Impact of Early Reversible Deafness and Cross-Modal Interactions on Adult Central Auditory Processing

By

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Abbreviations:

- A1: primary auditory cortex
- ABR: Auditory brainstem response
- BW20: Bandwidth 20dB above CF
- C: control
- CF: Characteristic frequency
- CHL: Conductive hearing loss
- CI: cochlear implant
- CP: Critical period
- D: Deaf
- dB: decibel
- DBR: Deaf-Blind group
- DNR: Deaf-Noise exposed group
- DR: Deaf-Recovered group
- EAC: External auditory canal
- EACL: external auditory canal ligation
- FM: Frequency modulated
- KHz: Kilo-Hertz
- ml: milliliter
- mm: millimeter
- MRI: Magnetic resonance imaging
- ms: millisecond
- P: post-natal day
- SLP: Sound pressure level

Thesis Abstract:

Background: Early auditory stimulation is essential for normal central auditory system maturation and language acquisition. Nevertheless, hearing loss is a widespread problem among young children, which is associated with poor auditory intelligence and academic performance despite the alleviation of the hearing loss.

Objectives:

- Create and validate an animal model for reversible hearing loss.
- Investigate the long-term impact of transient early-life deafness on the central auditory processing.
- Investigate the potential usefulness of dark rearing and noise exposure to promote recovery of auditory functions from early-life deafness.

Methods: A new technique of external auditory canal ligation and reopening is described. Auditory brainstem responses were used to measure both the degree of hearing loss induced by the external auditory canal ligation and the auditory thresholds following the hearing restoration procedure.

One control and four experiment groups were included to study the impact of early-life deafness and the potential usefulness of different interventions on recovery. Electrophysiological responses to auditory stimulation were recorded from the primary auditory cortex in all groups.

Results: External auditory canal ligation and reopening procedures had success rates of 81% and 78%, respectively. The average hearing threshold dropped by 40 decibels, with greater hearing loss for high frequencies.

An under-representation of high frequency sounds and altered electrophysiological responses to sound stimuli were consequences of early-life deafness and were shown to

persist following hearing restoration. While dark rearing failed to stimulate recovery, the noise exposure was found to reinstate a normal frequency distribution and central auditory processing within the primary auditory cortex.

Conclusion: Early-life deafness has a long-term impact on the central auditory processing. Visual deprivation failed to aid in recovery of function following hearing restoration. Continuous noise exposure, however, showed promising benefits for recovery. The mechanisms underlying this improvement remain unknown, further studies are recommended.

Résumé

Introduction: La simulation auditive est essentiel pour le développement normal du système auditif central ainsi que sa maturation et l'acquisition du langage. Malheureusement les pertes auditives sont fréquentes chez les jeunes enfants et sont associés à des problèmes auditifs ainsi que des problèmes scolaires même après que la surdité a été traité.

Objectifs:

- Créer et valider un modèle animal de la surdité réversible.
- Étudier l'impact à long terme d'une déficience auditive sur le fonctionnement auditive central.
- Étudier l'impact de l'élevage dans l'obscurité et dans le bruit sur la fonction auditive après une surdité précoce.

Méthodes: Une nouvelle technique a été développer pour ligaturer d'une manière réversible et pour plus tard rouvrir le conduit auditif externe. Les potentiels évoqués auditive du tronc cérébral ont été étudié afin de vérifier le niveau de surdité attend par la ligature du conduit auditif externe avant et après la réouverture du conduit.

Un groupe contrôle et quatre groupes expérimentaux ont été inclus dans cette étude sur la surdité a un bas âge et le potentiel de différentes interventions à pallier à ses ravages à long terme. Des mesures électrophysiologies ont été prises aux niveau du cortex cérébral auditive de chaque groupe.

Résultats: La ligature et la réouverture du conduit auditif externe avait un taux de succès de 81 % et de 78 % respectivement. Les seuils auditifs ont diminué de 40 dB avec la ligature. Nous avons noté une atteinte plus importante dans les hautes fréquences.

Nous avons observé une sous-représentation des hautes fréquences et une altérations électrophysiologique de réponse au son comme conséquence de surdité induite à un jeune âge. Elles ont persisté même après la réouverture du conduit. L'Élevage dans l'obscurité n'a pas aidé. Par contre, l'exposition au bruit continue a eu comme résultat une redistribution vers la normale dans le cortex auditive.

Conclusion: Exposition un bruit continue à démontrer certaines promesses. Le mécanisme de cette amélioration demeure inconnu et plus d'études seront nécessaires.

Preface:

This is a manuscript-based thesis comprised of two scientific papers. The first paper describes and validates a novel surgical technique of external auditory canal ligation and reopening in a rat model. This new technique offers a unique model for hearing loss research. The paper likewise compares various methods that have been used to induce hearing loss for auditory research, providing advantages and disadvantages of each. The second paper uses our new animal model in a well-structured scientific experiment to study the early and long-term impacts of early-life hearing loss on brain development. Furthermore, it investigates the potential usefulness of two methods to improve the functional recovery of auditory neurons following early-life deafness.

Contribution of Authors:

Dr. Ahmed Alkhateeb was responsible for the entire literature review, experiment design, data collection, and analysis of results of both manuscripts and the drafting of both manuscripts. The idea and technique of the surgical experiment were an exclusive effort of Dr. Alkhateeb. Animal care during the experiment, all surgical procedures, data collection and analysis, and extracting conclusions were the work of Dr. Alkhateeb. Dr. Etienne de-Villers-Sidani assisted in the conception and formulation of the research objectives. Dr. Etienne de-Villers-Sidani, Dr. Anthony Zeitouni and Dr. Patrice Voss provided supervision, guidance, clinical and basic science relevance expertise, and review of this thesis.

Claim of Originality:

The new techniques of external auditory canal ligation and reopening are novel methods for hearing loss research. To the best my knowledge, no prior study has

investigated the effect of visual deprivation nor noise exposure on the recovery of early-life deafness.

Acknowledgements:

This basic science thesis reflects more than two years of dedicated work of applying basic science knowledge in scientific experiments. I would like to express my sincere gratitude to my direct supervisors: Dr.Etienne de Villers-Sidani “Basic science supervisor” and Dr.Anthony Zeitouni “Clinical supervisor” for their continuous guidance and valuable contribution throughout the project.

I graciously thank Maryse Thomas “PhD candidate at the Montreal Neurology Institute” for her kindness and patience in teaching me many complex neurophysiological concepts of the central auditory system. Without her help in the data analysis, using the MatLab software, I wouldn’t be able to complete this project.

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Finally, Funding of this project was provided though Dr.de-Villers-Sidani’s laboratory at the Montreal Neurology Institute, for which I am deeply indebted.

Chapter 1: Introduction

1.1 Introduction and Rationale:

Conductive hearing loss is a widespread problem in young children due to the high prevalence of recurrent ear infections and middle ear effusion among this population. The auditory deprivation caused by conductive hearing loss was found to be associated with various sensory and cognitive difficulties such as long term auditory impairments and poor academic performance, that may persist despite the alleviation of the hearing loss. ([1], [2], [3], [4], [5] , [6], [7], [8]). This poor performance is thought to be a result of altered neural processing secondary to auditory deprivation during a critical period of development.

The brain is a fundamentally plastic organ with the ability to shape its circuits during development in order to improve the processing of available sensory inputs. In the absence of clear meaningful sensory inputs, as it is the case in congenitally deaf individuals, the deafferented cortical areas progressively retune themselves to sensory inputs from other sensory modalities ([9], [10], [11], [12], [13], [14], [15] , [16], [17], [18]). This phenomenon, known as cross-modal plasticity, was often linked to superior visual abilities in congenitally deaf individuals and superior tactile resolution in blind individuals using Braille language ([19], [20], [21], [22], [23],[24]). It is not known if the cross-modal reorganization is a potentially reversible process or if its plasticity has a critical period. To the best of my knowledge, there is no previous study that investigated the potential usefulness of visual deprivation or of noise exposure on the recovery of auditory function following early hearing loss during the critical period.

1.2 Objectives and Hypothesis:

In view of the above rational, this thesis has the following objectives:

1. Create and validate an animal model of reversible hearing loss.
2. Examine the impact of early auditory deprivation on the functioning of auditory cortical neurons following hearing restoration in adult rats.
3. Investigate the potential usefulness of visual deprivation on the post critical period recovery of auditory neural function following early-life deafness.
4. Investigate the impact of noise exposure on the recovery of auditory neural function after early-life deafness.

The above objectives was stated according to the following hypothesis:

1. External auditory canal ligation is a reliable technique that can induce significant yet reversible hearing loss.
2. Auditory deprivations during auditory developmental critical periods will have a prolonged impact on the electrophysiological responses to sounds within the auditory cortex.
3. Visual deprivation following hearing restoration might stimulate cross-modal re-reorganization and therefore improve the recovery of auditory functions.
4. Noise exposure following hearing restoration might help promote recovery of auditory neural functions.

1.3 Thesis Organization:

While the first paper describes a surgical technique used to create a model for reversible hearing loss, the second paper used this model to study the impact of early-life deafness on central auditory processing. This objective would have been difficult to achieve without restoring normal auditory input later in life. Therefore, it was imperative that the primary investigator, Dr. Alkhateeb, develop a novel technique of external auditory canal ligation and subsequent reopening. The two papers together

consist in a complete and integrated research project that started with creating a model, validating the model, and then finally using this model in a scientific experiment to address a research question.

Chapter 2: Literature Review:

The brain is a fundamentally plastic organ with the ability to shape its circuits during development in order to improve the processing of available sensory inputs. In the absence of clear meaningful sensory inputs, as it is the case in congenitally deaf individuals, the deafferented cortical areas progressively retune themselves to sensory inputs from other sensory modalities ([25], [26], [27], [28], [29], [30], [31] , [32], [33], [34]). This phenomenon, known as cross-modal plasticity, was often linked to superior visual abilities in congenitally deaf individuals and superior tactile resolution in blind individuals using Braille language ([35], [36], [37], [38], [39],[40]). It is not known if the cross-modal reorganization is a potentially reversible process or if its plasticity has a critical period. To the best of my knowledge, there is no prior study that investigated the potential usefulness of visual deprivation or of noise exposure on the recovery of auditory function following early hearing loss during the critical period.

Research investigating the impact of binaural auditory deprivation on the central auditory nervous system has been limited, in part because there is a lack of an ideal research model.

The laboratory rat has become an important animal model in the auditory neurophysiological research field. External auditory canal ligation (EACL), as a method to induce hearing loss in rats, was described earlier for hearing loss research ([41], [42], [43], [44], [45], [46], [47]). In this thesis, we are proposing a modified technique of EACL in rats that is easy, effective and, most importantly, reversible. We describe both the EACL and reopening procedures, and their respective success rates.

The literature related to cross modal plasticity and critical periods is reviewed in chapter four. The brain is a fundamentally plastic organ with the ability to shape its

circuits during development to improve the processing of available sensory inputs. In the absence of clear meaningful sensory inputs, as it is the case for congenitally deaf individuals, the deafferented cortical areas progressively retune themselves to sensory inputs from other sensory modalities ([48], [49], [50], [51], [52], [53], [54] , [55], [56], [57]). This phenomenon, known as cross-modal plasticity, tends to improve perception of intact sensory system. In many cases however, this adaptive "invasion" of cross-modal sensory inputs in the deafferented cortex results in reduced potential for learning to process the original inputs it was meant to process when they are restored, for example, by cochlear implantation. Congenitally deaf kids who received cochlear implants before 3.5 years of age had normal auditory cortical response latencies, whereas those who were implanted after 7 years of age failed to reach normal latencies, even after several years of implant use. ([58]). This result, along with those from several other studies ([59], [60], [61], [62] , [63]), suggest that sensory cortex is more plastic during a specific time window early in life, a window known as the critical period (CP).

The critical period theory states that brain development is very sensitive to external input within a specific timeframe ([64]).It is not known if the cross-modal plasticity that follows sensory loss is reversible, or if it is governed by a CP.

Chapter 3: External Auditory Canal Ligation: A Novel Technique to Induce Reversible Hearing Loss in Rats (Manuscript 1)

By

Ahmed Alkhateeb, Etienne de-Villers-Sidani, Anthony Zeitouni, Patrice Voss

A Manuscript in preparation for a thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master in Otolaryngology

3.1 Abstract

The rat has become an important model for auditory research. It has superior hearing to other rodents and a comparable auditory nervous system to other mammals. We propose external auditory canal ligation (EACL) as a procedure that is relatively easy and able to mimic congenital conductive hearing loss, and one that can be reversed. In the present article, we describe the surgical technique of both EACL, and the reopening procedure in rats, and evaluate the success rate of both techniques.

3.2 Introduction:

Conductive hearing loss (CHL) is a widespread problem in young children. Common causes include cerumen impaction, middle ear effusion, congenital ossicular fixation, and aural atresia ([65]). it has been demonstrated that auditory deprivation associated with conductive hearing loss is associated with sensory and cognitive difficulties such as long term behavioral problems and poor academic performance ([66, 67, 68, 69, 70 , 71, 72, 73]). Noteworthy, the pathophysiology underlying these issues remains unclear. Several anatomical and physiological central neural changes were reported secondary to unilateral conductive hearing loss. These changes include a decrease in neuronal size and of the size and density of the auditory nucleus and central pathway, a reduction in protein synthesis in the cochlear nucleus, and a decrease in the functional activity as shown by decrease in the uptake of 2-Deoxyglucose by the central auditory system following sound stimulation; these changes were generally thought to be the results of the imbalance of binaural auditory stimulation ([74, 75, 76, 77, 78, 79]). Research investigating the impact of binaural auditory deprivation on the central auditory nervous system processing has been limited, in part because there is a lack of an ideal research model.

The laboratory rat has become an important animal model in the auditory neurophysiological research field. It has a comparable hearing in its sensitivity and spectral acuity to other rodents ([80, 81, 82]), and shows a similar anatomical and functional organization of the central auditory system to other mammals ([83]). It also has a fast healing process and short life span, which made it feasible to study different interventions, scattered over different life developmental stages of the animal.

External auditory canal ligation (EACL) was described earlier for hearing loss research ([84, 85, 86, 87, 88, 89, 90]). In this article, we are proposing a modified technique of EACL in

rats that is easy, effective and, most importantly, reversible. We describe both the EACL and reopening procedures, and their respective success rates.

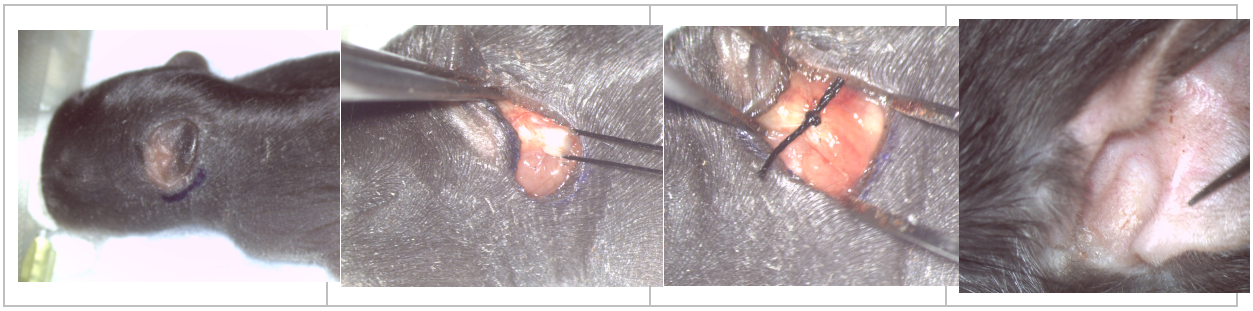
3.3 Methodology:

All procedures were performed in accordance with the Canadian Council on Animal Care guidelines. 84 neonatal ears had received a bilateral EACL procedure between postnatal day 7-10, before the external auditory canal (EAC) normally canalizes. Four weeks later, 54 ears underwent atresia repair.

3.3.1 External Auditory Canal Ligation Technique:

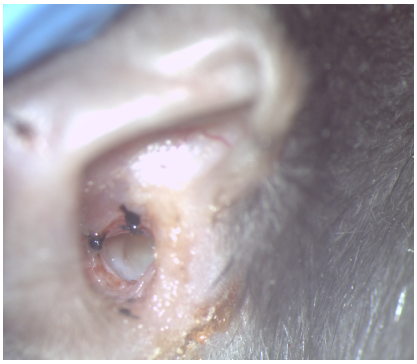
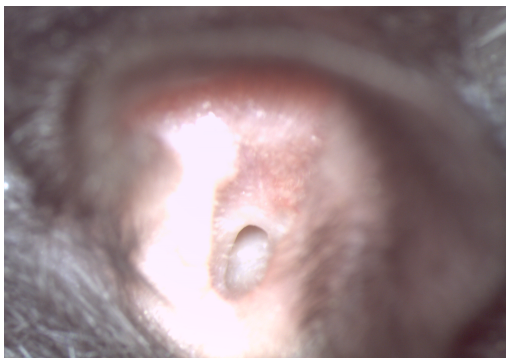
The procedure was done under inhalational anesthesia (isoflurane for vets at 4% for induction and 2% for maintenance with oxygen at 1.5 liter per minute) while the animal was spontaneously breathing. 0.1 ml of 1% Lidocaine with epinephrine at 1:100,000 normal saline was injected at the incision site. Pulling the auricle anteriorly and superiorly helps to see the bulge of the external auditory canal (EAC) cartilage which is the site of interest. Post auricular incision was made and the cartilaginous segment of the external auditory canal was identified as white and shiny. The distal portion of the EAC was isolated and ligated with 4-0 surgical silk suture, by passing the stitch around the EAC and tying it thus causing the cartilage to curl on itself. Placing the suture as lateral as possible was important for the reopening procedure. The wound is closed with 5-0 prolene in a simple interrupted fashion.

Figure 1: The site of the 3mm post auricular incision pre EACL	Figure 2: Magnified picture showing the suture around the EAC	Figure 3: Distally placed ligated suture	Figure 4: EAC atresia 2 weeks post EACL



3.3.2 EAC Reopening Technique:

The procedure was performed under isoflurane anesthesia. Initially, the condition of each ear was evaluated under a surgical microscope and each ear was found to maintain a complete atresia. After injecting 0.1 ml of 1% lidocaine with epinephrine 1:100,000, a post-auricular incision was made, and the previous silk suture was identified and removed. With meticulous dissection, the lateral end of the EAC was identified and opened, and was always found full of keratin. A window was made in the deep part of the concha, forming a superiorly based chondo-cutaneous flap, which was anastomosed with the newly opened EAC superiorly. Inferiorly, EAC was sutured to the conchal cartilage. Examining the newly created meatus should confirm direct alignment of the auditory canal. The site of anastomosis was stented with a 14-gauge cannula for seven days post operatively.

Figure 5: EAC immediately post reopening procedure	Figure 6: 2 weeks post reopening procedure
	

3.3.3 Auditory Brainstem Response Measurement:

Auditory brainstem response (ABR) was measured in a double-walled soundproof chamber. Animals were anesthetized with an isoflorane anesthesia. Tone pips (3 ms duration, 1.5 ms ramps) of 1,2, 4, 8, 12, 24, and 48 kilo Hertz (kH) at nine intensities (80-0 DeciBel (dB)) were presented via a speaker, fixed in a free field approximately 30cm from the rat. ABRs were recorded by placing silver wires subdermally at the scalp midline (negative), posterior to the ears bilaterally (positive), and on the midline of the back 1–2 cm anterior to the tail. ABR signals were acquired, filtered, amplified, and analyzed using the software OpenEx (Tucker-Davis Technology, Inc., Alachua, FL, USA). ABRs were obtained by averaging of 3000 recordings (10 per second) at different intensities. ABR thresholds were defined as the lowest sound intensity capable of eliciting a response pattern characteristic of that seen at higher intensities.

3.4 Results:

Out of 84 neonatal rat ears that underwent EACL procedure, 68 (81%) grew with complete aural atresia, 9 (11%) had meatal stenosis with variable degrees, 5 (6%) rejected the suture and healed with normal EAC, and one rat died during anesthesia induction. Rats with bilateral aural atresia (N=27) underwent bilateral ear reopening procedure. 42/54 (78%) ears had a successful EAC repair, as evaluated four weeks post stent removal, and finally 12/54 (22%) developed restenosis.

3 rats with bilateral EAC atresia underwent ABR measurements pre-ligation, post-ligation, and post-repair. Post ligation ABR revealed a hearing threshold average of 60dB sound pressure level (SPL) of frequencies 1, 2, 4, 8, 12, 24, and 48 kHz. There was more hearing loss in the high frequencies (12-48 kHz) than the low frequencies (1-3 kHz) *See table 1*. Post repair hearing threshold average was comparable to pre-ligation

threshold of 20 dB SPL cross all frequencies.

Table 1: The average post EACL hearing threshold for different frequency bins

Frequency Range	Hearing threshold Average
Low frequencies (1-3 kHz)	40 dB
Mid frequencies (3-12 kHz)	60 dB
High frequencies (12-48 kHz)	80 dB

3.5 Discussion:

Our technique of EACL was shown to be easy and reliable. Animals grew with complete cartilage and skin aural atresia, which caused changes in behavioral responses to sound stimuli in developing rats. Our technique consisted in placing a ligation suture around the cartilaginous canal distally to minimize the trauma to the EAC, to maintain the reopening procedure feasible. A well-developed—and full of keratin proximal to the suture—EAC with a normal tympanic membrane was seen in all animals. McGinn et al. 1984 showed that unlike in gerbils, EACL in rats induced keratin deposits in the ear canal ([91]). However, it did not cause any medial displacement of the tympanic membrane, cholesteatoma, or a bony erosion. EACL, as previously described, was performed by placing two sutures, one proximal and one distal in the EAC, and then by removing the skin in between the two sutures, allowing it to heal by fibrosis ([93, 94, 95, 96, 97, 98, 99]). Although the previous technique successfully induced atresia and hearing loss, it was not a reversible procedure. Instead of ligating the EAC, elevating a sleeve of skin over the cartilaginous canal and suturing it, has been proposed as a means to achieve a reversible method of CHL ([100]). In the former study, the ear closure was done at the age of 21 days, after the EACL was fully developed, whereas the repair was performed one

week later. Although the closure was made only with a skin sleeve, it induced a sufficient CHL to generate changes in the central auditory system.

Earplugs ([101, 102]), injecting silicone oil in the middle ear ([103]), and malleus removal ([104, 105]) are alternative methods that have been previously used. Although earplugs were simple and non-invasive, they couldn't be used in neonatal rats when the EACs were not yet canalized to imitate the effect congenital hearing loss. They weren't ideal for young, rapidly growing rats either, because the fit would not remain constant. In Hartley and Moore study ([106]), injecting high viscosity silicone oil in the middle ear raised the hearing threshold by 36dB. However, middle ear effusion tends to resolve spontaneously through a functioning Eustachian tube, which again makes the duration of auditory deprivation uncertain. Inducing Eustachian tube dysfunction has been described in the literature for otitis media with effusion research ([107]). However, it was irreversible and technically challenging. Malleus removal is an irreversible procedure, technically challenging, and is accompanied by the risk of injuring the other ossicles or the cochlea.

Table 2: Comparison of different induced hearing loss techniques

Technique	Hearing Loss	Reversible	Reliable
EACL	✅		✅
Cochlear ablation	✅		✅
Malleus removal	✅		✅
Ear plugs	✅	✅	
Intra-Tympanic silicone injection	✅	✅	
Modified EACL	✅	✅	✅

External Auditory Canal Ligation (EACL): Proximal suture with distal EAC injury to heal with fibrosis. Modified EACL: proximal suture with preservation of the EAC structure.

The EACL procedure described in this paper has limitations. First, as in any surgical procedure, anesthesia complications can occur. Second, the procedure does guarantee a successful CHL, as our results showed there was a small failure rate. Third, the reopening procedure was technically challenging, and the opening had tendency to reclose if the stent was removed too early or there was missing skin at the anastomosis site.

3.6 Conclusion:

Compared to other techniques, the EACL was an easy and reliable method to induce a reversible CHL. It had a very low risk of injuring vital structures or causing permanent damage. It can be applied to a seven-day old rat, before the external auditory canal naturally canalizes, and it shifts the hearing threshold up by 60dB SPL. It provides a novel animal model for studying congenital hearing loss that can be reversed.

After describing and validating a novel surgical technique of external auditory canal ligation and reopening in a rat model, a new technique that offers a unique model for hearing loss research, the second paper uses this new animal model in a well-structured scientific experiment to study the early and long-term impacts of early-life hearing loss on brain development. Furthermore, it investigates the potential usefulness of two methods to improve the functional recovery of auditory neurons following early-life deafness.

Chapter 4: Impact of Early Reversible Deafness and Cross-Modal Interaction on Adult Central Auditory Processing (Manuscript 2)

By

Ahmed Alkhateeb, Etienne de-Villers-Sidani, Anthony Zeitouni, Patrice Voss

A Manuscript in preparation for a thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master in Otolaryngology

4.1 Abstract:

The brain is a fundamentally plastic organ that interacts with the surrounding sensory inputs to mature. The absence of early auditory experience has long-term sequelae on language development and learning. Here we investigated the potential usefulness of two methods to improve the functional recovery of auditory neurons following early-life reversible deafness. We found that dark rearing adult rats following hearing restoration failed to reverse the impact of early-life deafness on the electrophysiological responses to auditory stimulation. We also found that exposing adult rats to broadband noise following hearing restoration resulted in comparable electrophysiological responses to auditory stimulations to those observed in control rats. The present findings demonstrate that the neural plasticity of the adult brain can be boosted with certain interventions to improve the functional recovery of neurons following early-life sensory impairments.

4.2 Introduction

Neural development is the result of a dynamic interplay between an inherited genetic code and extrinsic environmental factors. The brain is a fundamentally plastic organ with the ability to shape its circuits during development to improve the processing of available sensory inputs. In the absence of clear meaningful sensory inputs, as it is the case for congenitally deaf individuals, the deafferented cortical areas progressively retune themselves to sensory inputs from other sensory modalities ([108], [109], [110], [111], [112], [113], [114] , [115], [116], [117]). This phenomenon, known as cross-modal plasticity, tends to improve perception of intact sensory system. It has been shown that when a brain is deprived from auditory input, it often compensates by enhancing visual performance ([118], [119], [120], [121], [122]). Brain functional magnetic resonance imaging (MRI) studies of congenitally deaf participants showed activation in their superior temporal lobes, where the primary auditory cortex is located, in response to visual stimulations ([123], [124], [125]). Similarly, congenitally deaf cats exhibited supernormal abilities performing visual psychophysical tasks, including superior motion detection and localization in the peripheral visual field. Deactivation of specific areas of the congenitally deaf cats' auditory cortices eliminated the observed superiority in visual performance ([126]). In many cases however, this adaptive "invasion" of cross-modal sensory inputs in the deafferented cortex results in reduced potential for learning to process the original inputs it was meant to process when they are restored, for example, by cochlear implantation. Cochlear implant (CI) users with poor speech perception capacities showed a larger area of auditory cortical activity following visual stimulation despite a long period extensive speech rehabilitation. This poor speech performance was shown to be inversely proportional to the duration of deafness and the magnitude of cross-modal reorganization ([127],[128]). Dorman et al. reported that congenitally deaf kids who

received cochlear implants before 3.5 years of age had normal auditory cortical response latencies, whereas those who were implanted after 7 years of age failed to reach normal latencies, even after several years of implant use. ([129]). This result, along with those from several other studies ([130, 131, 132, 133 , 134]), suggest that sensory cortex is more plastic during a specific time window early in life, a window known as the critical period (CP).

The critical period theory states that brain development is very sensitive to external input within a specific timeframe ([135]). It was first proposed in the field of language acquisition by neurosurgeon Wilder Penfield and Lamar Roberts in their book Speech and Brain Mechanisms 1959, and popularized by the linguist Eric H. Lenneberg in 1967, who argued for the hypothesis based on evidence that children who experience brain injury early in life develop far better language skills than adults with similar injuries. That stimulated scholars, not only in linguistics, but also in neurology, cognitive behavior and psychology, to study and describe the hypothesis further. A good example of the CP in learning is the ease with which kids raised in different cultures can naturally acquire the language of their surroundings, whereas adults exhibit much greater difficulty when learning a second language. Children with bilateral hearing loss typically show significant long term communication and educational impairments, for which the underlying pathophysiology is mysterious ([136, 137, 138, 139, 140, 141, 142]). It is not known if the cross-modal plasticity that follows sensory loss is reversible, or if it is governed by a CP.

Our experiment was designed to investigate the potential usefulness of two methods to improve the functional recovery of auditory neurons following early-life reversible deafness. We first examined the effect of early-onset bilateral hearing loss, following

hearing restoration, on the electrophysiological responses to auditory stimulation in adult rats. Furthermore, we examined the effect of temporary visual deprivation following hearing restoration on the recovery of auditory neural functions. We hypothesized that if there is no CP for cross modal plasticity, the visual deprivation might enhance the recovery from deafness. We also investigated the effect of continuous broadband pulsed noise exposure as an intervention that could enhance auditory neuroplasticity and reopen a critical period for neural maturation.

4.3 Methodology

All procedures were approved by the Animal Care Committee of Montreal Neurological Institute and complied with guidelines of the Canadian Council on Animal Care. In total, twenty-five Long-Evan rats (11 males and 14 females) were used for electrophysiology experiments. Rats were randomly assigned to one control (N=5) and four experimental groups (N=20). Early auditory deprivation was achieved in all experimental groups by external auditory canal ligation (EACL) before the age of ten days; prior to this age the external auditory canal (EAC) is not yet naturally canalized. The EACL has been shown to increase the pure tone threshold average by 40 decibels sound pressure level (dB SPL) (See above: Paper I: Alkhateeb et al; External Auditory Canal Ligation: A Novel Technique to Induce Reversible Deafness in Rats). The EAC were opened in all experimental groups between postnatal days 40-50, and subsequently underwent auditory cortical mapping either on the same day or later between postnatal days 70-80 *(see figure 1)*.

1. **Deaf (D):** EAC opening and cortical mapping were performed on the same day (N=4). This group demonstrates the impact of auditory deprivation during the critical period on auditory cortical development.
2. **Deaf-Recovered (DR):** had cortical mapping thirty days after EAC opening (N=6). This group demonstrates if the impact of auditory deprivation during the critical period is long-lasting after post-CP hearing restoration.
3. **Deaf-Blind-Recovered (DBR):** reared in the dark for ten days after EAC opening, and then underwent cortical mapping twenty days later (N=6). This group was designed to test the effect of dark-rearing on auditory processing after post-CP hearing restoration.

4. **Deaf-Noise-Recovered (DNR):** exposed to a broadband noise for ten days, and then underwent cortical mapping twenty days later (N=4). This group was designed to investigate the effect of noise exposure, which promotes auditory cortical plasticity, on auditory processing following post-CP hearing restoration.
5. **Control (C):** rats in this group were raised in the same environment, but underwent no interventions until the day of cortical mapping (N=5).

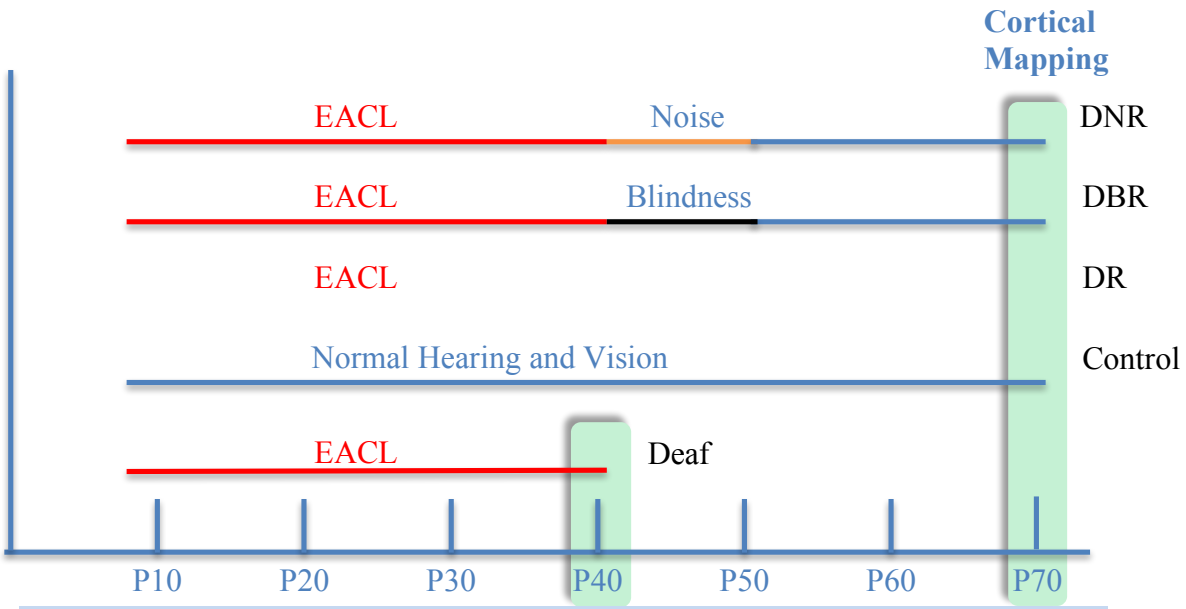


Figure 1: Demonstrates the timeline interventions for the experimental groups. EACL: External auditory canal ligation; DR: Deaf-Recovered; DBR: Deaf-Blind-Recovered; DNR: Deaf-Noise-Recovered; P: post-natal day.

4.3.1 External auditory canal ligation:

EACL was performed as described in paper I (See above: Alkhateeb et al; EACL: a novel technique to induce a reversible deafness in rats). The procedure was done under inhalational anesthesia (isoflurane for vets at 4% for induction and 2% for maintenance with oxygen at 1.5 liter per minute) while the animal was spontaneously breathing. A post auricular incision was made in one ear and the distal portion of the EAC was ligated with 4-0 surgical silk suture, by passing the stitch around the cartilage and tying it as a

surgical square knot. The procedure was then performed in both ears. The rats grew with bilateral complete atresia of the EAC. Two weeks post-ligation ABRs revealed pure tone hearing threshold average of frequencies 1,2,4,8,12,24,48 kilo-Hertz (kHz) at 60dB sound pressure level (SPL) which was equivalent to the attenuation produced by other ways of external auditory canal occlusion ([143], [144]).

4.3.2 External auditory canal reopening:

EAC reopening was performed as described in paper I (See above: Alkhateeb et al; EACL: a novel technique to induce a reversible deafness in rats). The procedure was performed under isoflurane anesthesia. After post auricular incision was made, the previous silk suture was identified and removed. The proximal segment of the EAC was opened and cleaned from keratin debris. No evidence of bony erosions or canal dilatation was seen in any animal. A window was made in the deep part of the concha, and was anastomosed with the newly opened EAC, creating a new auditory meatus. The meatus was stented with a 14-gauge cannula, sutured in place for seven days post operatively. Post EAC repair ABRs revealed a comparable pure tone hearing threshold average with those obtained from control animals (20dB SLP) at frequencies 1, 2, 4, 8, 12, 24, and 48 kHz.

4.3.3 Dark rearing:

DBR rats were kept within their own cage in a dark soundproof chamber for ten days after EAC repair. Rats were removed daily from their cage for less than five-minutes, to allow for ear and weight assessments.

4.3.4 Noise exposure:

DNR rats were placed in a soundproof chamber for noise exposure (24 hour per day). The continuous pulsed broadband noise was generated by a random-noise generator and amplified to a calibrated free-field sound level of 65 dB SPL, measured at the center

within the cage. The energy level for noise was essentially flat across a broad frequency spectrum (0.8–30 kHz). Rats had daily ear examination and their weights and activities were monitored. No abnormalities in the behavior of exposed rats could be detected during sound exposure and their weights were comparable with the naive rats, which indicated that the exposure stimuli were not overly stressful.

4.3.5 Auditory cortical mapping:

The left primary auditory cortex of rats was mapped as previously described ([145], [146]). Briefly, the rats were anesthetized with a cocktail of ketamine (50 mg/kg, intra-peritoneal), xylazine (5 mg/kg, intra-peritoneal), acepromazine (1 mg/kg, intra-peritoneal), and received an injection of the anti-inflammatory dexamethasone (0.2 mg/kg, intra-muscular) before surgery. Body temperature was maintained with a heating pad at 36 ± 1 degree Celsius. The EACs were examined to ensure their patency. A tracheostomy was made and connected to a ventilation machine where the rat's vital signs were monitored. The skull was kept fixed by a head holder, leaving the ears unobstructed. The cistern magna was drained of cerebrospinal fluid to minimize cerebral edema. The left temporalis muscle was dissected off the temporal bone and a craniotomy was made to expose the auditory cortex. The dura-mater was resected to broadly uncover the primary auditory cortex (A1) which was anatomically identified by the medial cerebral artery, dorsal to the rhinal fissure ([147]).

Multi-unit electrophysiological recordings were obtained using an array of 64 tungsten microelectrodes (8 × 8 electrodes). In the array, the distance between electrode columns was 375 μm, and between rows 500 μm. The array size was sufficient to encompass the whole cortical area corresponding to AI (3 × 4 mm). Microelectrodes were lowered perpendicular to the cortical surface to depths of 470–600 μm, targeting cortical layers IV and V. The preferential recording depth was 500 μm from pial surface,

where spontaneous neuronal activity is characteristic of a thalamo-cortical recipient layer. In all experiments, at least two penetrations of the microelectrode array were done in temporal cortex of each individual rat. In each array penetration, the rat's auditory system was stimulated by acoustic waves generated by TDT System III (Tucker-Davis Technologies, Inc., Alachua, FL, USA), through speakers that delivered sound to the contralateral ear in an open field mode in a soundproof chamber. Frequency-intensity receptive fields were reconstructed by presenting pure tones of 66 frequencies (1–70 kHz, 0.1-octave increments, 25-ms duration, 5-ms ramps) at eight sound intensities (0–70 dB SPL in 10-dB increments), in a rate of 2 stimuli per second. Neuronal responses were amplified (10,000×), filtered (0.3–3 kHz) and monitored online while recording (sampling rate of 20 kHz). We used the software OpenEx (Tucker-Davis Technology, Inc., Alachua, FL, USA) to generate acoustic stimuli, monitor cortical response properties online and store data for off-line analysis. Evoked spikes from multiple neurons were collected at each recording site of the array to reconstruct receptive fields.

4.3.6 Data analysis

A frequency tuning curve (intensity-frequency plot) was reconstructed and used to identify the characteristic frequency (CF) and the receptive field borders of each cortical site. The CF is the frequency with the lowest threshold to elicit a response from a given neuron. The tuning curves and tonotopic cortical maps were generated using custom-made MatLab routines. The center of each polygon in the map corresponds to the site of one microelectrode penetration and the colors represent the CF associated with neurons located in that site. Polygon area is proportional to the distance between neighboring penetrations. Boundaries of the primary auditory cortex were determined by the characteristic responses of the recorded neurons and by the topographic

organization of represented frequencies in the cortex. Primary auditory neurons generally have a continuous, single-peak shape tuning curve and their CFs are tonotopically organized with high frequencies represented rostrally and low frequencies represented caudally. To distinguish from posterior auditory field (PAF) and ventral auditory field (VAF), which are contiguous to AI, we considered their distinct tuning curves properties, as well as their particular topographical organization of responses to sounds of different frequencies ([148]). PAF was distinguished as a narrow band of cortex just caudal to AI, containing few and broad receptive fields, which exhibited discontinuous responses to sound frequencies. VAF, in the posterior ventral boundary, was identified by its patchy profile of responses to frequencies at low-threshold intensities (<30 dB SPL) but with little or no tone-evoked responses above this level. Recording sites outside AI were responsive only to higher intensity sounds, or were not reliably excited by tonal stimuli ([149]).

Tuning curves were classified as v-shaped and multi-peaked according to previous studies in AI ([150], [151], [152]). The receptive field irregularity index was used to quantify eventual differences between control and experimental animals. Higher irregularity index means that the tuning curve is less v-shaped. Single-peaked sites were identified as a well-defined v-shaped tuning curve, containing one apex or one CF. Tuning curves without apex (flat design) or containing more than one apex were seen as flat/multi-peaked sites. For flat-peaked tuning curves, the median frequency at minimal intensity was chosen as the CF. For tuning curves presenting multiple-peaks, the CF was defined at the apex with lowest threshold. BW20 was defined as the bandwidth of the tuning curve 20dB above CF and was measured in octaves (frequency response range). It provided an index of that frequency selectivity. For multi-peaked tuning curves, the BW20 was defined as the range of frequencies 20dB above the most sensitive peak, that

activated that cortical site. The response latency was defined as the time interval between the acoustic stimulus and the neuronal response. Characteristic frequencies, intensity thresholds, BW20, and latency were computed using custom-made Matlab routines (MathWorks, Natick, MA, USA). Percentages of sites described in our results were obtained from the total number of recorded sites inside AI.

4.3.7 Statistical Analysis

Data were analyzed using JMP version 11.2 software. Statistical significance was evaluated using Student's *t*-test. The significance level was set as $p < 0.05$.

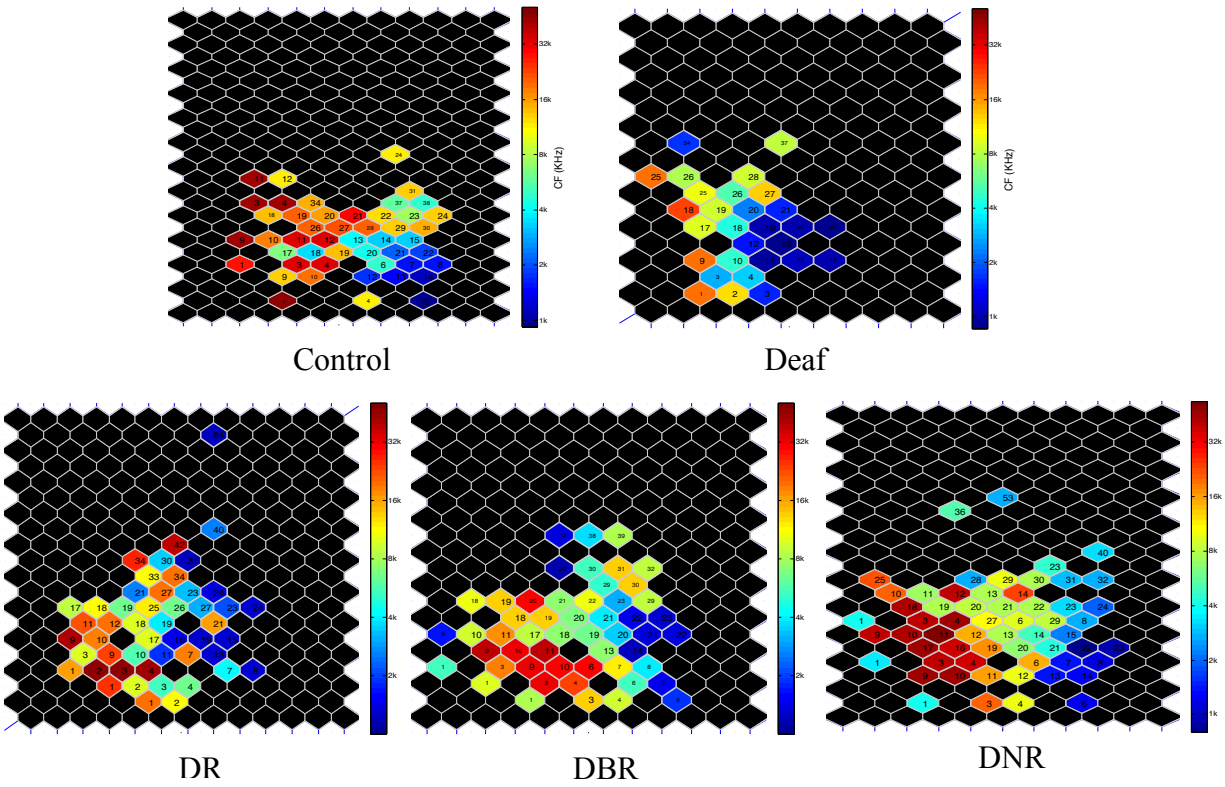


Figure 2: Representative A1 CF maps for different experimental groups. The color of each polygon in these maps indicates the CF for neurons recorded at that site (see color scale: Red color indicates high frequencies, Blue color indicates low frequencies).

4.4 Results:

Each animal underwent two recording sessions with a 64-channel electrode array, totalizing 128 recording sites per animal. Multiunit neural recordings were obtained from 934 electrode positions distributed along AI and the surrounding auditory cortical regions (See Table1). Only recordings from AI were included in the analysis.

Table 1: The summation of recording sites in each group. Each site represents a single or few adjacent recorded neurons.

Number of recorded positions	**Control**	**Deaf**	**DR**	**DBR**	**DNR**	**Total**
All sites	304	105	199	200	135	943
AI	136	86	98	121	100	541

4.4.1 The effect of CHL on the electrophysiological responses of A1:

The analysis started by generating CF maps of the primary auditory cortices of all groups. The comparison of cortical maps was done by calculating the percentage of the low CFs (1-3kHz), moderate CFS (3-12kHz), and high CFs (12-48kHz) sites of each group. The AI maps of deaf animals had significantly more sites with low CFs (Deaf 53%, Control 25%; p=0.009), and less with high CFs (Deaf 19%, Control 56%; p=0.020) than the controls. The average CF in D animals was significantly lower (D = 3585 Hz, Control = 3722 Hz; p<0.0001). The receptive fields in D animals were less tuned to its characteristic frequencies than those in controls, which was revealed by higher irregularity indices in these groups (D = 3.8, Control = 2.6; p<0.0001). Cortical frequency selectivity was examined by measuring the width of the receptive field 20 dB above the CF (BW20). D animals had a wider BW20 than the controls (D = 2.4 octaves, Control = 1.5 octaves; p<0.0001) which indicated less tuned receptive field to its CF.

Longer response latency was noticed in the D animals (D = 15.1millisecond, Control = 10.2 millisecond; $p<0.0001$).

D animals had higher hearing threshold than controls (D = 37.9 dB, Controls = 20.4 dB; $p<0.0001$). When braking down the data into frequency bins, we found a higher threshold at the sites of high CFs in D animals (high CFs threshold = 48.7dB, low CFs threshold = 34.3dB; $p=<0.001$), whereas the threshold was flat across the frequencies in controls (high CFs threshold = 20.9dB, low CFs threshold = 19.7dB; $p=0.95$). Noteworthy, the BW20 was wider (D = 2.3 octaves, Control = 1.6 octaves; $p<0.0001$), and latency was longer (D = 18.6 millisecond, Control = 9.5 millisecond; $p<0.0001$) in the high frequency bins, whereas in the low frequency bins there was no difference between D and control animals in the BW20 (D = 2.0 octaves, Control = 1.8 octaves; $p=0.61$), or in latency (D = 14.4 millisecond, Control = 11.2 millisecond; $p=0.053$).

4.4.2 The effect of hearing restoration on the electrophysiological responses of A1:

The average CF intensity threshold of the DR animals was lower (DR = 27.6, D = 37.1; $p<0.0001$; DR = 27.6 dB, Control = 20.4 dB; $p=0.0002$), though they maintained their down sloping configuration with higher threshold at high frequencies. The underrepresentation of high frequency sounds persisted, and the receptive fields remained poorly tuned as indicated by BW20 measures (DR = 1.95 octaves; Control = 1.53 octaves, $p<0.0001$; D = 2.43 octaves, $p=0.0015$). Response latencies were no different than in controls (DR = 11.8 millisecond, Control = 10.2 millisecond; $p=0.12$).

4.4.3 The effect of rearing in the dark on the recovery period:

DBR animals had comparable CF intensity thresholds (DBR = 25.8, DR = 27.6; $p=0.69$), average CF (DBR = 3678 Hz, DR = 3722 Hz; $p=0.99$) and BW20 values (DBR = 1.99

octaves, DR = 1.95 octaves; p=0.99) to DR animals. They maintained a down sloping of sound intensity thresholds and an underrepresentation of high frequencies. The DBR animals' response latency to sounds was longer than in the DR group, and comparable to the D animals (DBR = 14.2 millisecond; DR = 11.8 millisecond, p=0.0034; D = 15.1 millisecond, p=0.65). DBRs' receptive field borders were more irregular, as shown by a higher irregularity index than in DR animals (DBR = 3.2, DR = 2.7; p=0.0005).

4.4.4 The effect of noise exposure on the recovery period:

The tonotopic distribution and threshold configuration appeared to have recovered after noise exposure. The CF intensity thresholds (DNR = 27.9dB, DR = 27.6dB; p=0.99), and average CF (DNR = 3722 Hz, DR = 3734 Hz; p=0.97) were equivalent to the DR groups. Both the BW20 (DNR = 1.76 octaves, Control = 1.53 octaves; p=0.19) and the irregularity index (DNR = 2.85, Control = 2.65; p=0.43) were not significantly different in noise exposed animals than controls. Response latency was similar to that observed in DR animals (DNR = 12.3 millisecond; DR = 11.8 millisecond, p=0.93). This indicates that noise exposure was capable of inducing some neuroplasticity to retune the receptive fields and reorganize the tonotopy of AI.

Table 2: Comparison of electrophysiological responses in all experimental groups

	Control	**D**	**DR**	**DBR**	**DNR**
Threshold	20.9 dB	38.6 dB	27.6 dB	25.8 dB	27.9 dB
Average CF	3722 Hz	3585 Hz	3734 Hz	3678 Hz	3722 Hz
BW20	1.5 octaves	2.4 octaves	2.0 octaves	2.0 octaves	1.8 octaves
Latency	10.2 millisecond	15.1 millisecond	11.8 millisecond	14.2 millisecond	12.3 millisecond
Irregularity index	2.7	3.8	2.7	3.2	2.9

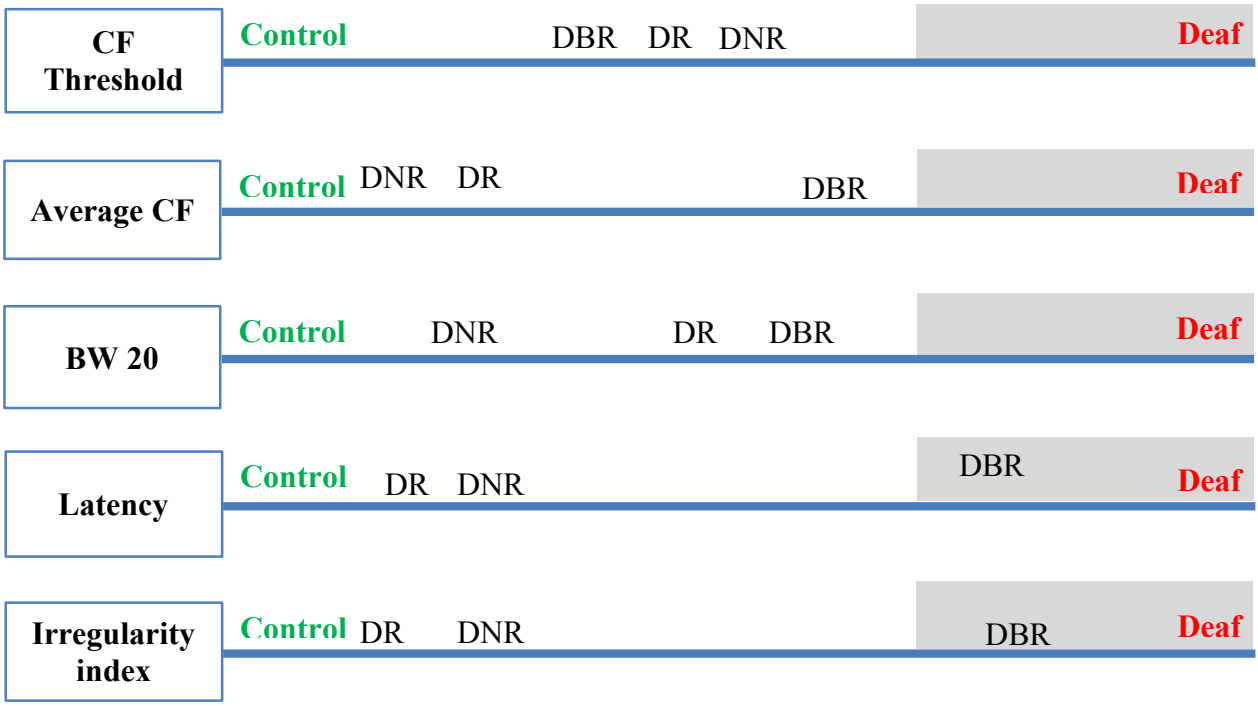


Figure 3: Schematic drawing showing a comparison of electrophysiological responses in different groups. The dotted zone indicates non-statistically significant from Control. The solid shadow zone indicates non-statistically significant from Deaf.

Table 3: Percentage of CF distribution in A1 in all groups. It showed under representation of high frequencies in the experiment groups secondary to EACL. Only the DNR group had restored normal CF distribution after hearing restoration.

	Low Frequency	Mid frequency	High frequency	p-value (control versus experiment)	p-value (Deaf versus intervention)
Control	19.4%	26.2%	54.4%		
Deaf	52.1%	28.2%	19.7%	< 0.001	
DR	33.9%	27.4%	38.7%	0.026	0.008
DBR	38.0%	29.8%	32.2%	0.001	0.012
DNR	25.2%	23.8%	51.0%	0.698	<0.001

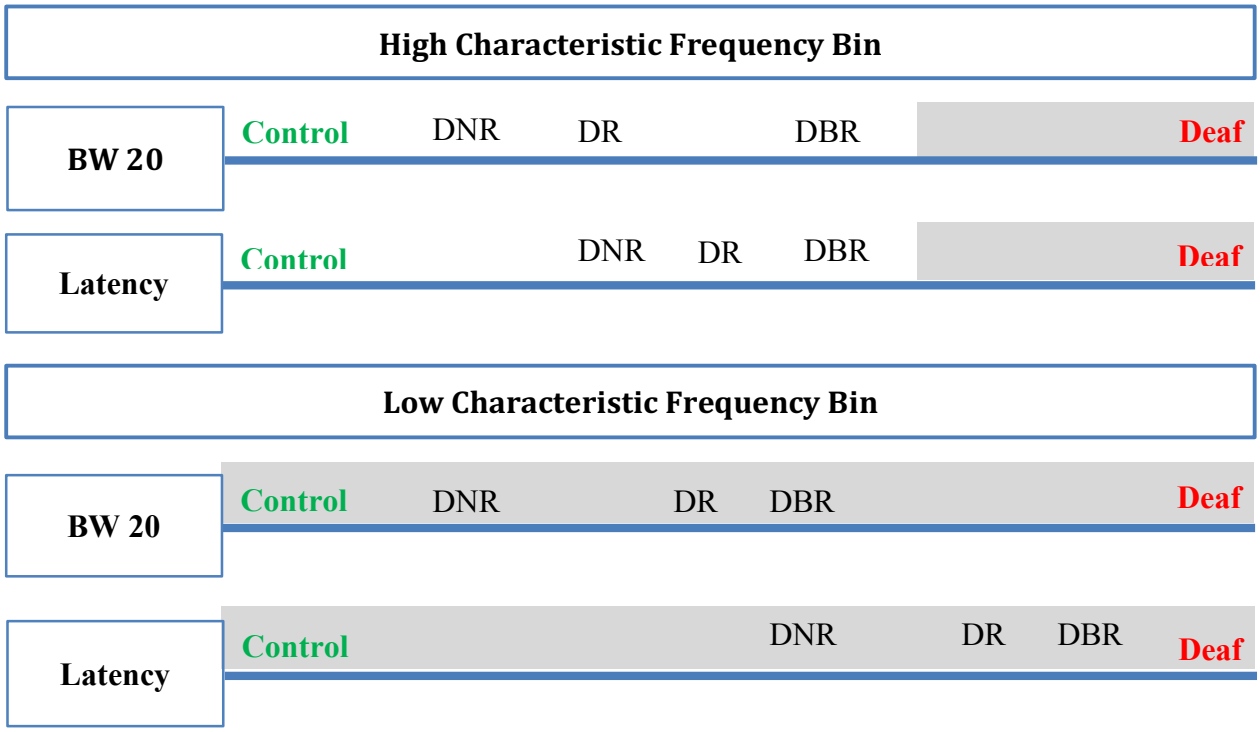


Figure 4: Schematic drawing showing a comparison of electrophysiological responses according to CF bins. The dotted zone indicates non-statistically significant from Control. The solid shadow zone indicates non-statistically significant from Deaf. It showed no statistical differences in BW20 and firing response latency among groups in the low CF distribution areas of A1, compared to high CF areas of AI, where were more affected by the EACL. DNR group recovered the BW20 after noise exposure.

4.5 Discussion:

4.5.1 Summary and Interpretation of Results:

In normal rats, receptive fields in the primary auditory cortex are organized in a continuous sound frequency "tonotopic" representation with responses distributed in a rostro-caudal gradient from low (~1 kHz) to high (~60 kHz) frequencies, with neurons firing at short latency (6–20 millisecond) after stimulus onset and sharply tuned (v-shaped tuning curves) to a particular CF ([153], [154], [155], [156]). Consistent with previous studies, the primary auditory cortex of our control animals showed orderly, continuous tonotopic responses to low-high frequency gradient from rostral to caudal sites. Receptive fields in AI showed a sharply tuned, v-shaped responses with latency to

neuronal firing within ~10ms of sound presentation and low-intensity thresholds. These responses were compatible with those obtained for animals of similar age ([157, 158]).

Our findings showed that EACL caused more hearing loss of for high frequency sounds. Rats raised with bilateral EACL had an under-representation of high frequency sounds in their primary auditory cortices. They had higher intensity thresholds, particularly for high frequencies, broadly tuned receptive fields, and delayed neuronal firing in response to sound stimulation. We believe these electrophysiological changes resulted from a lack of auditory stimuli during the critical period of auditory development. After hearing restoration and a recovery period, hearing thresholds dropped, but maintained the down sloping pattern. A1 sustained distorted spatial distribution of frequencies, in addition to broad and poorly tuned receptive fields, which highlights the consequences of auditory deprivation during early development. Dark rearing failed to reorganize neural circuits between the auditory and visual cortices and, therefore, did not assist hearing recovery. In fact, it resulted in more biased electrophysiological responses. This may be because of lack of normal interaction between rats in the dark or because of the combination of two major afferent neural stimuli deficiencies. Interestingly, noise exposed rats had equivalent electrophysiological responses to controls, which means that the chronic impact of auditory deprivation has to some degree reversed.

4.5.2 The Long-Term Impact of Auditory Deprivation During Critical Period on Central Auditory Processing:

Our findings, consistent with other studies, show that lack of afferent stimulation of the nervous system during a critical period of development results in an abnormal neurophysiological response to that specific stimulation. Continuous pure tone

exposure to rats between post-natal day 11-13 results in profound alterations in sound representations in their primary auditory cortices (AI) ([159]). Passive exposures of a variety of simple e.g. pure tune to more complex e.g. Frequency modulated (FM) sweeps stimuli, have demonstrated the existence of different plasticity CP windows in A1 for each sound stimulus. Although these CPs are mostly overlapping, FM sweep selectivity tuning is biased only if the exposure occurs between P25-P40, suggesting that stable representations of basic sound parameters in AI, such as frequency tuning, are a prerequisite for the establishment of FM sweep selectivity ([160]). This representational distortion resulting from CP exposure proved to be long lasting and persists for a significant portion of the rat's life ([161],[162]). Moore et al showed that unilateral conductive hearing loss using ear-plugs in infant ferrets impaired binaural hearing during and post ear-plug removal. Although, those ferrets weren't exposed to binaural auditory stimulation pre-ear-plugging, they showed improvement in their binaural task performance few weeks after their first experience of normal hearing, and were equivalent to control animals several months later ([163]). Studies of early unilateral conductive or sensory hearing loss, both showed different brain histological alterations and behavioral changes toward unilateral cerebral dominance ([164], [165], [166], [167]). Hutson et al found that gerbils exposed to a short period of unilateral conductive hearing loss had asymmetric response to binaural stimulation early after ear plug removal, however it recovered one week later ([168]). Congenitally bilaterally deaf children who received unilateral cochlear implantation showed more activation of the contralateral auditory cortex. Children who received a second cochlear implant more than 1.5 years following the first implant, had persisted unilateral cortical dominance to binaural stimulation, which was assocaited with poorer speech perception despite 3 years of rehabilitation, ([169]). Adult rats that had single sided deafness showed a disappearance of unilateral

cerebral dominance few weeks post cochlear ablation ([170]). This means that their adult brains, shaped with normal auditory experience maintained plasticity enough to rewire its circuits "cross-modal reorganization" in order to improve auditory performance. The previous studies conclude that the developing brain has its extreme plasticity to learn certain tasks during early critical periods that diminishes, but probably does not vanish, after closure of this critical period. Early auditory exposure, during CP, maintains neural plasticity that helps overcome the influence of auditory deprivation later in life.

4.5.3 Cross-Modal Reorganization Between Auditory and Visual Cortices:

Cross modal reorganization between the auditory and visual cortices was evident in several clinical and animal studies ([171], [172], [173], [174], [175], [176]). It's believed to be an adaptive mechanism of an extremely plastic developing brain to the environmental stimulation. It is not known if this reorganization of neural circuits is completely reversible or has a critical period. In our experiment, dark rearing the animals during the recovery period from bilateral conductive hearing loss did not help to reverse the impact of deafness. Although we can't claim with certainty, we hypothesize that the poor central auditory processing of these animals was due to closure of the critical period for cross-modal reorganization or for auditory cortical maturation, or both. This interaction between the auditory and visual system based on the sensory exposure is supported by the study of Mowery et al., which showed that very early visual experience leads to an early closure of the critical period in animals recovering form conductive hearing loss, and caused long-term effects on auditory processing. It has been hypothesized that hearing loss prevented this adaptive mechanism of cross-modal reorganization in dark reared animals, and therefore, maintained longer a critical period for recovery from hearing loss ([177]). Brief monocular visual deprivation shifted the ocular dominance of the

binocular visual cortex in juvenile rats but was ineffective in adult rats ([178]). Dark rearing of adult rats prior to monocular deprivation resulted in a juvenile like response ([179]). These previous studies indicate that early dark rearing could stimulate plasticity and reopen a critical period for neural circuit reorganization, which was not observed in our study.

4.5.4 Noise Exposure and Reopening of Critical Period:

Our noise exposed group showed complete recovery of electrophysiological responses to auditory stimulation and normal tonotopic organization of CFs within the primary auditory cortex. Similarly, Zhou et al. found that noise exposed adult rats recovered the overrepresentation of certain frequencies caused by pure tone exposure early in life ([180]). In both studies, exposing adult rats to a broadband noise induced neural plasticity after development. These findings agree with the body of evidence derived from several studies on the visual nervous system where an intervention could successfully induce plasticity and reopen critical periods for further development ([181], [182], [183]). We concluded that an abrupt, consistent change in the environment might promote plasticity toward an early stage of neural maturation. Further studies are needed to help explain the underlying mechanisms.

4.6 Conclusion:

Auditory deprivation during the critical period of auditory development was shown to have a long-term impact on the electrophysiological responses to auditory stimulation within the primary auditory cortex. Visual deprivation after hearing restoration failed to promote recovery from early-life deafness, which might indicate the presence of a critical period for cross-modal reorganization to occur. Continuous pulsed broadband noise exposure, however, was found to help restore normal characteristic frequency

distribution and electrophysiological responses to auditory stimulation. Further studies are required to ascertain the underlying pathophysiology.

Chapter 5: Overall discussion:

Our technique of EACL was shown to be easy and reliable. Animals grew with complete cartilage and skin aural atresia, which caused changes in behavioral responses to sound stimuli in developing rats. Our reopening procedure successfully restored the continuity and patency of the external auditory canal, and therefore a normal hearing threshold. The ingenuity in our model that it is both reliable and reversible, which aren't seen in comparative methods of hearing loss research.

Our experiment showed that rats raised with bilateral EACL had an under-representation of high frequency sounds in their primary auditory cortices. They had higher intensity thresholds, particularly for high frequencies, broadly tuned receptive fields, and delayed neuronal firing in response to sound stimulation. We believe these electrophysiological changes resulted from a lack of auditory stimuli during the critical period of auditory development. After hearing restoration and a recovery period, hearing thresholds dropped, but maintained the down sloping pattern. A1 sustained distorted spatial distribution of frequencies, in addition to broad and poorly tuned receptive fields, which highlights the consequences of auditory deprivation during early development. The long-term impact of auditory deprivation during a critical period has been shown in previous research [184,185,186,187].

Cross modal reorganization between the auditory and visual cortices was evident in several clinical and animal studies [188, 189, 190]. It's believed to be an adaptive mechanism of an extremely plastic developing brain to the environmental stimulation. It is not known if this reorganization of neural circuits is completely reversible or has a critical period. In our experiment, dark rearing failed to reorganize neural circuits between the auditory and visual cortices and, therefore, did not assist hearing recovery.

In fact, it resulted in more biased electrophysiological responses. This may be because of lack of normal interaction between rats in the dark or because of the combination of two major afferent neural stimuli deficiencies. Interestingly, noise exposed rats had equivalent electrophysiological responses to controls, which means that the chronic impact of auditory deprivation has to some degree reversed. In other words, consistent change in the environment might promote plasticity toward an early stage of neural maturation. Further studies are needed to help explain the underlying mechanisms.

Chapter 6: Overall Conclusion and Future Direction:

Early-life deafness has a long-term impact on the central auditory processing. Visual deprivation after hearing restoration failed to aid in recovery of function following hearing restoration, which could be related to closure of a critical period of central auditory system development. Continuous noise exposure, however, showed promising benefits for recovery. The mechanisms underlying this improvement remain unknown, further studies are recommended.

External auditory canal ligation is an easy and reliable method to induce a reversible hearing loss. It can be applied to a young rat, before the external auditory canal naturally canalizes, and the brain receives normal auditory stimulation. It provides a novel animal model for reversible congenital hearing loss research.

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