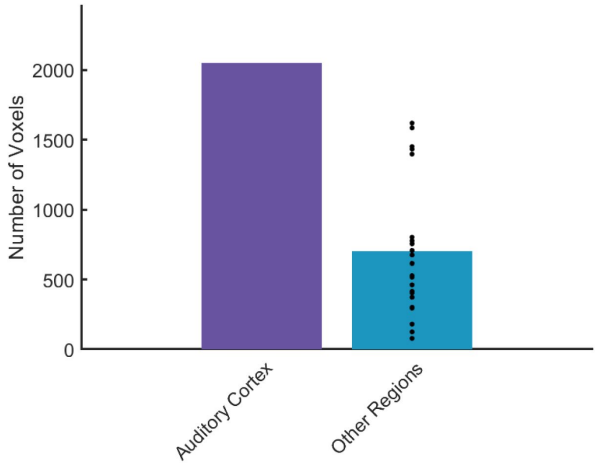
Declaration of interests

Yanchao Bi: Senior Editor, eLife. The other authors declare that no competing interests exist.

Supplementary figures and tables

Supplementary Figure 1.

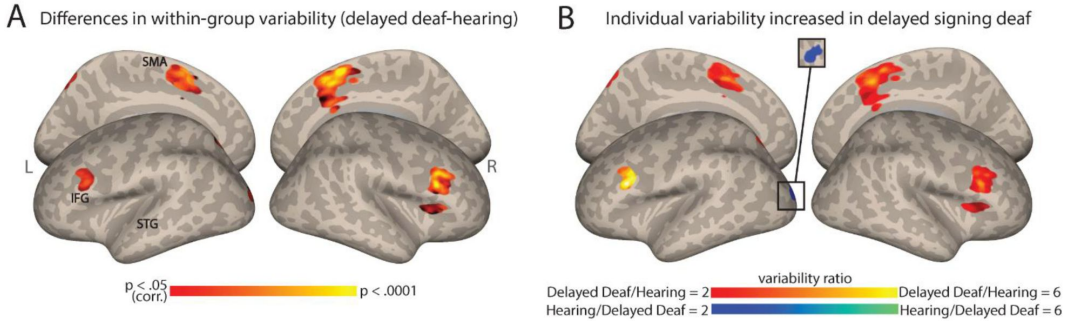
To test if increased variability in deafness was unique to the auditory system, we calculated inter-individual variability using all regions from the Harvard-Oxford Atlas, excluding auditory and language regions, as control seed regions for the functional connectivity (FC) analysis. FC variability from the seed areas to the whole brain was calculated for the Auditory Cortex (AC) and control regions. The plot shows the number of voxels with a significant Brown-Forsythe effect (change in variability) for the AC region (purple bar) and the average number of significant voxels using the control regions (blue bar). The black dots represent the number of significant voxels for each individual control region. The connectivity variability from the AC is uniquely increased compared to these control regions (X^2 = 2303.18, p < .0001).



Supplementary Figure 2.

Auditory Cortex functional connectivity (AC-FC) variability difference between delayed signing deaf and hearing individuals.

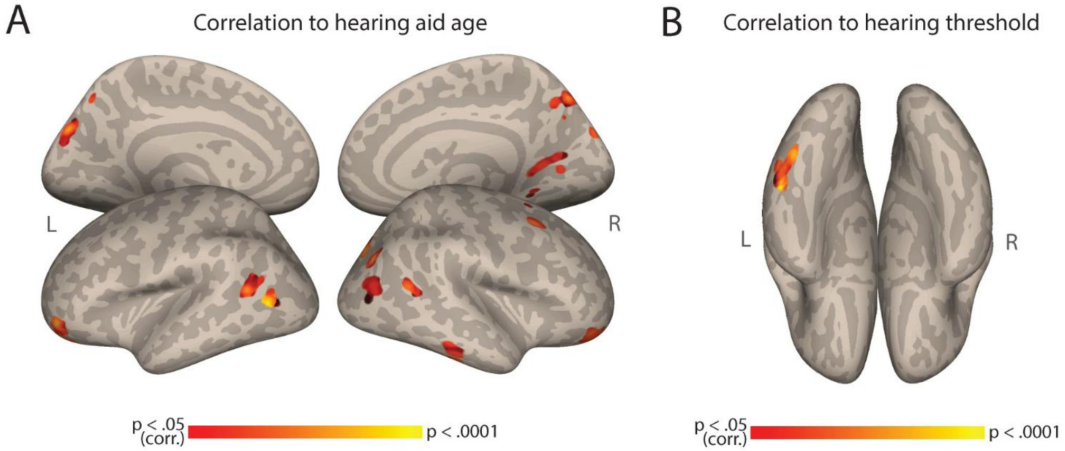
A. Differences in delayed signing deaf subgroup and hearing group in their interindividual variability of the auditory cortex FC values (p < .05, cluster-corrected for multiple comparisons). **B**. The ratio of the variability of auditory cortex FC between the delayed signing deaf and hearing (within areas showing variability difference between the groups) replicates the effects seen when comparing the hearing and mixed deaf group.
Anatomical marks: SMA = Supplementary Motor Area; IFG = Inferior Frontal Gyrus; STG = Superior Temporal Gyrus.



Supplementary Figure 3.

Correlation to hearing aid age and hearing threshold.

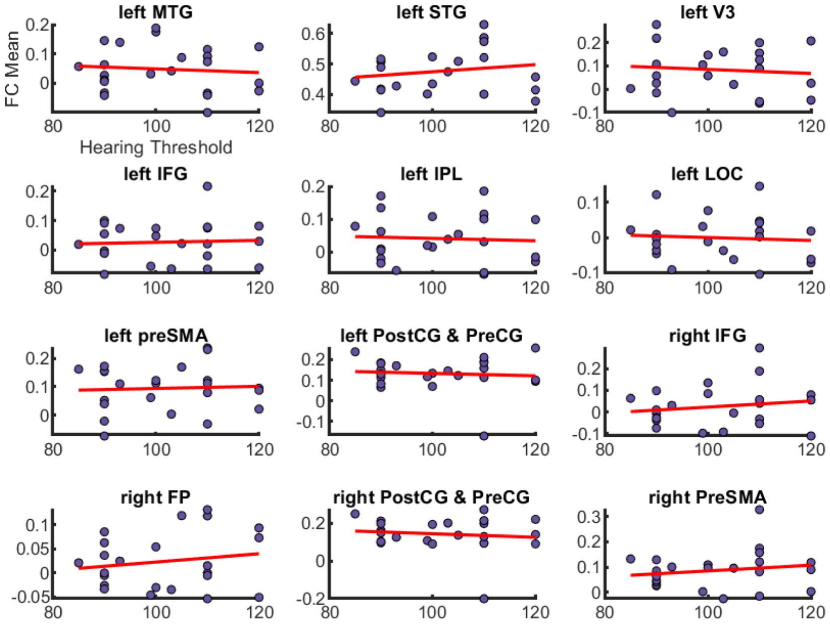
A. Auditory Cortex Functional Connectivity (AC-FC) to different regions is positively correlated to the age of deaf participants started using hearing aids (N=28). **B.** AC-FC to the fusiform gyrus is positively correlated to the hearing threshold of deaf participants (N = 23). Both maps are cluster-corrected for multiple comparisons, p < .05.

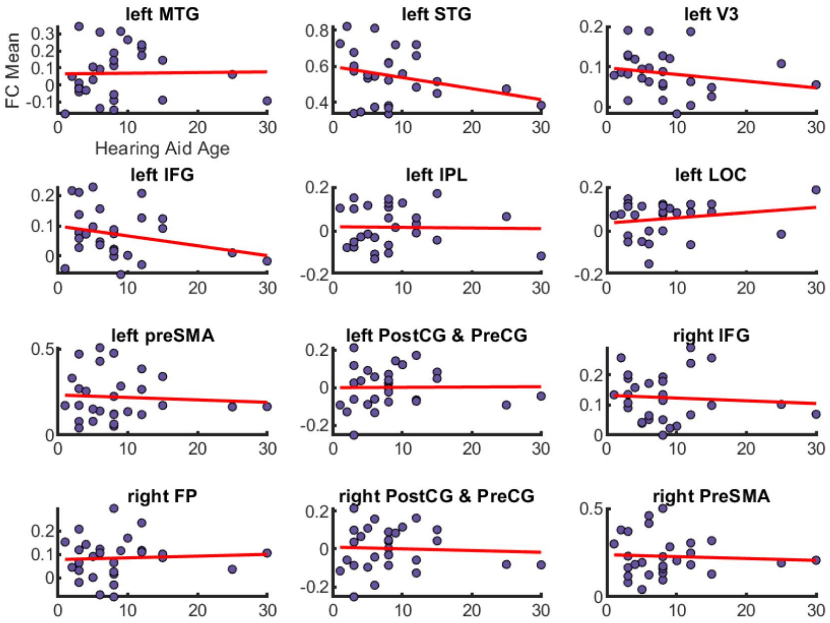


Supplementary Figure 4.

Scatter plots depicting the correlation between auditory cortex functional connectivity (AC-FC) mean values and hearing thresholds across various brain regions in deaf participants.

The x-axis represents the hearing threshold (in decibels), while the y-axis represents the AC-FC mean value for the cluster. Each purple dot indicates individual participant data points, and the red line represents the trend line (linear regression) for each region. No significant correlations were found between FC mean and hearing threshold (all p > .05).
MTG = middle temporal gyrus, STG = superior temporal gyrus, V3 = visual area 3, IFG = inferior frontal gyrus, IPL = inferior parietal lobe, LOC = lateral occipital cortex, preSMA = pre supplementary motor area, postCG = postcentral gyrus, preCG = precental gyrus, FP = frontal pole.

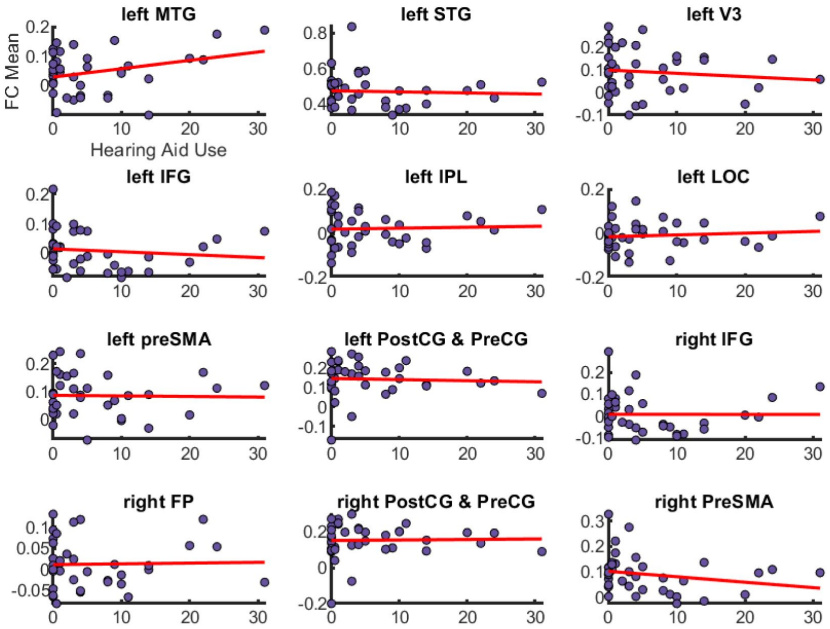




Supplementary Figure 5.

Scatter plots depicting the correlation between auditory cortex functional connectivity (AC-FC) mean values and age at which hearing aids were first used across various brain regions in deaf participants.

The x-axis represents the hearing aids age (in years while the y-axis represents the AC-FC mean value for the cluster. Each purple dot indicates individual participant data points, and the red line represents the trend line (linear regression) for each region. No significant correlations were found between FC mean and hearing aids age (all $p > .05$).
MTG = middle temporal gyrus, STG = superior temporal gyrus, V3 = visual area 3, IFG = inferior frontal gyrus, IPL = inferior parietal lobe, LOC = lateral occipital cortex, preSMA = pre supplementary motor area, postCG = postcentral gyrus, preCG = precental gyrus, FP = frontal pole.



Supplementary Figure 6.

Scatter plots depicting the correlation between auditory cortex functional connectivity (AC-FC) mean values and duration of hearing aids use across various brain regions in deaf participants.

The x-axis represents the duration of hearing aids use (in years), while the y-axis represents the AC-FC mean value for the cluster. Each purple dot indicates individual participant data points, and the red line represents the trend line (linear regression) for each region. No significant correlations were found between FC mean and hearing aids use (all $p > .05$). MTG = middle temporal gyrus, STG = superior temporal gyrus, V3 = visual area 3, IFG = inferior frontal gyrus, IPL = inferior parietal lobe, LOC = lateral occipital cortex, preSMA = pre supplementary motor area, postCG = postcentral gyrus, preCG = precental gyrus, FP = frontal pole.

		Deaf vs. Hearing (peak F-value) Fig. 1A	Deaf Native vs. Hearing (peak F-value) Fig. 1C	Deaf Delayed vs. Hearing (peak F-value) Fig. S2	Deaf Delayed vs. Deaf Native (peak F-value) Fig. 3A
Left Hemisphere	*Superior Temporal Gyrus*	-56 -16 4 (15)	-64 -28 0 (15.7)		
	Middle Temporal Gyrus	-64 -44 0 (16.3)	-66 -46 2 (17.2)		
	Inferior Frontal Gyrus	-40 20 24 (15.5)	-46 28 20 (30.4)	-40 20 24 (13.2)	
	Middle Frontal Gyrus		-36 30 36 (19.3)		
	Posterior Middle Frontal Gyrus				-54 -4 48 (13.7)
	Visual V3	-20 -100 14 (18.1)		-14 -94 20 (15.7)	
	Lateral Occipital Cortex (dorsal stream)	-16 -66 50 (17.5)		-16 -66 50 (14.7)	
	Supramarginal Gyrus				-50 -42 34 (21.1)
	Precuneus				-18 -64 26 (12)
	Inferior Parietal Lobe	-38 -84 32 (11.9)			
	Precentral Gyrus	-2 -26 72 (10.9)	-4 -26 66 (10.9)		
	Pre-Supplementary Motor Area	-2 8 56 (14.9)		-2 6 56 (18.2)	
Right Hemisphere	*Postcentral Gyrus*	12 -42 70 (21.6)			
	Precentral Gyrus		4 -14 56 (23.4)		
	Pre-Supplementary Motor Area	4 10 60 (20.9)		4 12 58 (31.9)	
	Inferior Frontal Gyrus	46 22 24 (27.6)	44 24 24 (20.2)	44 20 26 (29.1)	
	Anterior Inferior Frontal Gyrus			42 28 8 (14.6)	44 34 -8 (16)
	Middle Frontal Gyrus				
	Frontal pole	48 48 12 (17)	46 48 12 (19.3)		
	Supramarginal gyrus		66 -38 40 (14.9)		
	Superior Parietal Lobe		6 -76 48 (13.7)		

Supplementary Table 1.

MNI coordinates (x, y, z) for the FC variability analyses.

Sub no.	Age	Sex	Years of education	Age of deafness (months)	Hearing loss-left ear (dB)	Hearing loss-right ear (dB)	Age of sign language acquisition	Hearing aid use	Age of hearing aid	Hearing aid use duration (years)
34	22	female	15	0	120	110	7	used in the past	8	14
35	27	male	16	0	120	100	5	uses currently	3	24
36	22	female	14	0	110	110	8	used in the past	8	1
37	23	female	14	0	71-90	71-90	8	used in the past	15	0.5
38	22	male	12	0	120	120	8	never used	-	0
39	22	male	12	0	>90	>90	10	never used	-	0
40	23	male	15	0	71-90	71-90	9	used in the past	15	1
41	24	male	16	0	>90	>90	7	never used	-	0
42	23	female	15	0	71-90	71-90	4	uses currently	12	11
43	22	female	14	0	71-90	71-90	6	used in the past	8	9
44	24	female	16	0	105	105	7	uses currently	2	22
45	20	female	12	0	>90	>90	6	used in the past	6	14
46	24	male	16	0	95	90	5	used in the past	6	8
47	31	male	16	12	99	100	8	never used	-	0
48	42	male	16	6	93	93	7	used in the past	8	3
49	39	male	16	0	100	90	5	used sporadically	10	Unknown
50	31	female	16	0	>90	>90	5	used in the past	5	20
51	34	female	16	0	>90	71-90	5	used in the past	12	10
52	33	male	16	0	100	90	9	used in the past	3	5
53	29	female	16	3	110	110	6	uses currently	25	4
54	30	male	16	0	>90	>90	8	never used	-	0
55	30	male	16	0	110	110	7	used in the past	3	5
56	26	female	16	0	71-90	>90	9	used in the past	9	3
57	23	male	14	0	71-90	>90	0	never used	-	0
58	21	male	13	0	103	105	0	used in the past	3	10
59	23	male	15	0	120	120	0	never used	-	0
60	23	male	15	0	120	120	0	used in the past	1	4
61	23	male	14	0	>90	>90	0	used in the past	8	8
62	28	female	15	0	>90	>90	0	never used	-	0
63	39	female	16	0	90	110	0	never used	-	0
64	21	male	12	0	90	91	0	never used	-	0
65	37	female	15	36	85	92	0	used in the past	6	1
66	22	male	9	0	120	120	0	used in the past	8	2
67	34	male	16	0	100	100	0	uses currently	3	31
68	30	female	16	0	110	110	0	used in the past	5	4
69	44	male	9	0	>90	>90	0	used in the past	30	0.5
70	25	male	16	0	90	90	0	used in the past	4	0.5
71	32	male	16	0	110	110	0	never used	-	0
72	31	female	15	0	>90	>90	0	used in the past	12	3

Supplementary Table 2.

Additional characteristics of deaf participants.

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Editors

Reviewer #1 (Public review):

This experiment sought to determine what effect congenital/early-onset hearing loss (and associated delay in language onset) has on the degree of inter-individual variability in functional connectivity to the auditory cortex. Looking at differences in variability rather than group differences in mean connectivity itself represents an interesting addition to the existing literature. The sample of deaf individuals was large, and quite homogeneous in terms of age of hearing loss onset, which are considerable strengths of the work. The experiment appears well conducted and the results are certainly of interest.

https://doi.org/10.7554/eLife.96944.2.sa2

Reviewer #2 (Public review):

Summary:

This study focuses on changes in brain organization associated with congenital deafness. The authors investigate differences in functional connectivity (FC) and differences in the variability of FC. By comparing congenitally deaf individuals to individuals with normal hearing, and by further separating congenitally deaf individuals into groups of early and late signers, the authors can distinguish between changes in FC due to auditory deprivation and changes in FC due to late language acquisition. They find larger FC variability in deaf than normal-hearing individuals in temporal, frontal, parietal, and midline brain structures, and that FC variability is largely driven by auditory deprivation. They suggest that the regions that show a greater FC difference between groups also show greater FC variability.

Strengths:

The manuscript is well-written, and the methods are clearly described and appropriate. Including the three different groups enables the critical contrasts distinguishing between different causes of FC variability changes. The results are interesting and novel.

Weaknesses:

Analyses were conducted for task-based data rather than resting-state data. The authors report behavioral differences between groups and include behavioral performance as a nuisance regressor in their analysis. This is a good approach to account for behavioral task differences, given the data. Nevertheless, additional work using resting-state functional connectivity could remove the potential confound fully.

The authors have addressed my concerns well.

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Author response:

The following is the authors' response to the original reviews.

> ***Reviewer #1 (Public Review):***
>
> *(1) This experiment sought to determine what effect congenital/early-onset hearing loss (and associated delay in language onset) has on the degree of inter-individual variability in functional connectivity to the auditory cortex. Looking at differences in variability rather than group differences in mean connectivity itself represents an interesting addition to the existing literature. The sample of deaf individuals was large, and quite homogeneous in terms of age of hearing loss onset, which are considerable strengths of the work. The experiment appears well conducted and the results are certainly of interest. I do have some concerns with the way that the project has been conceptualized, which I share below.*

Thank you for acknowledging the strengths and novelty of our study. We have now addressed the conceptual issues raised; please see below in the specific comments.

> *(2) The authors should provide careful working definitions of what exactly they think is occurring in the brain following sensory deprivation. Characterizing these changes as 'largescale neural reorganization' and 'compensatory adaptation' gives the impression that the authors believe that there is good evidence in support of significant structural changes in the pathways between brain areas - a viewpoint that is not broadly supported (see Makin and Krakauer, 2023). The authors report changes in connectivity that amount to differences in coordinated patterns of BOLD signal across voxels in the brain; accordingly, their data could just as easily (and more parsimoniously) be explained by the unmasking of connections to the auditory cortex that are present in typically hearing individuals, but which are more obvious via MR in the absence of auditory inputs.*

We thank the Reviewer for the suggestion to clarify and better support our stance regarding reorganization. We indeed believe that the adaptive changes in the auditory cortex in deafness represent real functional recruitment for non-auditory functions, even in the relatively limited large-scale anatomical connectivity changes. This is supported by animal works showing causal evidence for the involvement of deprived auditory cortices in non-auditory tasks, in a way that is not found in hearing controls (e.g., Lomber et al., 2010, Meredith et al., 2011, reviewed in Alencar et al., 2019; Lomber et al., 2020). Whether the word "reorganization" should be used is indeed debated recently (Makin and Krakauer, 2023). Beyond terminology, we do agree that the basis for the changes in recruitment seen in the brains of people with deafness or blindness is largely based on the typical anatomical connectivity at birth. We also agree that at the group level, there is poor evidence of large-scale anatomical connectivity differences in deprivation. However, we think there is more than ample evidence that the unmasking and more importantly re-weighting of non-dominant inputs gives rise to functional changes. This is supported by the relatively weaker reorganization found in late-onset deprivation as compared to early-onset deprivation. If unmasking of existing connectivity without any functional additional changes were sufficient to elicit the functional responses to atypical stimuli (e.g., non-visual in blindness and non-auditory in deafness), one would expect there to be no difference between early- and late-onset deprivation in response patterns. Therefore, we believe that the fact that these are

based on functions with some innate pre-existing inputs and integration is the *mechanism* of reorganization, not a reason not to treat it as reorganization. Specifically, in the case of this manuscript, we report the change in variability of FC from the auditory cortex, which is greater in deafness than in typically hearing controls. This is not an increase in response per se, but rather more divergent values of FC from the auditory cortex, which are harder to explain in terms of 'unmasking' alone, unless one assumes unmasking is particularly variable. The mechanistic explanation for our findings is that in the absence of auditory input's fine-tuning and pruning of the connectivity of the auditory cortex, more divergent connectivity strength remains among the deaf. Thus, auditory input not only masks non-dominant inputs but also prunes/deactivates exuberant connectivity, in a way that generates a more consistently connected auditory system. We have added a shortened version of these clarifications to the discussion (lines 351-372).

> *(3) I found the argument that the deaf use a single modality to compensate for hearing loss, and that this might predict a more confined pattern of differential connectivity than had been previously observed in the blind to be poorly grounded. The authors themselves suggest throughout that hearing loss, per se, is likely to be driving the differences observed between deaf and typically-hearing individuals; accordingly, the suggestion that the modality in which intentional behavioral compensation takes place would have such a large-scale effect on observed patterns of connectivity seems out of line.*

Thank you for your critical insight regarding our rationale on modality use and its impact on connectivity patterns in the deaf compared to the blind. After some thought, we agree that the argument presented may not be sufficiently strong and could distract from the main findings of our study. Therefore, we have decided to remove this claim from our revised manuscript.

> *(4) The analyses highlighting the areas observed to be differentially connected to the auditory cortex and areas observed to be more variable in their connectivity to the auditory cortex seem somewhat circular. If the authors propose hearing loss as a mechanism that drives this variability in connectivity, then it is reasonable to propose hypotheses about the directionality of these changes. One would anticipate this directionality to be common across participants and thus, these areas would emerge as the ones that are differently connected when compared to typically hearing folks.*

We are a little uncertain how to interpret this concern. If the question was about the logic leading to our statement that variability is driven by hearing loss, then yes, we indeed were proposing hearing loss as a mechanism that drives this variability in connectivity to the auditory cortex; we regret this was unclear in the original manuscript. This logic parallels the proposal made with regard to the increased variability in FC in blindness; deprivation leads to more variable outcomes, due to the lack of developmental environmental constraints (Sen et al., 2022). Specifically, we first analyzed the differences in within-group variability between deaf and hearing individuals (Fig. 1A), followed by examining the variability ratio (Fig. 1B) in the same regions that demonstrated differences. The first analysis does not specify which group shows higher variability; therefore, the second analysis is essential to clarify the direction of the effect and identify which group, and in which regions, exhibits greater variability. We have clarified this in the revised manuscript (lines 125-127): "To determine which group has larger individual differences in these regions (Figure 1B), we computed the ratio of variability between the two groups (deaf/hearing) in the areas that showed a significant difference in variability (Figure 1A)". Nevertheless, this comment can also be interpreted as predicting that any change in FC due to deafness would lead to greater variability. In this case, it is also important to mention that while we would expect regions with higher variability to also show group differences between the deaf and the hearing

(Figure 2), our analysis demonstrates that variability is present even in regions without significant group mean differences. Similarly, many areas that show a difference between the groups in their FC do not show a change in variability (for example, the bilateral anterior insula and sensorimotor cortex). In fact, the correlation between the regions with higher FC variability (Figure 1A) and those showing FC group differences (Figure 2B) is significant but rather modest, as we now acknowledge in our revised manuscript (lines 324-328). Therefore, increased FC and increased variability of FC are not necessarily linked.

> *(5) While the authors describe collecting data on the etiology of hearing loss, hearing thresholds, device use, and rehabilitative strategies, these data do not appear in the manuscript, nor do they appear to have been included in models during data analysis. Since many of these factors might reasonably explain differences in connectivity to the auditory cortex, this seems like an omission.*

We thank the Reviewer for their comment regarding the inclusion of these variables in our manuscript. We have now included additional information in the main text and a supplementary table in the revised manuscript that elaborates further on the etiology of hearing loss and all individual information that characterizes our deaf sample. Although we initially intended to include individual factors (e.g., hearing threshold, duration of hearing aid use, and age of first use) in our models, this was not feasible for the following reasons: 1) for some subjects, we only have a level of hearing loss rather than specific values, which we could not use quantitatively as a nuisance variable (it was typical in such testing to ascertain the threshold of loss as belonging to a deafness level, such as “profound” and not necessarily go into more elaborate testing to identify the specific threshold), and 2) this information was either not collected for the hearing participants (e.g., hearing threshold) or does not apply to them (e.g., age of hearing aid use), which made it impossible to use the complete model with all these variables. Modeling the groups separately with different variables would also be inappropriate. Last, the distribution of the values and the need for a large sample to rigorously assess a difference in variability also precluded sub-dividing the group to subgroup based on these values.

Therefore, we opted for a different way to control for the potential influence of these variables on FC variability in the deaf. We tested the correlation between the FC from the auditory cortex and each of these parameters in the areas that showed increased FC in deafness (Figures 1A, B), to see if it could account for the increased variability. This ROI analysis did not reveal any significant correlations (all $p > .05$, prior to correction for multiple comparisons; see Figures S4, S5, and S6 for scatter plots). The maximal variability explained in these ROIs by the hearing factors was $r2=0.096$, whereas the FC variability (Figure 1B) was increased by at least 2 in the deaf. Therefore, it does not seem like these parameters underlie the increased variability in deafness. To test if these variables had a direct effect on FC variability in other areas in the brain, we also directly computed the correlation between FC and each factor individually. At the whole-brain level, the results indicate a significant correlation between AC-FC and hearing threshold, as well as a correlation between AC-FC and the age of hearing aid use onset, but not for the duration of hearing aid use (Figure S3). While these may be interesting on their own, and are added to the revised manuscript, the regions that show significant correlations with hearing threshold and age of hearing aid use are not the same regions that exhibit FC variability in the deaf (Figures 1A, B).

Overall, these findings suggest that although some of these factors may influence FC, they do not appear to be the driving factors behind FC variability. Finally, in terms of rehabilitative strategies, only one deaf subject reported having received long-term oral training from teachers. This participant started this training at age 2, as now described in the participants’ section. We thank the reviewer for raising this concern and allowing us to show that our findings do not stem from simple differences ascribed to auditory experience in our participants.

> ***Reviewer #2 (Public Review):***
>
> *(1) The paper has two main merits. Firstly, it documents a new and important characteristic of the re-organization of the brains of the deaf, namely its variability. The search for a welldefined set of functions for the deprived auditory cortex of the deaf has been largely unsuccessful, with several task-based approaches failing to deliver unanimous results. Now, one can understand why this was the case: most likely there isn't a fixed one well-defined set of functions supported by an identical set of areas in every subject, but rather a variety of functions supported by various regions. In addition, the paper extends the authors' previous findings from blind subjects to the deaf population. It demonstrates that the heightened variability of connectivity in the deprived brain is not exclusive to blindness, but rather a general principle that applies to other forms of deprivation. On a more general level, this paper shows how sensory input is a driver of the brain's reproducible organization.*

We thank the Reviewer for their observations regarding the merits of our study. We appreciate the recognition of the novelty in documenting the variability of brain reorganization in deaf individuals.

> *(2) The method and the statistics are sound, the figures are clear, and the paper is well-written. The sample size is impressively large for this kind of study.*

We thank the Reviewer for their positive feedback on the methodology, statistical analysis, clarity of figures, and the overall composition of our paper. We are also grateful for the acknowledgment of our large sample size, which we believe significantly strengthens the statistical power and the generalizability of our findings.

> *(3) The main weakness of the paper is not a weakness, but rather a suggestion on how to provide a stronger basis for the authors' claims and conclusions. I believe this paper could be strengthened by including in the analysis at least one of the already published deaf/hearing resting-state fMRI datasets (e.g. Andin and Holmer, Bonna et al., Ding et al.) to see if the effects hold across different deaf populations. The addition of a second dataset could strengthen the evidence and convincingly resolve the issue of whether delayed sign language acquisition causes an increase in individual differences in functional connectivity to/from Broca's area. Currently, the authors may not have enough statistical power to support their findings.*

We thank the Reviewer for their constructive suggestion to reinforce the robustness of our findings. While we acknowledge the potential value of incorporating additional datasets to strengthen our conclusions, the datasets mentioned (Andin and Holmer, Bonna et al., Ding et al.) are not publicly available, which limits our ability to include them in our analysis. Additionally, datasets that contain comparable groups of delayed and native deaf signers are exceptionally rare, further complicating the possibility of their inclusion. Furthermore, to discern individual differences within these groups effectively, a substantially larger sample size is necessary. As such, we were unfortunately unable to perform this additional analysis. This is a challenge we acknowledge in the revised manuscript (lines 442-445), especially when the group is divided into subcategories based on the level of language acquisition, which indeed reduces our statistical power. We have however, now integrated the individual task accuracy and reaction time parameters as nuisance variables in calculating the variability analyses; all the results are fully replicated when accounting for task difficulty. We also report that there was no group difference in activation for this task between the groups which could affect our findings.

We would like to note that while we would like to replicate these findings in an additional cohort using resting-state, we do not anticipate the state in which the participants are scanned to greatly affect the findings. FC patterns of hearing individuals have been shown to be primarily shaped by common system and stable individual features, and not by time, state, or task (Finn et al., 2015; Gratton et al., 2018; Tavor et al., 2016). While the task may impact FC variability, we have recently shown that individual FC patterns are stable across time and state even in the context of plasticity due to visual deprivation (Amaral et al., 2024). Therefore, we expect that in deafness as well there should not be meaningful differences between resting-state and task FC networks, in terms of FC individual differences. That said, we are exploring collaborations and other avenues to access comparable datasets that might enable a more powerful analysis in future work. This feedback is very important for guiding our ongoing efforts to verify and extend our conclusions.

> *(4) Secondly, the authors could more explicitly discuss the broad implications of what their results mean for our understanding of how the architecture of the brain is determined by the genetic blueprint vs. how it is determined by learning (page 9). There is currently a wave of strong evidence favoring a more "nativist" view of brain architecture, for example, face- and object-sensitive regions seem to be in place practically from birth (see e.g. Kosakowski et al., Current Biology, 2022). The current results show what is the role played by experience.*

We thank the Reviewer for highlighting the need to elaborate on the broader implications of our findings in relation to the ongoing debate of nature vs. nurture. We agree that this discussion is crucial and have expanded our manuscript to address this point more explicitly. We now incorporate a more detailed discussion of how our results contribute to understanding the significant role of experience in shaping individual neural connectivity patterns, particularly in sensory-deprived populations (lines 360-372).

> ***Reviewer #3 (Public Review):***
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> *Summary:*
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> *(1) This study focuses on changes in brain organization associated with congenital deafness. The authors investigate differences in functional connectivity (FC) and differences in the variability of FC. By comparing congenitally deaf individuals to individuals with normal hearing, and by further separating congenitally deaf individuals into groups of early and late signers, the authors can distinguish between changes in FC due to auditory deprivation and changes in FC due to late language acquisition. They find larger FC variability in deaf than normal-hearing individuals in temporal, frontal, parietal, and midline brain structures, and that FC variability is largely driven by auditory deprivation. They suggest that the regions that show a greater FC difference between groups also show greater FC variability.*
>
> *Strengths:*
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> *- The manuscript is well written.*
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> *- The methods are clearly described and appropriate.*
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> *- Including the three different groups enables the critical contrasts distinguishing between different causes of FC variability changes.*
>
> *- The results are interesting and novel.*

We thank the Reviewer for their positive and detailed feedback. Their acknowledgment of the clarity of our methods and the novelty of our results is greatly appreciated.

> *Weaknesses:*
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> *(2) Analyses were conducted for task-based data rather than resting-state data. It was unclear whether groups differed in task performance. If congenitally deaf individuals found the task more difficult this could lead to changes in FC.*

We thank the Reviewer for their observation regarding possible task performance differences between deaf and hearing participants and their potential effect on the results. Indeed, there was a difference in task accuracy between these groups. To account for this variation and ensure that our findings on functional connectivity were not confounded by task performance, we now included individual task accuracy and reaction time as nuisance variables in our analyses. This approach allowed us to control for any performance differences. The results now presented in the revised manuscript account for the inclusion of these two nuisance variables (accuracy and reaction time) and completely align with our original conclusions, highlighting increased variability in deafness, which is found in both the entire deaf group at large, as well as when equating language experience and comparing the hearing and native signers. The correlation between variability and group differences also remains significant, but its significance is slightly decreased, a moderate effect we acknowledge in the revised manuscript (see comment #4). The differences between the delayed signers and native signers are also retained (Figure 3), now aligning better with language-sensitive regions, as previously predicted. The inclusion of the task difficulty predictors also introduced an additional finding in this analysis, a significant cluster in the right aIFG. Therefore, the inclusion of these predictors reaffirms the robustness of the conclusions drawn about FC variability in the deaf population.

We would like to note that while we would like to replicate these findings in an additional cohort using resting-state if we had access to such data, we do not anticipate the state in which the participants are scanned to greatly affect the findings. FC patterns of hearing individuals have been shown to be primarily shaped by common system and stable individual features, and not by time, state, or task (Finn et al., 2015; Gratton et al., 2018; Tavor et al., 2016). While the task may impact FC variability, we have recently shown that individual FC patterns are stable across time and state even in the context of plasticity due to visual deprivation (Amaral et al., 2024). Therefore, we expect that in deafness as well there should not be meaningful differences between resting-state and task FC networks, in terms of FC individual differences. We have also addressed this point in our manuscript (lines 442-451).

> *(3) No differences in overall activation between groups were reported. Activation differences between groups could lead to differences in FC. For example, lower activation may be associated with more noise in the data, which could translate to reduced FC.*

We thank the reviewer for noting the potential implications of overall activation differences on FC. In our analysis of the activation for words, we found no significant clusters showing a group difference between the deaf and hearing participants ($p < .05$, cluster-corrected for multiple comparisons) - we also added this information to the revised manuscript (lines 542-544). This suggests that the differences in FC observed are not confounded by variations in overall brain activation between the groups under these conditions.

> *(4) Figure 2B shows higher FC for congenitally deaf individuals than normal-hearing individuals in the insula, supplementary motor area, and cingulate. These regions are all associated with task effort. If congenitally deaf individuals found the task harder (lower performance), then activation in these regions could be higher, in turn, leading to FC. A study using resting-state data could possibly have provided a clearer picture.*

We thank the Reviewer for pointing out the potential impact of task difficulty on FC differences observed in our study. As addressed in our response to comment #2, task accuracy and reaction times were incorporated as nuisance variables in our analysis. Further, these areas showed no difference in activation between the groups (see response to comment #3 above). Notably, the referred regions still showed higher FC in congenitally deaf individuals even when controlling for these performance differences. Additionally, these findings are consistent with results from studies using resting-state data in deaf populations, further validating our observations. Specifically, using resting-state data, Andin & Holmer (2022), have shown higher FC for deaf (compared to hearing individuals) from auditory regions to the cingulate cortex, insular cortex, cuneus and precuneus, supramarginal gyrus, supplementary motor area, and cerebellum. Moreover, Ding et al. (2016) have shown higher FC for the deaf between the STG and anterior insula and dorsal anterior cingulated cortex. This suggests that the observed FC differences are likely reflective of genuine neuroplastic adaptations rather than mere artifacts of task difficulty. Although we wish we could augment our study with resting-state data analyzed similarly, we could not at present acquire or access such a dataset. We acknowledge this limitation of our study (lines 442-451) in the revised manuscript and intend to confirm that similar results will be found with resting state data in the future.

> *(5) The correlation between the FC map and the FC variability map is 0.3. While significant using permutation testing, the correlation is low, and it is not clear how great the overlap is.*

We acknowledge that the correlation coefficient of 0.3, while statistically significant, indicates a moderate overlap. It's also worth noting that, using our new models that include task performance as a nuisance variable, this value has decreased somewhat, to 0.24 (which is still highly significant). It is important to note that the visual overlap between the maps is not a good estimate of the correlation, which was performed on the unthresholded maps, to estimate the link not only between the most significant peaks of the effects, but across the whole brain patterns. This correlation is meant to suggest a trend rather than a strong link, but especially due to its consistency with the findings in blindness, we believe this observation merits further investigation and discussion. As such, we kept it in the revised manuscript while moderating our claims about its strength.

> ***Reviewer #1 (Recommendations For The Authors):***
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> *(1) Page 4: Does auditory cortex FC variability..." FC is not yet defined.*

Corrected, thanks.

> *(2) Page 4: "It showed lower variability..." What showed this?*

Clarified, thanks.

> *(3) Page 11: "highlining the importance" should read "highlighting the importance".*

Corrected, thanks.

> *(4) Page 11: Do you really mean to suggest functional connectivity does not vary as a function of task? This would not seem well supported.*

We do not suggest that FC doesn't vary as a function of task, and have revised this section (lines 447-451).

> *(5) Page 12: "there should not to be" should read "there should not be".*

Corrected, thanks.

> *(6) Page 12: "and their majority" should read "and the majority".*

Corrected, thanks.

> ***Reviewer #2 (Recommendations For The Authors):***
>
> *Major*
>
> *(1) Although this is a lot of work, I nonetheless have another suggestion on how to test if your results are strong and robust. Perhaps you could analyze your data using an ROI/graph-theory approach. I am not an expert in graph theory analysis, but for sure there is a simple and elegant statistic that captures the variability of edge strength variability within a population. This approach could not only validate your results with an independent analysis and give the audience more confidence in their robustness, but it could also provide an estimate of the size of the effect size you found. That is, it could express in hard numbers how much more variable the connections from auditory cortex ROI's are, in comparison to the rest of the brain in the deaf population, relative to the hearing population.*

We thank the Reviewer for suggesting the use of graph theory as a method to further validate our findings. While we see the potential value in this approach, we believe it may be beyond the scope of the current paper, and merits a full exploration of its own, which we hope to do in the future. However, we understand the importance of showing the uniqueness of the connectivity of the auditory cortex ROI as compared to the rest of the brain. So, in order to bolster our results, we conducted an additional analysis using control regions of interest (ROIs). Specifically, we calculated the inter-individual variability using all ROIs from the CONN Atlas (except auditory and language regions) as the control seed regions for the FC. We showed that the variability of connectivity from the auditory cortex is uniquely more increased on deafness, as compared to these control ROIs (Figure S1). This additional analysis supports the specificity of our findings to the auditory cortex in the deaf population. We aim to integrate more analytic approaches, including graph theory methods, in our future work.

> *Minor*
>
> *(1) Some citations display the initial of the author in addition to the last name, unless there is something I don't know about the citation system, the initial shouldn't be there.*

This is due to the citation style we're using (APA 7th edition, as suggested by eLife), which requires including the first author's initials in all in-text citations when citing multiple authors with the same last name.

> ***Reviewer #3 (Recommendations For The Authors):***
>
> *(1) I recommend that the authors provide behavioral data and results for overall neural activation.*

Thanks. We have added these to the revised manuscript. Specifically, we report that there was no difference in the activation for words ($p < .05$, cluster-corrected for multiple comparisons) between the deaf and hearing participants. Further, we report the behavioral

averages for accuracy and reaction time for each group, and have now used these individual values explicitly as nuisance variables in the revised analyses.

> *(2) For the correlation between FC and FC variability, it seemed a bit odd that the permuted data were treated additionally (through Gaussian smoothing). I understand the general logic (i.e., to reintroduce smoothness), but this approach provides more smoothing to the permutation than the original data. It is hard to know what this does to the statistical distribution. I recommend using a different approach or at least also reporting the p-value for non-smoothed permutation data.*

In response to this suggestion and to ensure transparency in our results, we have now included also the p-value for the non-smoothed permutation data in our revised manuscript (still highly significant; $p < .0001$). Thanks for this proposal.

> *(3) For the map comparison, a plot with different colors, showing the FC map, the FC variability map, and one map for the overlap on the same brain may be helpful.*

We thank the Reviewer for their suggestion to visualize the overlap between the maps. However, we performed the correlation analysis using the unthresholded maps, as mentioned in the methods section of our manuscript, specifically to estimate the link not only between the most significant peaks of the effects, but across the whole brain patterns. This is why the maps displayed in the figures, which are thresholded for significance, may not appear to match perfectly, and may actually obscure the correlation across the brain. This methodological detail is crucial for interpreting the relationship and overlap between these maps accurately but also explains why the visualization of the overlap is, unfortunately, not very informative.

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