Mapping the Tonotopy of the Mouse Brain at UHF MRI

Design and application of an auditory stimulation setup for functional studies

Guilherme Blazquez Freches

Thesis to obtain the Master of Science Degree in

Biomedical Engineering

Supervisor(s): Dr. Noam Shemesh
Prof. Patrícia Margarida Piedade Figueiredo

Examination Committee

Chairperson: Prof. Ana Luísa Nobre Fred
Supervisor: Dr. Noam Shemesh
Members of the Committee: Dr. Fernando Henrique Lopes da Silva

November 2016
Acknowledgments

I would like to deeply thank: my friends, who kept me sane during this process; my mother, who every day called me to know how it was going; my colleagues in the Shemesh lab who saved me from magnet quenches and other dangers on a daily basis; my girlfriend, who watched over me as I wrote this document and kept me from procrastinating; and finally, my supervisor, Noam Shemesh, for the trust he put in me, the guidance he provided me, and the constant encouragement words.
Abstract

**Background:** The rodent auditory system has been a popular research subject for electrophysiological studies for its complexity, fine tuning and adaptability. Also, and more recently, some studies on auditory Functional Magnetic Resonance Imaging (fMRI) in rats have surfaced, hoping to unravel the intricacies of this system by relying on its larger field of view (FOV). In mice models, fewer studies have been made using MRI, and a real-time characterization of the auditory response on a global scale is yet to be made.

**New method:** An auditory stimulation system, capable to delivering sounds up to 65 kHz directly to a mouse undergoing a MRI scan was created and validated with two studies.

**Results:** The auditory stimulation system proved highly reliable for providing high quality pure tones to anesthetized mice in a MR scanner albeit some limitations. Tonotopy maps were computed for low frequency signals (5, 12 and 20 kHz) as well as for ultrasonic frequencies (35-39 kHz) and ROI analysis for low frequencies confirmed the validity of the mapping obtained.

**Comparison with existing method(s):** fMRI using auditory stimulation had been performed previously, but not in a mouse model.

**Conclusion:** The system can be used to study auditory function in mice, hoping to shed some light on this system in humans.

Keywords

fMRI, Auditory Stimulation, Tonotopy
Resumo

**Contexto:** O sistema auditivo de roedores tem sido um tema de investigação muito popular com vários estudos electrofisiológicos focados na sua complexidade, estrutura fina e adaptabilidade. Mais recentemente, alguns estudos em estimulação auditiva em ressonância magnética funcional em ratos têm vindo à superfície. Estes estudos esperavam revelar as particularidades do sistema auditivo ao terem um maior campo de visão que as técnicas previamente utilizadas. Em modelos de ratinho, menos estudos têm sido feitos com recurso à ressonância magnética funcional e uma caracterização da resposta auditiva em tempo real não é todavia uma realidade.

**Método novo:** Foi criado e validado um sistema de estimulação auditiva capaz de emitir sons até 65 kHz directamente a um ratinho durante um exame de ressonância magnética funcional.

**Resultados:** O sistema de estimulação auditiva provou ser altamente confiável para produzir tons puros de alta qualidade a ratinhos anestesiados numa máquina de ressonância magnética apesar de algumas limitações. Foram traçados mapas de tonotopia para frequências baixas (5,12 e 20 kHz) e para frequências ultrasónicas (35-39 kHz). Foram também análises específicas do sinal em regiões especialmente localizadas para provar a validade dos mapas observados.

**Comparação com métodos existentes:** Existiam estudos de função auditiva com recurso à ressonância magnética funcional mas não no modelo de ratinho.

**Conclusão:** O sistema pode ser usado para estudar a função auditiva em ratinho, podendo trazer á luz descobertas em seres humanos.

Palavras Chave

fMRI, Estimulação Auditiva, Tonotopia
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Abbreviations

MRI  Magnetic Resonance Imaging
BOLD  Blood Oxygenation Level Dependant
fMRI  functional Magnetic Resonance Imaging
MEMRI  Manganese Enhanced Magnetic Resonance Imaging
CNC  Cochlear Nucleus Complex
SOC  Superior Olivary Complex
NLL  Nucleus of the Lateral Lemiscus
MTz  Medial Nucleus of the Trapzeoid Body
IC  Inferior Colliculus
MG  Medial Geniculate Body
AC  Auditory Cortex
IHC  Inner Hair Cell
OHC  Outer Hair Cell
VCN  Ventral Cochlear Nucleus
DCN  Dorsal Cochlear Nucleus
MSO  Medial Superior Olive
LSO  Lateral Superior Olive
VLL  Ventral Nucleus of the Lateral Lemniscus
DLL  Dorsal Nucleus of the Lateral Lemniscus
CIC  Central Nucleus of the Inferior Colliculus
DCIC  Dorsal Cortex of the Inferior Colliculus
LCIC  Lateral Cortex of the Inferior Colliculus
MGD  Dorsal Division of the Medial Geniculate Body
MGM  Medial Division of the Medial Geniculate Body
MGV  Ventral Division of the Medial Geniculate Body
UF   Ultrasonic field
DP   Dorsoposterior field
MR   Magnetic Resonance
SNR  Signal to Noise Ratio
RF   Radiofrequency
FOV  Field of View
ATP  Adenosine Triphosphate
CBF  Cerebral Blood Flow
CBV  Cerebral Blood Volume
HRF  Hemodynamic Response Function
EPI  Echo Planar Imaging
bSSFP balanced Steady State Free Precession
GLM  General Linear Model
SPM  Statistic Parametric Mapping
TE   Echo Time
TR   Repetition Time
UHF  Ultra High Field
SPL  Sound Pressure Level
FISP Fast Imaging with steady state Precession
ROI  Region of Interest
RMS  Root Mean Square
RARE Rapid Acquisition with Refocused Echoes
This chapter provides a context to the project. It states the motivation behind it, the objectives it hopes to achieve and introduces the reader to the essential concepts that form the theoretical basis sustaining this undertaking.

1.1 Motivation

Evolutionarily, the auditory system has been proven as an essential tool for survival across a wide range of species despite having evolved in distinct ways for each of them. Its complexity - it can deconstruct a specific sound into its basic characteristics: e.g frequency and amplitude; fine tuning - its ability to distinguish two or more very similar sounds; and flexibility - it adapts to extreme situations; makes it an enticing study subject.

These features of the auditory system have different manifestations for distinct species. For example, in rodent models, neuroplasticity in the system is evident in complete recovery following deep injury [1] or adaptation to adverse conditions.[2],[3]. In humans, the fact that thousands of different languages coexist, each with its unique phonemes and construction rules (grammar) that one can learn, shows an instance of neuroplasticity.[4]

Historically, researchers have focused heavily on the rodent auditory system resorting to electrophysiological and microscopical techniques. Some, motivated by the thought of getting to the root of the capabilities briefly described above[1],[5],[6],[7] and others, focused in specific morphologies in the system that make exquisite synaptic models.[8],[9]

More recently, some studies began using Magnetic Resonance Imaging (MRI), and, more specifically, Blood Oxygenation Level Dependent functional Magnetic Resonance Imaging (BOLD fMRI) in conjunction with auditory stimulation in rats, hoping that the larger field of view (FOV) of this technique, compared to others used previously could unravel more of the intricacies of this system.[10],[11],[12],[13],[14],[15] Despite these advances, few efforts have been made to translate these findings to other models. Although a number of studies have been made [3],[16],[17] using Manganese Enhanced MRI (MEMRI) they couldn’t report the fast dynamics present in the auditory system.

The possibilities are immense. Auditory stimuli have a much more rich dimension space (Amplitude, Frequency, Timbre) than others commonly employed: e.g Electrical stimuli such as forepaw stimulation. Furthermore, the representation of each of these dimensions in the brain can, in theory, be probed both in normal conditions or post-lesion for better understanding the cascade of events underlying plasticity across the whole auditory pathway (the elaboration of neurotrophic factors, the stabilization of synapses and the elaboration of myelin). Additionally, using mice allows for the use of simultaneous stimuli (electrical, visual, olfactory) in conjunction with the auditory cues. Not only that, also the simultaneous use of optogenetics becomes easier and more reliable, since this technique, first described in 2005 [18] is in a much more advanced stage at this point in this model.[19]

From what is described above, one can realize that there is a need for the characterization of the mouse auditory system using [BOLD][MRI]. The (relative) fast dynamics it can capture, its large field of view giving us a complete overview of the entire auditory pathway and the possibility of combining this
imaging modality with optogenetic stimulation can offer unique insights on neuronal organization and neuroplasticity of the auditory pathway.

1.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a three-dimensional imaging technique with great soft-tissue contrast, high spatial resolution (<1 mm) and good temporal resolution (~1s). This section will provide the reader with an introduction to the technique, including magnetic resonance(MR) physics and the image formation process.

1.2.1 Magnetic Resonance Physics

Not all nuclei can be used to generate MR signals. For a nucleus to be useful for MRI, it must have odd numbers of protons or neutrons because it is not possible to distribute either the electric charge or the atomic mass evenly in these nuclei. All nuclei with an odd atomic weight and/or an odd atomic number possess a fundamental quantum mechanical property termed "spin". In MRI the most important nucleus is the hydrogen nucleus, or proton due to the natural abundance of water and, consequently, hydrogen nuclei in biological systems. This property of spin is analogous to the proton spinning around an internal axis of rotation and, given that it has mass, giving it a certain value of angular momentum \( P \). Because the proton is a charged particle, this rotation gives the proton a magnetic moment \( \mu \).

\[
|\mu| = \gamma |P| \quad (1.1)
\]

Where \( \mu \) is the gyromagnetic ratio of the nucleus and has a distinct value for all nuclei. For protons, this magnetic moment \( \mu \) can also be defined as:

\[
|\mu| = \frac{\gamma h \sqrt{3}}{4\pi} \quad (1.2)
\]

where \( h \) is Planck’s constant.

If in the presence of a strong magnetic field \( (B_0) \) along the z-axis, the spins align with the \( (B_0) \) field and start precessing at an angle around its axis, either in the same sense - parallel, or in the opposite one - dubbed the antiparallel. In this case the interaction energy \( (E) \) between the magnetic field and the \( z \)-component of the magnetic moment of the proton can be defined as:

\[
E = -\mu_z B_0 \quad (1.3)
\]

Combining equations 1.2 and 1.3 we can conclude that:

\[
E = \pm \frac{\gamma h B_0}{4\pi} \quad (1.4)
\]

Equation 1.4 shows that two energy states exist: One higher energy state, in which the proton's magnetic moment aligns antiparallel with \( B_0 \) and a lower energy state, where the proton's magnetic moment aligns parallel with \( B_0 \). This indicates that the population of protons parallel with \( B_0 \) should be higher than the opposite case, since this state has a lower interaction energy. That is indeed the case,
and after some manipulations, Equation 1.4 can show that the difference in populations is proportional to the strength of $B_0$. This population difference is the basis of the MR signal which means that, the higher the strength of $B_0$, the higher the Signal to Noise ratio (SNR) will be. Under normal conditions there are more parallel spins (in the more stable low-energy state) than antiparallel spins (in the high-energy state), and thus there will always be a net magnetization. [20]

**Figure 1.1:** Spins distribution in the presence of a strong $B_0$ field. It shows that more spins are likely to align parallel to the field since that energy state is lower. Adapted from [20]

Since the MR signal is dependent on population differences between states, it becomes clear that in order to obtain signal, changes in this ratio must be induced. This is achieved by giving energy to the system equivalent to the energy difference between the states ($\Delta E$).

When a spin changes states it will either emit or absorb energy in the form of an electromagnetic pulse. The frequency $\nu$ of this electromagnetic pulse is determined by the energy difference between the states, as given by the Bohr relation:

$$\Delta E = h\nu$$  \hspace{1cm} (1.5)

Combining equations 1.4 and 1.5 leads to:

$$\nu = \frac{\gamma}{2\pi} B_0$$ \hspace{1cm} (1.6)

This frequency is called the Larmor frequency. It defines the frequency of the electromagnetic radiation needed during excitation to make spins change to a high-energy state, as well as the frequency emitted by spins when they return to the low-energy state. It is also equivalent to the frequency the spins precess around $B_0$.

### 1.2.1.1 Radiofrequency pulse application

As mentioned in subsection 1.2.1, in order to provoke a population switch between the antiparallel and parallel energy states, electromagnetic energy must be supplied to the spins. This is accomplished through the use of radiofrequency (RF) pulses. To explain the role of these pulses, it is important to first define the concept of net magnetization.

Net magnetization can be considered as the sum of all the magnetic moments present in the system. As previously stated, in the presence of a $B_0$ field aligned with the z-axis, it can be assumed that all spins precess around the z-axis (parallel or anti parallel sense). This means that the net magnetization of a system will only be different than 0 in the z-axis called the longitudinal axis and, again will be proportional to the $B_0$ field strength as seen in Figure 1.2. The xy-plane is defined as transverse.
When a radiofrequency pulse is applied - also called $B_1$ field (by what is called a transmit coil), the net magnetization "cone" as seen in Figure 1.3 is tipped towards the axis of the radiofrequency pulse. Typically, a $90^\circ$ pulse is applied, tipping the magnetization to the xy-plane.

To simplify the visualization of the evolution of the net magnetization over time, the concept of a "rotating reference frame" ($x', y', z'$) must be introduced. In this frame the $z'$ axis is the same as in the frame ($x, y, z$) used previously, but the $x'$ and $y'$ axis rotate around the $z'$ axis at the Larmor frequency.

Signal detection is possible through what is called a detection coil. This coil senses the variation of magnetic flux through it and gives rise to an electromotive force. The MR signal is the electromotive force caused by the changing magnetic field across the detector coil which is placed in relation to the system such as only changes in the transverse plane contribute to the MR signal.

### 1.2.1.B Excitation and Relaxation

When a RF pulse is applied, the system does not remain indefinitely in the exited state. When the excitation pulse is over, the spin system starts to gradually lose the energy absorbed during the excitation. This happens through two processes: longitudinal relaxation and transversal relaxation.

In longitudinal relaxation the spins in the high-energy antiparallel state gradual go back to their original parallel state. This is called spin lattice interaction. As increasing numbers of individual spins return to their low-energy state, the net magnetization returns to a direction that is parallel with the main field. The time constant that describes the recovery of the longitudinal component of net magnetization over
time is called $T_1$ and the net longitudinal magnetization following the $90^\circ$ pulse can be described as:

$$M_z(t) = M_0(1 - e^{-t/T_1})$$

(1.7)

In which $M_0$ is the initial net magnetization.

In transverse relaxation, the following happens: After the net magnetization is tipped into the transverse plane by an excitation pulse, it is initially coherent. This means that all of the spins in the sample are precessing around the main field at about the same phase. Over time, the coherence between the spins is gradually lost and they fall out of phase. There are two main causes for transverse relaxation, one intrinsic and the other extrinsic. The intrinsic cause is due to the so called spin-spin interaction. When many spins are excited at once, there is a loss of coherence caused by their effects on one another. The signal loss that results from this intrinsic mechanism, which is irreversible, is characterized by the time constant $T_2$.

$$M_{xy}(t) = M_0 e^{-t/T_2}$$

(1.8)

The extrinsic source is due to local variations in magnetic field. These can be caused by different magnetic susceptibilities of different tissues or spatial variations of the field strength. The combined effects of spin-spin interaction and field inhomogeneities lead to signal loss known as $T_2^*$ decay, characterized by the time constant $T_2^*$. $T_2^*$ is always shorter than $T_2$. [20]

All of these time constants ($T_1$, $T_2$ and $T_2^*$) can be measured easily, they are distinct for each substance and they depend on the magnetic field $B_0$. They have to be taken into account when designing sequences so the correct contrasts are chosen. Typically ($T_1 » T_2 > T_2^*$).

### 1.2.1.C Signal from MR

Very succinctly, the electric signal generated in the receiver coil by the exitation/relaxation phenomena is demodulated (reduced to a lower frequency), amplified and Fourier transformed. The final result is what is called an MR spectrum. This spectrum is location inespecific, meaning one cannot discern the locations of the elements detected looking at the spectra.

### 1.2.2 Magnetic Resonance Imaging

The MR signal described so far is simply the sum of the individual signals from each proton: there is no feature that allows for distinction between the signals from protons located at different locations. The development of MRI resulted from the realization by Lauterbur in 1973 that using gradient coils, that is, coils that produced a uniform gradient of magnetic field strength, originated specific ressonance frequencies for protons related to their position in the sample. [22]

In modern MRI scans, these gradient fields are created by what is called gradient coils: one for each direction $(x,y,z)$ which, in conjuncture with the $B_0$ field, make all protons in the scanned object precess at a spacially dependent frequency at all times.
1.2.2.A Slice Selection

The MRI process begins with the application of the slice-selective RF pulse. The objective of this pulse is to tip the magnetization of the spins present in the slice, leaving spins outside the slice unaffected. The slice thickness is dependent on the bandwidth of the RF pulse (thicker if the bandwidth is wider and inversely) and on the slope \((G)\) of the spatial variation of the gradient coil corresponding to the slice plane (thinner if the slope is larger and inversely).

![Slice selection sequence](image)

**Figure 1.4:** Slice selection sequence. A frequency-selective RF pulse is applied simultaneously with a gradient along the z-direction (conventional axis for slices). This gradient is applied with a positive \(G\) during the pulse duration followed by shorter negative application - to counteract the effects of the RF pulse on the gradient field felt by the protons inside the slice. Adapted from [21]

1.2.2.B Frequency and Phase Encoding

After having selected a slice, the other two-dimensions must be encoded in order to form a two-dimensional image. One of these directions is encoded by imposing a spatially dependent phase on the signal from the precessing protons (Phase encoding), and the other by creating a spatially dependent precessional frequency during signal acquisition (Frequency Encoding). The difference between these two encoding schemes is that the phase is encoded by a gradient turning on and off before data acquisition begins, and a number of different values of this phase-encoding gradient must be used. In contrast, the frequency-encoding gradient is turned on during data acquisition and has always the same value.[20]

![Complete acquisition sequence](image)

**Figure 1.5:** Complete acquisition sequence. A frequency-selective RF pulse is applied simultaneously with a gradient along the z-direction (conventional axis for slices). Then, a Phase encoding gradient is turned on and off to give protons different phases in the phase encoding axis (by convention the y axis). Finally a frequency encoding gradient is turned on simultaneously with the acquisition. This process is repeated until sufficient Phase encoding steps have been acquired. Adapted from [21]
At this stage, it is possible to prove that the MR signal $S(t)$ is equal to:

$$S(t) = \int_x \int_y \int_z M_{xy} e^{-\gamma t \int_0^t (G_x(\tau)x + G_y(\tau)y + G_z(\tau)z) d\tau}$$

(1.9)

In which $G_x$ represents the slope of the gradient of the field along direction $x$. In two dimensions, equation (1.9) is equivalent to:

$$S(t) = \int_x \int_y M_{xy} e^{-\gamma t \int_0^t (G_x(\tau)x + G_y(\tau)y) d\tau}$$

(1.10)

### 1.2.2. C K-space formalism

A useful model for understanding exactly how the acquired data matrix is transformed into the final image is the "k-space" formalism. If we consider that:

$$k_x(t) = \frac{\gamma}{2\pi} \int_0^t G_x(\tau) d\tau$$

(1.11)

and that:

$$k_y(t) = \frac{\gamma}{2\pi} \int_0^t G_y(\tau) d\tau$$

(1.12)

Equation (1.10) can be represented as:

$$S(t) = \int_x \int_y M_{xy} e^{-2\pi k_x(t)x} e^{-2\pi k_y(t)y} dx dy$$

(1.13)

So, by creating the variables $k_x$ and $k_y$ which are scaled integrals of the $G$ values, we arrive to the conclusion that the image space - $S(t)$ and k-space are the 2-D Fourier Transform of one another.

![Figure 1.6:](image)

**Figure 1.6:** (Left) Acquisition sequence as seen and explained in Figure 1.5. (Right) Pattern that this sequence goes through in k-space. Each line of k-space is acquired following a separate excitation, then the application of $G_y$ at a particular strength (causing an upward or downward motion in k-space), then the application of $G_x$ at a constant strength and duration (first - a small negative gradient to move to the left in k-space and then a twice as long positive gradient causing a rightward motion in k-space). Adapted from [21]

In conclusion, the raw MRI signal, $S(t)$ is a set of points acquired through the k-space. This signal can be broken into two dimensions, according to $k_x$ and $k_y$, to allow for a 2-D inverse Fourier transform. Decreasing the separation between adjacent data points in k-space increases the FOV in image space. and increasing the extent of k-space decreases the voxel (three-dimensional image point) size in image space. Finally, two parameters, echo time (TE) - the time between the first RF pulse and acquisition and repetition time (TR) - the time between successive RF pulses will affect the final images by defining the type of contrast that is evidenced.
1.2.2.D Spin-Echo vs Gradient Echo

In Figure 1.6, a typical gradient echo sequence is shown. A different sequence scheme exists - called spin echo - that can overcome some hurdles sometimes present on gradient echo sequences. Namely, the fact that local field inhomogeneities will cause a loss of phase coherence over time, as some spins have fast precession frequencies and some have slower precession frequencies. When \(180^\circ\) refocusing pulse is presented at time \(T_E\) (along with a slice selective gradient again), the precession direction will be flipped. At the precise time \(T_E\), all of the spins will have their original phases and the sequence will no longer be influenced by \(T_2^*\) but by \(T_2\).

![Typical Spin-Echo Sequence](image)

Figure 1.7: Typical Spin-Echo Sequence. Adapted from [21]

1.3 Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) is a technique designed to image function in a body of study using MRI. Although promising new techniques are being developed in this field, the technique that has been used far more often aiming to image function uses a contrast called Blood Oxygen Level Dependent (BOLD). In this subsection, the BOLD contrast will be presented: its origin, how it is imaged and analyzed and what it can and can’t tell us about function in the brain.

1.3.1 The Blood Oxygen Level Dependent Signal

Following Pauling’s discovery that deoxyhemoglobin was paramagnetic and oxyhemoglobin was diamagnetic [23], Ogawa and colleagues hypothesized that manipulating the proportion of blood oxygen would affect the visibility of blood vessels on T2*-weighted images. They found that deoxygenated blood decreased the measured MR signal in T2* images while oxygenated blood had no effect [24]. They hypothesized that this contrast in signal could be used to image neuronal activity (albeit indirectly) and proposed two independent mechanisms. First, neuronal activity would increase oxygen consumption, which would increase the amount of deoxygenated hemoglobin, given a constant blood flow. Alternatively, increased blood flow in the absence of increased metabolic activity might decrease the amount of deoxygenated hemoglobin. Their assumption proved with time to be correct but the mechanism behind the BOLD contrast showed, with time, to be more nuanced and is still today not completely understood [25].
1.3.1.A From Neuronal Activation to Hemodynamic Effects

Neuronal activity can be characterized as either integrative or signaling. Integrative activity receives inputs from other neurons through connections on both its dendrites and cell body. Signaling activity refers to transmitting the outcome of integrative activity to other neurons. This transfer of information between neurons occurs at specialized junctions called synapses, where an axon terminal from one neuron is located adjacent to the postsynaptic membrane of the dendrite or soma of another neuron. The synaptic process begins with the axon of the presynaptic neuron releasing neurotransmitters - chemicals that diffuse across the synaptic cleft and interact with receptors on the postsynaptic membrane that gate ion channels. These interactions may open normally closed ion channels, which depending on the polarity of the ions that enter or leave the postsynaptic neuron, make the interactions excitatory or inhibitory. Restoring the basal concentration of ions in the intracellular and extracellular mediums (either for excitatory or inhibitory interactions) requires active transport (i.e. transport of ions against their concentration gradient) which demands consumption of energy.

It is now known that the brain has no energy reserves which means that a continuous flow of energy, proportional to the metabolic needs (more activity -> more synapses -> increased energy consumption) of the local neurons must be maintained in order for all these processes to occur normally. The main energy currency for cells in the human body is adenosine triphosphate, or ATP. For ATP to be produced in the Mitochondriae of cells through aerobic respiration, two things are required: Glucose and Oxygen. This means that a steady stream of Glucose and Oxygen must reach the neurons. This is accomplished by the perfusion of the brain by blood vessels.

In the adult human brain, about 54 mL of blood flows through each 100 g of tissue every minute. This adds up to about 800 mL/min for the average 1400 g brain and accounts for 20% of the total blood flow in the body.

The vasculature network in the brain is highly complex. The oxygenated blood comes from the heart.
and enters the brain via the internal carotid artery. In the brain, the basilar artery and the right and left
internal carotids fuse in order to form the circle of Willis, a major circulatory structure which, in its turn,
diverges bilaterally as the anterior, middle, and posterior cerebral arteries. Each of these major cerebral
arteries supplies blood to a distinct region of the brain.

The blood supply to the cerebral cortex comes from meningeal arteries that traverse the cortical
surface. In the cortex, many conducting arteries run along the sulci. From these conducting arteries,
many much smaller, distributing arteries branch out forming what are called the pial arterioles. Finally,
these arterioles branch into smaller arterioles (labeled Penetrating arterioles) that penetrate the cortex at
a 90° angle. The density of vascularization is not uniform across the different cortical layers with denser
vascularization reported in locations with higher of neural cell bodies. Finally these small arterioles
perfuse the cortex with a high number of capillaries. These capillaries enable the diffusion of oxygen
and glucose from the blood to the neurons and the removal of waste carbon dioxide in its surface. The
venous blood is then drained via the ascending and pial veins.

The control of blood flow to the capillaries is achieved at the level of this pial arterioles which trans-
form the pulsatile-like flow from the heart into a steady flow. Astrocytes play a crucial role in this flow
regulation. The process can be summarized as follows: neurotransmitters released from active neurons
evoke Calcium increases in astrocytes, leading to the release of vasoactive metabolites from astrocytes
onto blood vessels. This fact highlights one of the disadvantages of BOLD-MRI. While the epicen-
ter of the response is in the region of increased synaptic activity, arteriolar dilation and increased blood
flow are also observed away from this center - where there is no synaptic activity - which means the
observed signal will always be dependent on the microvasculature architecture.

1.3.1. BOLD Hemodynamic Response Function

Having defined how neuronal activation leads to changes in the vasculature, it is important now to
define what are the effects of those changes in terms of measured signal. Three variables are important
in this domain: Cerebral Blood Volume (CBV), Cerebral Blood Flow (CBF) and BOLD signal. The BOLD
hemodynamic response function (HRF) refers to the variation of MR signal in T2* weighted images. It is
influenced by the local CBV and CBF and has three distinct phases:

- The initial dip - a initial undershoot in the BOLD signal caused by an early increased metabolic
extraction of blood oxygen that increases the quantity of deoxyhemoglobin locally (therefore reducing
The signal in that region. It has been proposed that this dip is more specific to voxels actively involved in
the neuronal activity and other hypothesis about its genesis exist.\cite{27}

The peak - caused by the inflow of oxygenated blood provoked by the increased metabolic demands
due to the increased neuronal activity over baseline levels. This results in a decrease in the amount of
deoxyhemoglobin within the voxel and makes the signal peak. If the stimulus is prolonged in time, the
peak becomes a plateau, stabilizing at values slightly lower than the peak.

The post-stimulus undershoot - caused by a rapid reduction in CBF post-stimulus that is not accom-
panied by as fast a drop in CBV which increases the proportion of deoxyhemoglobin locally making the
signal undershoot.\cite{21}

These three stages are present in Figure 1.10 but it should be stated that the HRF has been
shown to differ wildly between species, age\cite{30}, location in the brain\cite{31}, and type of anesthesia
used.\cite{32,31,33}

A final mention should be made in respect to a characteristic of the BOLD HRF - its linearity. If the
HRF following neuronal activity were to be linear that would mean that a twice as strong stimulus would
produce a twice as high HRF and that two stimuli separated by a short interval of time would produce an
HRF mathematically equal to the sum of the two individual HRFs. This is not the case and numerous
studies have shown it. When similar stimuli are separated by a short interval of time, the BOLD effect
measured is lower than what would be expected if the system were linear - dubbed habituation. For
this reason it is important to take these considerations into account when designing experiments and
especially when analysing data from said experiments.\cite{34}

1.3.1.C Temporal and Spatial Resolution

The spatial resolution of an fMRI study - its ability to resolve differences between adjacent spatial
locations, depends on several factors. One important one is the voxel size which, as mentioned in
Section 1.2.2, is dependent in its turn on the FOV and the number of acquired points in k-space (also
called Matrix Size) and the slice thickness. If a voxel is the same size in the three dimensions it is
deemed isotropic.

It would seem reasonable to think that fMRI would call for the best spatial resolution possible in order
to detect very localized signal changes. That is not always the case since the signal from a voxel is the
summation of the signal of all protons in that voxel. This means that if a voxel is half as big, it will also have its \( \text{SNR} \) cut in half. Additionally, smaller voxels (for the same \( \text{FOV} \)) ask for a larger data matrix which increases acquisition time. For T2* mapping, which is the case in most MRI studies this timing issue is crucial: during the acquisition period, the spins are continuously undergoing T2* decay, which means that if the acquisition period is too long compared with the T2* value of the tissues being imaged, points collected toward the end of the acquisition period will have almost no signal, which results in blurred MRI images. In opposition, using voxels that are too large can also reduce sensitivity since they suffer from partial volume effects meaning that they may contain multiple tissue types, each contributing differently to the total MR signal from that voxel. [35]

Temporal resolution is limited by \( \text{TR} \) which can be viewed as an inverse of the sampling rate. Again, using very short \( \text{TRs} \) introduces some problems. For typical gradient-echo sequences with long \( \text{TRs} \), a flip angle of $90^\circ$ can be used to recover maximal MR signal. But at shorter \( \text{TRs} \), a smaller flip angle must be used to make sure the signal is sampled with the same flip angle at each sample. As a result, the amplitude of the transverse magnetization following excitation will be reduced, and less MR signal will be measured. A clever strategy to avoid this effect is to sample the HRF with different delays from the stimulation at each stimulation although this means that less samples at the same point will be acquired, reducing the precision of the estimated HRF.

So it seems temporal and spatial resolution are fundamentally connected and changes in one of them will usually affect the other. Some new strategies have been trying to overcome this unspoken rule such as compressed sensing. [36]

1.3.2 Imaging the BOLD signal

In section 1.2.2, the spin-echo and gradient-echo sequences were introduced in the context of anatomical imaging. In these anatomical images, contrast is more important than speed of acquisition, since structural parameters usually don’t change during the scanning period.

Inversely, when looking at function, images should be acquired at approximately the same rate as the changes of interest. Fast pulse sequences have been developed that can be used to acquire very large numbers of images within short periods of time. These sequences typically use variants of the gradient-echo approach described in Section 1.2.2 and are sensitive to T2* contrast. More recently, and responding to persisting problems in T2* weighted images such as their oversensitivity to oxygenation changes in large draining veins located far away to the site of activation, and their inability to accurately describe regions near strong field inhomogeneity like air filled compartments, the switch has been made towards T2-weighted images (spin-echo). [37] Furthermore, the use of spin-echo has been shown to eliminate the non-specific signals from large vessels while preserving the small-vessel specific signal which is more likely to be activity related. [38] Until the advent of ultra high-field MRI, the loss of BOLD sensitivity caused by the use of spin-echo sequences was fatal for functional imaging but, currently, high-resolution, highly specific images can be acquired. This section describes two of the mostly used sequences used at UHF. One is a spin-echo sequence - spin echo echo planar imaging (spin echo EPI) and the other is technically a gradient-echo sequence but shares great similarities to
1.3.2.A Spin Echo Planar Imaging

The first MRI scanners were very conditioned in terms of both the strength of the gradients they
could produce, and the speed in which gradients would change. This limited the speed of high quality
acquisitions for a long time. In the late 1980’s and 1990’s the continuous improvement of gradient
technology made possible the advent of fast imaging in MRI. The standard fast acquisition sequence
is spin echo Echo Planar Imaging (EPI). It is distinct to the sequences described in section 1.2.2 since
it acquires multiple lines (or even all the lines in single-shot mode) of the k-space in just one excitation.
This drastically reduces the time it takes to acquire an image making artifacts caused by movement less
likely.

This gain in speed is obtained by the faster filling of the k-space. To achieve this goal, EPI uses an
unconventional pattern in which alternating lines are scanned in opposite directions. This zigzag like
approach burdens the gradient hardware heavily, since different sets of gradients must be rapidly turned
on and off to enable the 90° turns in the k-space pattern. This pattern is also inefficient since data
collected while transitioning from one line of k-space are not used in the image-creation process.

The main advantages of this sequence are, as mentioned before, its low scanning time (TR<1s) and,
comparing it with gradient echo EPI its relative insensitivity to spin-spin interactions and although it has
been heavily used for MRI studies it is highly sensitive to field inhomogeneities, eddy currents caused
by the rapid switching of gradients and has poor spatial resolution.

1.3.2.B Balanced Steady State Free Precession

If a sequence of RF pulses is applied with a very short TR the same sequence of rotations, preces-
sion and relaxation repeats across cycles forming what is called a steady state, where the magnetization
at some point in the sequence is the same from one repetition to the next. In a special case when
TR < 2T1 and TR < 2T2, both longitudinal and transverse magnetization reach a non-zero steady
state. This allows for a very fast T2/T1 weighted acquisition with very high SNR. In BSSFP the net integral of the gradient fields is 0 in one acquisition giving it its name - balanced.

1.3.2.C Limitations of BOLD

BOLD fMRI signal, as the name indicates, measures the blood oxygenation of a certain region, at a certain point in time. It cannot be directly attributed to neural activity. It is usually alleged that cognitive capacities reflect a local processing of inputs and outputs, by the means of patterns of action potentials. Although this may apply for some subcortical nuclei, to make this assumption for cortical circuits is a gross oversimplification. For instance, it is now known that subcortical input to cortex is weak, the feedback is massive, and that the output reflects changes in the balance between excitation and inhibition, rather than plain feedforward integration of subcortical input. All of these activities require oxygen and glucose to occur, which makes the task of mapping task related activation a quite cumbersome one. When possible, inferences from studies should refrain from claims not yet observed in electrophysiological studies. It is essential to keep in mind that the signal cannot distinguish task related activity or neuromodulation, between feedforward and feedback signals, or between excitatory and inhibition. This limitation does not lie from poor scanner or sequence design, but rather with the underlying subject of imaging in BOLD fMRI.

1.3.3 Experimental Design

Experiments must be designed in such a way that they can answer a certain question. These questions can be broad such as "Which areas of the rodent model are associated with the auditory pathway" or as specific as "Does the Inferior Colliculus modulate responses on the Auditory Cortex?". A good experimental design is essential to a study. Especially in experiments, given the extensive resources they require in collecting and subsequently analysing data.

Two paradigms of experimental designs have become the norm in studies. They are the blocked design and the event related design.

1.3.3.A Blocked Design

Block designs are well suited to localize functional areas associated to a certain task. Succinctly, conditions A and B are alternated in distinct blocks. For example one could imagine a block of 40s with no stimulation intertwined with a block of 40s listening to a certain sound.

Block designs are powerful in terms of detection, meaning they are suited to determine which voxels are activated in a certain task. On the other hand, because of summation of the hemodynamic responses in time, block designs have a poor estimation power meaning they cannot accurately estimate the shape of the hemodynamic function.

1.3.3.B Event-Related Design

In event related design, each event is separated in time by a varying inter-stimulus interval. They assume that that neural activity will occur for short intervals of time and try to measure transient changes
in brain activity. Unlike blocked designs, the events are presented in a random order which offers a higher flexibility in experimental design.

The estimation power of event-related design is often quite good since they allow to determine the hemodynamic shape for each condition and compare parameters such as the amplitude or the timing between conditions. On the contrary, the detection power is relatively weak in comparison with the blocked design. This happens because experimental power depends on the number of events that are averaged and event related designs usually have less events that block design ones. [21]

1.3.4 Analysing BOLD data

1.3.4.A Preprocessing of fMRI data

MR images are very prone to noise. Noise can be defined as non-sample specific signal and it can be measured as ratio with the sample-specific signal by what it is called Signal to noise ratio (SNR).

In the case of fMRI, another ratio (Functional SNR) can be computed. It reflects the signal variability when there is no expected activity versus the magnitude of the signal in stimulus related areas.

Both these ratios are affected by several factors and noise sources. The main factor of influence is field strength since a higher magnitude in \( B_0 \) means that there will be a greater difference between the signal of deoxyhemoglobin and oxyhemoglobin. [40]

The sources of noise can be internal to the sample or external. As internal sources, movement, respiration and cardiac pulsation have manifested as the most nuisant to subsequent data analysis. As external sources of noise we can account for thermal noise (heat related motion of electrons within the subject and the scanner) and system noise (static field inhomogeneities due to imperfect shimming, nonlinearities in the gradients or scanner drift). Additionally, when performing an auditory experiment, the actual scanner acoustic noise must be taken into consideration.

These noise sources make for the case of a very thorough preprocessing of fMRI data. The first step of this preprocessing should be always a visual analysis of the data. Looking at the composite film of acquisition can easily and rapidly detect bad quality images caused by, for example radiofrequency leakage. This can also be complemented by a visual analysis of the frequency spectrum of a certain voxel. A trained eye will quickly detect certain peaks in the spectra corresponding to task related signal or to some noise source and apply the appropriate filters.

Following visual inspection of the data, many preprocessing steps can be applied such as slice timing correction (correcting for the acquisition of slices at different time points - irrelevant for very fast acquisitions), spatial smoothing and head motion correction.

Head motion correction is one of the major issues in fMRI studies since a slight head movement greatly alters the spins’ precession rates and consequently the image itself. Additionally the signal coming from the inside of samples is largely superior to the outside, which means that even slight movements, can create huge variations in signal in the borders that can be confused by activity. These movements are accounted for by including a measure of difference to a mean image as a regressor for the final signal. Usually 6 degrees of freedom are used: 3 for translation and 3 for rotation.
Finally and to be able to analyse data from different subjects in the same referential, all the data is mapped to a reference image, a process called spatial normalization.

1.3.4.B Analysis of fMRI data

1.3.4.C General Linear Model

In very simplistic terms, the General Linear Model (GLM) is a regression model tries to find a set of weights $\beta$ that minimize the distance (error) $\epsilon$ of the predicted signal given a set of regressors $X$ and the observed signal $Y$.

$$Y = \beta X + \epsilon \quad (1.14)$$

The GLM is elegant in its simplicity. After the data is obtained and the regressors have been established, it is only a matter of minimizing $\epsilon$. The regressors in the design matrix $X$ represent the hypothetical contributors to the fMRI time course. In a perfect situation, the only regressor of interest would be the convolution of the experiment design with the HRF such as the one in 1.10. This HRF is usually modeled using a double gamma function so that it can represent the initial peak and the subsequent undershoot.

In an experimental setup, it is practically impossible to only use the convolution between our paradigm and a HRF since other factors affect the fMRI signal. These are usually called nuisance regressors. There is no consensus about which nuisance regressors should be included in design matrices although the most usual ones to be added are head motion parameters, typically six regressors representing three directions of translation and three axes of rotation. The value of each regressor at each point in time reflects the accumulated movement along that direction or around that axis. Other often used regressors include, for example, heart rate or respiration rate. It is very important to check at this point if all the regressors are orthogonal, so that the variance of fMRI signal related to a certain regressor is independent from all the others. [21]

Using the general linear model, a researcher constructs a design matrix consisting of a set of regressors, and then determines how strongly each of those regressors relate to changes in the measured BOLD signal. Regressors that explain much of the variance of the signal will have high-magnitude parameter weights but regressors that explain little of the measured signal will have parameter weights near zero.

To test an experimental hypothesis, the researcher evaluates whether the experimental design caused a significant change in those parameter weights. This is performed through the means of what is called a contrast. A contrast is simply a vector input that hypothesizes the weight of the regressors. For example, if a certain design matrix had one regressor for the paradigm-HRF convolution and two for head motion, then the contrast to be tested would be $c = [1 \ 0 \ 0]$. The statistical software used would then evaluate...
(usually with a t-test) that value against a null hypothesis.

\[ t = \frac{c^T \beta}{\text{std}(c^T \beta)} \] (1.15)

All of these steps are done in a voxel-by-voxel basis and produce at the end of the pipeline, a map with t-values for each voxel, representing how well they fit the predicted response according to the regressors.

The GLM makes some assumptions which have to be considered when using it to analyse data:

One assumption is the use of the same design matrix throughout the brain. Although each voxel will have a different calculated set of parameter weights, the model (regressors) used to calculate those weights is the same despite the properties of the hemodynamic response differing across brain regions.

One other assumption the GLM makes is that the noise sources do not vary across time, which is obviously highly unlikely and should be noted with care.

Finally, the GLM considers that all voxels represent independent statistical tests, even though adjacent voxels tend to have similar properties and behaviours.

All these assumptions of the model highlight the importance of designing an efficient design matrix so that the measured statistics correspond to actual task-related variations in the data rather than just noise or consequences of poor modeling.

1.3.4.D Statistical Parametric Mapping

Statistical Parametric Mapping (SPM) is a open source software developed and maintained by members and collaborators of the Wellcome Trust Centre for Neuroimaging. It is used to analyse neuroimaging data (EEG, [MRI] and PET) and includes all the steps described previously such as registration of images to the mean of their series, spatial smoothing, spatial normalization, building the design matrix, estimating the parameters and building contrasts.

![Figure 1.13: SPM graphical user interface. All the processing and estimating options are shown.](image)
1.4 The Mouse Auditory System

The ability of understanding the characteristics and diseases of the Human Auditory System and their molecular and cellular causes is of vital interest for modern auditory research. Comparative studies are essential to this goal, due to the fact that animal models can be developed, evaluated and eventually applied to clinical cases.

In the inner ear, receptor cells are preferentially activated by different frequencies of sound depending on their position along the basilar membrane of the cochlea. The resulting gradient in frequency tuning gives rise to a topographic representation of sound frequency, or tonotopic organization, that is maintained in a point-to-point fashion from the first auditory relay center, the cochlear nucleus complex, throughout the whole auditory pathway, up to the auditory cortex.\[6\] Due to the recent advances in gene targeting technologies, the mouse model has reemerged as a valid framework to study Human disease mechanisms.

This section provides the reader with an introduction to the anatomical and functional organization of the mouse auditory pathway.

Figure 1.14: Ascending Auditory Pathway in the mouse brain. Sagital view of the simplified pathway in the Mouse adapted from \[41\]. Relevant abbreviations can be found in the Abbreviations section.

1.4.1 Sound Transduction

Vibrating objects such as vocal cords or speaker membranes create sound waves or pressure waves in the air. When these pressure waves reach the ear, they are transformed into a nervous stimulus (electrical signal). In general terms, the mechanic energy of the vibrating tympanum due to the arrival of sound waves is transferred to a chain of three small ossicles (the malleus, incus and stapes). The final ossicles, the stapes, insert into a fine diaphragm, called the oval window, which is the outermost structure of the inner ear. It is also important to mention that, since the surface area of the foot plate of the stapes is considerably smaller than the tympanic membrane, the impedance of the air filling the outer and middle ear and the fluid in the inner ear is matched. \[42\]

In the inner ear, the oval window is adjacent to the vestibular duct of the cochlea. The cochlea as can be seen in Figure [1.16] is a spiral-shaped organ with three fluid-filled compartments: The vestibular duct (Scala vestibuli), the cochlear duct (Scala media) and the tympanic duct (Scala Tympani).
Figure 1.15: Ascending Auditory Pathway in the mouse brain. Schematic view adapted from [42]. Relevant abbreviations can be found in the Abbreviations section.

Figure 1.16: Organization of the mammalian Cochlea and organ of Corti (a) and closeup the hair cells on the basilar membrane (b). Adapted from [43]

In this structure, sound constitutes a traveling wave that propagates from the base to the apex, growing in amplitude and slowing in velocity until reaching a point of maximal displacement; the latter is determined by the frequency of the sound and persists vibrating strongly as long as the sound lasts. High frequency sounds reach the point of maximal amplitude of vibration near the base and low frequency ones reach this point near the apex. This results in a spectral decomposition otherwise known as tonotopy.

Due to the different anchor points of the basilar and the tectorial membranes, the vertical component of the basilar membrane vibration is translated to a shearing movement of the membranes. This causes receptor potentials to be produced in the IHC by means of deflections of their stereocilia. This signal is
then conducted to the Auditory Nerve (AN) and then propagated to the rest of the ascending pathway.

The Outer Hair Cells (OHC) also play a very important role in sound processing. They receive input via the descending pathway from the Superior Olivary Complex (SOC) which trigger active vibrations of their cell body. This drives oscillations in the cell’s length, which occur at the frequency of the incoming sound and provide mechanical feedback amplification. The effect of this system is to non-linearly amplify quiet sounds more than large ones so that a wide range of sound pressures can be reduced to a much smaller range of hair displacements. [44] [45]

### 1.4.2 The Cochlear Nucleus Complex

The Cochlear Nucleus Complex (CNC) is the site of convergence of all the fibers of the AN which makes it the first synaptic center of the ascending auditory pathway. It is divided into the Ventral Cochlear Nucleus (VCN) and the Dorsal Cochlear Nucleus (DCN).

![Image of Cochlear Nucleus Complex](image)

**Figure 1.17:** Organization of the cat CNC tonotopy. Adapted from [46]. Relevant abbreviations can be found in the Abbreviations section.

The CNC also shows tonotopic anatomy as can be shown in Figure 1.17. The VCN coding preferably for lower frequencies and the DCN for higher frequencies.

The ascending projections of the CNC can be seen in Figure 1.15 and will be discussed in detail when the target nuclei are described. Here it suffices to emphasize that in addition to ascending projection to higher centers, the right and left CNC are interconnected and that it also receives descending projections from the auditory cortex (AC), the inferior colliculus (IC), the nucleus of the lateral lemniscus (NLL), and the superior olivary complex (SOC)[42].

### 1.4.3 The Superior Olivary Complex

The SOC consists in a set of neuronal groups located in the ventral tegmentum of the caudal pons. It is quite variable among species although three main nuclei are consistently identified: the lateral superior olive (LSO) the medial superior olive (MSO), and the medial nucleus of the trapezoid body (MTz).

One consistent feature of the SOC is the relationship of the LSO and MTz and the MSO. This link becomes apparent from the relative sizes of these structures, when they are compared to the animal’s audiogram. The LSO and MTz are well developed in species that have high frequency hearing, such as
rodents and carnivores, whereas they are relatively small in animals with low frequency hearing – such as the human. The MSO is small in mice, but well developed in humans. The inference is that the MSO is more important for the localization of low-frequency sounds, whereas the LSO-MTz may code for all frequencies. [47]

The SOC is believed to play several roles in the auditory function.

1.4.3.A Lateral Superior Olive

The LSO of the SOC has been implicated as the initial site of Interaural Level Difference (ILD) processing. [48] Many neurons in the LSO are excited by inputs from the ipsilateral ear and inhibited by inputs from the contralateral ear. This is the case because the MTz receives input from the contralateral CNC (via thick axons that terminate as large axosomatic calyces of Held in a one to-one relationship; these calyces constitute the largest synaptic terminals in the mammalian brain) and projects to the LSO via glycinergic inhibitory projection. In an opposite fashion, the connection between the LSO and the ipsilateral CNC is direct and excitatory. [10]

The LSO projects bilaterally to the central nucleus of the inferior colliculus (CIC). The ipsi- and contralateral projections are provided by distinct cell types. Most of the ipsilaterally projecting cells are glycinergic. The contralaterally projecting cells are excitatory. The LSO also projects to the dorsal nucleus of the lateral lemniscus (DLL). [42]

1.4.3.B Medial Superior Olive

The MSO is located between the LSO and the MTz. It is small in the mouse (contains only about 200 neurons) and is tonotopically organized with low frequency responsive neurons in its dorsal part and high frequency responsive neurons in its ventral part with an imbalance favoring low frequency responsive neurons. The MSO receives input from the CNC bilaterally. Unlike the LSO, the MSO receives direct and excitatory input from both sides, but the inputs remain separated: the lateral neurons receive input from the ipsilateral side and the medial neurons receive input from the contralateral side. This direct bilateral input scheme suggests that the MSO neurons, are ideally suited to measure interaural phase or time differences. The MSO projects to the ipsilateral DLL and CIC and their projections are excitatory. [42]
1.4.4 The Nuclei of the Lateral Lemniscus

The NLL is constituted by two main neuronal groups: the ventral nucleus of the lateral lemniscus (VLL) and a dorsal nucleus of the lateral lemniscus (DLL). The VLL receives inputs mainly from the contralateral ear, unlike the DLL which receives inputs from both ears.\[49\]

1.4.4.A Ventral Nucleus of the Lateral Lemniscus

The afferent projections to the VLL arise mainly from the contralateral CNC and ipsilateral MTz. The VLL appears to show topographic projections to frequency representations in the CIC, which would imply a tonotopic organization although some argue that this is not the case.\[42\]

1.4.4.B Dorsal Nucleus of the Lateral Lemniscus

The DLL is a distinctive group of neurons within the dorsal part of the lateral lemniscus. In opposition to the VLL, it receives input from both ears. Inputs arrive from both CNC and SOC and in turn projects to the homonymous contralateral nucleus and to the IC bilaterally.\[50\] Studies have shown that the DLL refines the binaural response properties of the IC neurons and contributes to accurate sound localization.\[51\]

1.4.5 The Inferior Colliculus

The IC is among the largest auditory nuclei in the vertebrate brain and it is an almost obligatory synaptic relay for ascending input to the MG. Its size and its many connections suggest that it has critical roles in both the ascending and descending pathways of the auditory system. Also, the IC has the most varied set of projection of all of the auditory pathway. It receives input from almost all parts of the CNC from much of the SOC from each of the NLL and from every auditory cortical area. Apart from these external inputs, there is a variety of commissural and self projections. It projects to almost all brain stem nuclei that project to it, and it sends axons bilaterally to the MG. Most of these connections are topographic which perhaps means they might be related to processes embedded in the tonotopic arrangement of characteristic frequency neurons.

One feature of the IC organization that has attracted considerable attention is the structural basis for frequency–specific arrangements, the isofrequency or frequency-band lamina, which contains thousand of neurons with a similar characteristic frequency (CF). This observation holds for several imaging modalities like MRI or electrophysiological and microscopical methods.

The IC contains three principal divisions: central nucleus (CIC), lateral nucleus (LCIC), and dorsal cortex (LCIC). These divisions differ in neuronal structure, their connections and, consequently, in their functional roles. The central nucleus is solely an auditory convergence center and it is essential for normal hearing; the lateral nucleus is multisensory, and the target of considerable nonauditory input and the dorsal cortex receives most of its projections from the cerebral cortex. Its role in hearing is still unknown.
1.4.6 The Medial Geniculate Body

The auditory thalamus was, for a long time, seen as a simple relay of information to the cerebral cortex but the last decade has proven that its functional nature has a lot more sophistication. Recent research shows complicated circuits and rich sets of membrane properties. It is now clear that the Medial Geniculate Body (MG) is not solely a conveyor of information to the cortex, but instead engages in many modulating processes that significantly alter the nature of the information. For example, the fact that the strength of corticothalamic input to the MG is much greater than that of the corresponding corticocollicular projection, implies a tighter coupling between thalamic and cortical oscillatory behavior than between the cortex and the auditory midbrain suggesting a complex role for the MG. More roles were discovered including routine auditory processing steps such as active amplification but also changes involved in learning and memory. These parallel roles highlight the significance of the thalamus in the sensory processing pathway.

The MG is divided into three parts according to their location in the complex and their anatomical and functional properties: ventral (MGV), dorsal (MGD), and medial (MGM) divisions.

1.4.6.A Ventral Division of the Medial Geniculate Body

The main sensory nucleus of the MG is its ventral division - MGV. The primary input to this division arises from the ipsilateral CIC and from the AC. Its main projection is to the layers III and IV of the primary AC. This division preserves, to a certain degree, a tonotopic gradient similar to its primary input, the CIC. No clear hypothesis regarding the nature and purpose of functional transformation at this
1.4.6.B Dorsal Division of the Medial Geniculate Body

The main sources of ascending auditory inputs to the MG are the DCIC and LCIC where tonotopy maps are hard to find. The MGD also projects mainly to several areas of the nonprimary AC where tonotopy is weak or absent. Tonotopic organization in the MGD is not apparent from recordings in any species but, in opposition, responsiveness to structured signals, like vocalizations show more prominence in the dorsal division than responses to simpler stimuli.

1.4.6.C Medial Division of the Medial Geniculate Body

The MGM has a coarse tonotopic organization and its afferent input includes nonauditory sources such as the vestibular system as well as fibers from the LCIC. The medial division has the widest spectrum of outputs in the MG, it projects to every part of AC (layers I and VI), to nonauditory cortex and to the amygdala. This thalamoamygdaloid pathway is unique to the auditory system and it is essential for learning based on auditory cues.

1.4.7 The Auditory Cortex

The cerebral cortex is a structure whose internal order, and high degree of local microcircuitry (the cortex is interconnected extensively with itself in recurrent and feedforward networks) makes it a fascinating research subject. Furthermore, its laminar organization distinguishes it from all the subcortical centers. Each layer has specific neuronal organization, inputs and outputs. This suggests that layer specific processes may occur similarly to what happens in the subdivisions of the subcortical auditory centers. Like all of the neocortex, it has six layers and although ipsilateral corticocortical connections exist in conjuncture with interhemispheric, a huge portion of all the connections are within layers. The complexity of the neural networks involved in this brain region explains why it is so difficult to explain cortical function, as some connections seem to act in a highly spreadout fashion, whereas others are much more localized.
In the mouse, the AC is situated on the caudal half of the parietal cortex. Five regions have been identified within it based on tonotopy, spontaneous activity and the general characteristics of responses to pure tones, noise bursts and frequency sweeps. These fields include the primary auditory field (AI), the anterior auditory field (AAF), the ultrasonic field (UF), the secondary auditory field (AII) and the dorsoposterior field (DP). AI and AAF are tonotopically organized in opposition to UF and DP that show no characteristic tonotopic gradient. These regions can be seen in Figure 1.21.

As mentioned in subsection 1.4.6, the AC receives differentiated input from the MG, meaning that different nuclei of the MG project to different layers of the AC. As will be discussed in the next subsection, the AC projects to nearly every subcortical auditory center.

### 1.4.8 The Descending Pathway

In parallel with the ascending auditory pathway, a second stream of information, also known as the descending pathway, takes place. This descending feedback circuit is also essential for auditory processing and is as complex as the ascending pathway. Three important circuits are worth mentioning: The corticofugal, the coliculofugal and the olivocochlear circuits.

#### 1.4.8.A Corticofugal Circuit

The mouse AC projects to a wide range of subcortical targets in the auditory pathway. The projections to the auditory thalamus (MG) and midbrain (IC) are the strongest and largest but there are also the projections to subcollicular nuclei like the CNC or the SOC. The corticothalamic projections are often studied due to their diversity.

As mentioned in section 1.4.6, the connections between AC and MG can be reciprocal or not. In the case of the reciprocal connections, one can say that they engage mainly in feedback communications, and that the non-reciprocal communications serve the purpose of communication between different cortical areas. This means that the AC can use the MG as a relay for communication to other cortical regions. It is important to mention that this corticofugal control is not limited to primary areas. Indeed, studies of these projections from nonprimary, nontonotopic areas show that they are as strong, focal, and topographic, as that of their primary, tonotopic counterparts. The exact role of this corticofugal circuit is not yet pinpointed but several studies have suggested it may have very fine and precise functions such as species-specific vocalizations.

#### 1.4.8.B Coliculofugal Circuit

Studies in the rat and guinea pig have shown that the IC provides descending projections to the NLL, SOC and CNC. Their role is still uncertain.

#### 1.4.8.C Olivocochlear Circuit

As mentioned in Section 1.4.1, the olivocochlear circuit provides the organ of Corti with its efferent innervation. This circuit is unique among sensory systems because hair cell receptors receive direct projections from the brain. Physiological studies in several species have shown that that the SOCC
neurons that engage in this circuit are sharply tuned with a wide dynamic range that may enhance transduction or signal detection by regulating the slow motility of the OHCs and thereby the stiffness of the basilar membrane. The SOC activity can also reduce temporary threshold shift by inhibiting cochlear sensitivity and thereby protecting the inner ear from acoustic injury. In summary, the olivocochlear system plays a critical role in maintaining the normal operating of the cochlea and introduces non-linear dynamics into the auditory system.

1.4.9 Neuroplasticity in the Auditory System

Neuroplasticity is a term with many meanings, but mainly refers to the capacity of the brain to reorganize its networks when provided with a new environment. This is accomplished, by rearranging the connections between neurons. Changes in gene expression cause neuronal, glial and vascular changes at the molecular, cellular, and tissue levels. Ultimately, new brain structures form or are eliminated (synapses for example), and the process can be local or it can affect whole areas. It may emerge quickly or slowly, be permanent or temporary, and may reflect a shift in the influence of excitatory or inhibitory events.

One of the characteristics of the auditory system that has motivated many studies is its capability to adapt to extreme conditions. In other words, its neuroplasticity. Neuronal adaptation to changing conditions or injuries has been seen in all of the auditory centers. From the CNC to the AC.

Figure 1.22: Descending Auditory Pathway in the mouse brain. Schematic view adapted from [42]. Relevant abbreviations can be found in the Abbreviations section.

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1.4.10 Electrophysiology

A vast bibliography on electrophysiological studies on the mouse auditory system exists and was already mentioned in some sections of this chapter but its richness must be explored in a more condensed way so that a proper overview of existing knowledge is made.

Electrophysiological studies usually take some time as they are technically very difficult. Despite this, the results they provide far outperform fMRI studies in terms of spatial and temporal resolution but are unpractical when trying to map different structures that participate in a given system.

This modality of imaging has been used for several purposes. For example, studies like [46] on the CNC and [47] on the SOC have vastly contributed for the idea that all structures in the Auditory Pathway across a wide range of animal models have a clear tonotopic distribution. Additionally, the spatial resolution of the technique reveals some inhomogeneities in the tonotopy maps that other imaging modalities fail to capture [5]. In [64] the development of tonotopic maps in the IC of aging Mice was studied to ascertain its modifications through time showing not only inhomogeneities of the tonotopic maps but also it's variance in even very short time windows (days).

Other electrophysiological studies have focused more on the circuitry itself. For example in [58] the MG was shown to act as a relay for communication among cortical regions. In [59] the SOC activity was shown to inhibit cochlear sensitivity and thereby protecting the inner ear from acoustic injury when sounds go above a threshold of pain.

1.5 Rodent Auditory fMRI state of Art

Rodent auditory fMRI is a relatively new field. It appeared in 2005 when Yu and colleagues used MEMRI to map the auditory circuit and show tonotopy in the IC [16]. Building on this study, the same team produced two subsequent studies [3], [17] in which they showed instances of neuroplasticity and the linear dependence of the signal with the SPL of the auditory stimulus. So far, these have been the only studies on mouse auditory fMRI - meaning that no BOLD fMRI study has yet been made with this animal model. At the time, they stated that BOLD fMRI would not be a good candidate for auditory pathway mapping since the sequences existing at the time were acoustically very loud and that this sound would interfere with the task. Later research on this field learned to rely on sparse sampling paradigms or constant noise sequences such as FISP so that the task would not be affected by the background acousting noise.

In 2012, Wu and colleagues showed the first auditory BOLD fMRI study in rodents (Sprague-Dawley Rats). They used a waveform generator, that connected to an ultrasonic speaker. This speaker was coupled to a progressively thinner tube, that eventually entered the rat's ear delivering sound stimuli at the same time as the scanner was acquiring. In this study, Wu and his colleagues mapped the auditory circuit and showed tonotopy in the IC [11]. Since then, this team has successfully used slight variations of the same auditory stimulation system to reveal very unique features of the auditory system. They have shown very precise tonotopic maps in the IC using DSSFP [12], sound pressure level encoding in the IC [65], the interaural difference encoding in the auditory system [10], the influence of the IC in...
deviant sound detection [13], the ultrahigh frequency encoding in the IC [14], the effect of AC and visual cortex in IC processing [15], and the effects of long term exposure to certain types of sounds [2, 63]

1.6 Thesis Outline

Although the Auditory Pathway and its features have been extensively studied in rodents using electrophysiological probes, few of these dimensions have been analysed using fMRI. Its larger FOV and the fact that it is non-invasive means that it is adequate for longitudinal studies looking at the global interactions of the system. Particularly in the mouse, no study has yet used real-time auditory fMRI. The difficulty lies in the smaller size of the mouse brain compared to rats, in conjunction with the technical difficulty of building a MRI-safe mouse auditory stimulation system.

The main objective of this work is therefore to build and validate a MRI-safe auditory stimulation system. To reach this goal, more specific goals will be aimed at. Specifically:

- To build a cheap, open-source MRI-safe auditory stimulation system capable of reliably producing signals (electrical and sound pressure waves) up to 65 kHz
- To validate the system by accessing the tonotopy in the mouse brain in several experiments and comparing the results with those coming from electrophysiological studies.
Materials and Methods

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2.2 Auditory Stimulation Experiments .......................................... 35
2.1 Auditory Stimulation System

This section introduces the logic behind the construction of the MRI safe auditory stimulation system. The system can be seen in the following figure and will described in detail in the following subsections.

![Figure 2.1: Schematic of the Auditory Stimulation system used](image)

2.1.1 Playback Equipment

Mice can hear sounds up to 85 kHz \(^{[66],[67]}\). For this reason, the playback system selected had to be able to sample signals up to this frequency, so that the total hearing range of mice could be probed. Previous investigations with rodents have classically relied in waveform generators \(^{[63],[10]}\) since these devices can usually sample signals effectively until the MHz range and with a very good dynamic range. Their only shortcoming is its price which is typically in the range of the thousands of euros. The low-cost alternative found was a soundboard (YAMAHA AG-03, Japan), commonly used by musicians for playback and recording. The board costs less than one hundred euros and allows for playback of sounds up to 96 kHz (sample rate of 192 kHz) with a dynamic range of 24 bits and 2.451 V\(^{\text{RMS}}\) output before clipping. It has two independent out channels allowing for simultaneous binaural stimulation if needed and can also serve as a sampler for a calibrating microphone. It is USB powered and can be controlled by its native software or by any programming language with a soundboard interface. The performance

![Figure 2.2: Playback equipment used in the Auditory Stimulation System](image)
of the electric signal output of the board can be seen in Table A.1 measured with an oscilloscope (TEXTRONIC TPS 2000, United States)

### 2.1.2 Amplification

The exit voltage of 2.541 V RMS is still low for typical ultrasonic speakers and as such, an amplification stage had to be included in the circuit. The chosen solution was an in-house designed voltage amplifier (Hardware Platform - Champalimaud Foundation) capable of a 3x amplification up to 24 Vpp. The board is powered by an electric socket and has one in-channel and one out-channel.

![Amplification Board used](image)

### 2.1.3 Speakers

As previously mentioned, Mice can hear sounds up to 85 kHz. At this point in the circuit, the electric signal generated in the Yamaha AG03 board has been amplified and has to be transformed into a sound wave. Few speakers can handle ultrasonic frequencies but two categories stand out: piezoelectric speakers and electrostatic speakers. In essence, piezoelectric speakers rely on the vibration of a piezoelectric crystal and electrostatic on the vibration of an electrically charged thin membrane with electrostatic speakers being a lot more expensive than their piezoelectric counterparts. The choice was made of using a piezoelectric speaker (L010 KEMO, Germany). This speaker has been shown to be able to produce ultrasonic sounds at high output levels (+100 dB) with a relatively flat frequency response up to 75 kHz and was already used in previous similar studies with rats. The speaker needs an input of 16 V or more to function at maximum performance. It is also small (36mm diameter) and lightweight (10 g) which makes the system flexible. The performance of the speakers at the level of the ear of mice can be seen in Table A.2

### 2.1.4 Tubing

Having produced sound waves, the next step becomes guiding the sound waves up to the mouse ear in an efficient and spacially economic way. The space in the magnet is very limited and especially in the
mouse-coil interface when a very tight fit between the mouse head and the coil is needed for maximal SNR. As such, along the path from the speaker to the mouse ear tubes of different calibers have to be used. The main part of the tubing consists of a rigid nylon tubing with a 14 mm inner diameter. The tube that enters the mouse ear is a semi-rigid tygon tube with an inner diameter of 1.6mm.

The first step is the interface between the speaker and the main tube. This is accomplished by a pair of 3-D printed custom parts designed in Autodesk Fusion 360 software for students.

At the end of the main tube, the circuit had to be tapered down to the width of the final semi rigid tube that inserts into the ear of the mouse. This coupling was also ensured by a screw lock mechanism identical to the one in Figure 2.5.

Using these specially engineered parts, the sound produced in the speaker was guided to the mouse ear with minimal dissipation. The total length of the tubing was 88 cm (including 6 cm of tygon tubing).

### 2.1.5 Sound Calibration

Having the sound delivery sound built, it became then important to measure its performance. This was accomplished by using a free-field microphone (Brüel and Kjær 4939-A-011, Denmark) with a Type 2670 preamplifier that connected directly into the Yamaha Soundboard. This way, the performance of the system and other measures such as scanner noise could be performed. Scanner noise was measured 30 cm away from the bore of the magnet and the performance of the sound stimulation system was measured 1mm away from the tip of the semi rigid tube. Results of these measurements can be seen in
2.1.6 Integration with the Scanner and scripts

Integration with the scanner was made using an Arduino microcontroller as a trigger detector. In the scanner, the sequences could be programmed to send triggers when a sound cue was due. This trigger would be detected by the Arduino (ARDUINO, United States), which would then activate the soundboard to deliver the sound. This whole process lasted less than 50 msec and was globally controlled by a Python script.

2.2 Auditory Stimulation Experiments

Two different experiments were performed. Both of them served two purposes: accessing the quality of the Auditory Stimulation System built and mapping tonotopy in the mouse brain using [MRI] for the first time. In the first experiment (herein defined as Low-Frequency), 6 male C57BL/6 mice, aged between 7 and 8 weeks old and weighing between 21 and 25 g were used. The second experiment (defined as High-Frequency), used 7 female C57BL/6 mice, aged between 7 and 8 weeks old and weighing between 17 and 21 g.

2.2.1 Ethics Statement

All aspects of this study were approved by the local animal ethics committee.

2.2.2 Animal Preparation

For both experiments the preparation protocol was identical:

- Mice were weighted and their weight was registered.
- Each animal was anesthetized with 4% isofluorane (VIRBAC, France) for 3 minutes in a custom build plastic anesthetizing box mixed with ambient air. The air-isofluorane mixture was maintained
by a vaporizer (VETEQUIP, United States). After this amount of time, the Isofluorane was lowered to 3% and a timer would be started. After each interval of 6 minutes passed, the anesthesia was lowered 1% until it reached 0%. Oxygen level was maintained at 27% with the aid of a oxygen sensor (Viamed, United Kingdom)

- Once sedated, animals were placed in the prone position on an animal bed (Bruker BioSpin, Germany) with a nose cone and tooth bar to limit motion

- After placement in the bed, the mice would receive an infusion of medetomidine (DORMITOR, Pfizer, United States of America) for the remainder of the experiment. A bolus of 0.4 mg/kg was injected, followed 2 minutes after by a constant infusion of 0.8 mg/kg/h. The medetomidine was diluted 1:10 in saline and was injected in the animal with a syringe pump (GenieTouch, KentScientific, United States of America). This anesthesia protocol was similar to the one found in [68]

- Temperature and respiration sensors were placed for monitorization of the condition of the mouse. (Model 1030 Monitoring Gating System, SAII, United States of America). Warm water was circulated under the mouse so that its temperature was kept at 37°C. Vital sign measurements were monitored in real time but were not available for analysis.

- The flexible tygon tube was inserted into the mouse ear. The tube was held in place and external sounds were isolated by occluting both mice ears with semi-liquid (50%) Parafin(Sasolwax 5803 SASOL, Germany).

![Figure 2.7](image)

Figure 2.7: Schematic of a mouse placed in the bed with the full experimental setup. All the components can be seen: the sound tube entering the mouse’s left ear, the medetomidine infusion, the rectal thermometer and the respiration sensor

- The entire assembly was placed inside a 9.4 T MRI scanner (Biospec, Bruker, Germany) and warm water was circulated while continously monitoring rectal temperature and vital signs

Every experimental session lasted about 1.5 h. Approximately 30 min was used for animal preparation, 30 min was spent on acquisition of preparatory [MRI] scans, and the remaining time was used for acquiring [MRI] data during hindpaw stimulation. After the experiments, time for recovery from anesthesia and atipamezole hydrochloride (SEDASTOP, AnimalCare, United Kingdom) at 0.1 mg/kg administration was provided for all the animals.

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2.2.3 **fMRI Acquisition**

Experiments were performed on a Bruker BioSpec 9.4T AVANCE3HD scanner, equipped with a gradient system capable of producing up to 660 mT/m in all directions. An 86mm resonator volume coil was used for transmittance, and a 4-channel array cryocool was used for reception. In particular, the cryoprobe serves to enhance the signal-to-noise ratio by a factor typically between 2 and 3. Once the animal was properly positioned in the scanner, scout images were acquired to determine the coronal and sagital planes of the brain. A $B_0$ map was acquired to allow for a proper shimming. Six parallel 0.65 mm thick slices, separated by 0.15 mm (Bregma -5.80mm to -1.40mm), were oriented orthogonal to the sagital plane and used for anatomical reference. The parameters for the anatomical scans were: Turbo RARE sequence, RARE factor=8, Number of averages: 6, TR/TE=2000/10ms, FOV=15x15 mm2, data matrix=200x200. Slice acquisition order was interleaved with the order being [1 3 5 2 4 6].

![Figure 2.8: Example of the anatomical images acquired](image)

After the anatomical image was acquired the fMRI acquisitions could begin. All fMRI acquisitions were made with the following parameters: True FISP sequence, TR/TE=2.8/1.4ms, Effective repetition time:1.307s, FOV=15x15 mm2, data matrix=100x100, flip angle: 30°. Slice acquisition order was interleaved with the order being [1 3 5 2 4 6]. The slices were acquired in the same position as in the anatomical scans and the total number of volumes acquired was always equal to 334 which made all runs last 7m16s736ms.

2.2.4 **Auditory Stimulus**

In the Low-frequency experiment, three pure tones were used (5 kHz, 12 kHz and 20 kHz). Each mouse had one run listening to one of the three frequencies in a random fashion with a total of three runs per mouse. The frequency spectra of these frequencies at the level of the mice ear can be seen in Figure [A.1](image)

In the High-frequency experiment, five pure tones were used (35 kHz, 36 kHz, 37 kHz, 38 kHz and 39 kHz). The frequency spectra of these frequencies at the level of the mice ear can be seen in Figure [A.2](image) In this experiment, each mouse received five different sounds in one run (presented in a random
order), with an unbalance of runs across the 8 mice due to some of them waking up mid-experiment. In total 26 runs were acquired for the 7 mice, with two mice having 1 run, one mouse with 2 runs, 3 mice with 6 runs and 1 mouse with 5 runs.

2.2.5 Stimulation Paradigm

Figure 2.10: Experimental paradigm used in both experiments - 34 volumes of rest and 16 volumes of activation

In both experiments, the experimental paradigm was as depicted in Figure 2.10. Cycles of 50 volumes in which sound was off for 34 volumes and on in 16 (20.912s). In the low frequency experiment, the mice always heard the same frequency in one run. In the high frequency experiment, the mice heard all frequencies (presented in a random order) in one run. The first 50 volumes acquired were always discarded since it was hypothesized that the magnetization still hadn’t reached a steady state by then. This made each run have 5 presentations of sounds. All sounds were presented to the left ear of the mouse. The frequency spectra of the FISP sound can be seen in Figure A.3.

2.2.6 Data Analysis

All data was analysed using the SPM package for MATLAB and a Grafical User Interface designed for simplifying the connection between MATLAB and SPM, dubbed fMRAT. The data was first realigned to the mean image of each run and the realignment parameters were kept to be later used as regressors in the GLM model. The images were then spatially smoothed using a Gaussian Filter with a FWHM of 0.3 mm. Then the Design matrix was built using an HRF peaking at 3 seconds, the parameters were estimated voxel-wise and the contrasts were built. When building global maps of activation all the images were coregistered to one representative animal.

ROI analysis was also performed for the low frequency experiment. To this end, the realigned images were used (unsmoothed) to reduce interference by motion. ROIs were drawn manually and all runs of
Figure 2.11: Hemodynamic Response function used to model the BOLD response following activation each frequency were detrended, temporally filtered (notch at 0.2 Hz) averaged across mice. Then each representative run was also averaged so that one representative cycle could be shown. ROIs were only drawn for regions shown to be activated by the activation maps.
3 Results

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3.1 Low Frequency Experiment

3.1.1 SPMt Maps

At the end of the SPM pipeline, SPMt maps are obtained. These are basically 3-D matrices with the t-values of each voxel, resulting from the voxel by voxel t-tests performed by SPM and according to the GLM. From now on, these t-maps are shown with a threshold of p<0.001 and a minimum cluster of 12 voxels passing the threshold.

For the Low Frequency experiment, it was possible to compute individual maps for the frequencies 5, 12 and 20 khz as well as global maps of activation for each mouse and for all mice. Global maps of activation refer to voxels activated in all frequencies considered. Tonotopy maps were also computed. These consist of the voxels maximally activated for each frequency (the 20th percentile of voxels by their t-score) overlayed in the same anatomical image.

3.1.1.A Mouse 1

![Image of SPMt Maps for Mouse 1]

Figure 3.1: Map of activation across all frequencies for Mouse 1. Colorbar in t-values
Figure 3.2: Map of activation for 5kHz for Mouse 1. Colorbar in t-values

Figure 3.3: Map of activation for 12kHz for Mouse 1. Colorbar in t-values
Figure 3.4: Map of activation for 20kHz for Mouse 1. Colorbar in t-values.

Figure 3.5: Tonotopy map for Mouse 1. Colorbars in t-values and ordered so that Red - 5 kHz, Green - 12 khz and Blue - 20 kHz.
3.1.1.B  Mouse 2

Figure 3.6: Map of activation across all frequencies for Mouse 2. Colorbar in t-values

Figure 3.7: Map of activation for 5kHz for Mouse 2. Colorbar in t-values
**Figure 3.8:** Map of activation for 12kHz for Mouse 2. Colorbar in t-values.

**Figure 3.9:** Map of activation for 20kHz for Mouse 2. Colorbar in t-values.
Figure 3.10: Tonotopy map for Mouse 2. Colorbars in t-values and ordered so that Red - 5 kHz, Green - 12 kHz and Blue - 20 kHz
3.1.1.C Mouse 3

Figure 3.11: Map of activation across all frequencies for Mouse 3. Colorbar in t-values

Figure 3.12: Map of activation for 5kHz for Mouse 3. Colorbar in t-values
Figure 3.13: Map of activation for 12kHz for Mouse 3. Colorbar in t-values

Figure 3.14: Map of activation for 20kHz for Mouse 3. Colorbar in t-values
Figure 3.15: Tonotopy map for Mouse 3. Colorbars in t-values and ordered so that Red - 5 kHz, Green - 12 kHz and Blue - 20 kHz
3.1.1.D  Mouse 4

Figure 3.16: Map of activation across all frequencies for Mouse 4. Colorbar in t-values

Figure 3.17: Map of activation for 5kHz for Mouse 4. Colorbar in t-values
Figure 3.18: Map of activation for 12kHz for Mouse 4. Colorbar in t-values

Figure 3.19: Map of activation for 20kHz for Mouse 4. Colorbar in t-values
Figure 3.20: Tonotopy map for Mouse 4. Colorbars in t-values and ordered so that Red - 5 kHz, Green - 12 kHz and Blue - 20 kHz
3.1.1.E Mouse 5

Figure 3.21: Map of activation across all frequencies for Mouse 5. Colorbar in t-values

Figure 3.22: Map of activation for 5kHz for Mouse 5. Colorbar in t-values
Figure 3.23: Map of activation for 12kHz for Mouse 5. Colorbar in t-values

Figure 3.24: Map of activation for 20kHz for Mouse 5. Colorbar in t-values
Figure 3.25: Tonotopy map for Mouse 5. Colorbars in t-values and ordered so that Red - 5 kHz, Green - 12 kHz and Blue - 20 kHz.
3.1.1.F Mouse 6

Figure 3.26: Map of activation across all frequencies for Mouse 6. Colorbar in t-values

Figure 3.27: Map of activation for 5kHz for Mouse 6. Colorbar in t-values
Figure 3.28: Map of activation for 12kHz for Mouse 6. Colorbar in t-values

Figure 3.29: Map of activation for 20kHz for Mouse 6. Colorbar in t-values
Figure 3.30: Tonotopy map for Mouse 6. Colorbars in t-values and ordered so that Red - 6 kHz, Green - 12 kHz and Blue - 20 kHz
3.1.1.G Global

Figure 3.31: Map of activation across all frequencies with region labeling. Colorbar in t-values

Figure 3.32: Map of activation for 5kHz. Colorbar in t-values
Figure 3.33: Map of activation for 12kHz. Colorbar in t-values

Figure 3.34: Map of activation for 20kHz. Colorbar in t-values
Figure 3.35: Tonotopy map. Colorbars in t-values and ordered so that Red - 6 kHz, Green - 12 kHz and Blue - 20 kHz.
3.1.2 ROI

After obtaining the SPMt maps, it became interesting to plot some ROI's to found out more about the evolution of the signal along time in different regions. The individual SPMt maps were used to plot the ROI's depicted in Figure 3.36. The AC region was not actually studied since it seldom appeared in the SPMt maps (only in two out of six subjects) but is depicted in the figure as well. A control region inside of the sample but out of expected activated regions was drawn as well.

![Slice images](image1)

**Figure 3.36:** ROI's drawn according to their location in each SPMt map and the existing literature and their respective label

Then, the signals obtained in the ROI were detrended and averaged across mice. The result of an individual signal (only detrended) in the IC can be seen in Figure 3.37.

![Signal plot](image2)

**Figure 3.37:** Signal in the IC of a representative mouse. Stimulation happens during the highlighted bars
The result of the averaged (across mice) signal in the IC can be seen in Figure 3.38.

Figure 3.38: Averaged signal in the IC across mice. Stimulation happens during the highlighted bars.

This signal still seemed to be characterized by a constant and unexpected oscillation and, for that reason, a Fast Fourier Transform was performed on the signal to try to pinpoint the source of this noise. The result can be seen in Figure 3.39.

Figure 3.39: Fast Fourier Transform of the average data, showing a peak in the frequency of the experiment - expected and indicated and another at around 0.2 Hz - unexpected.
A notch filter was applied in the data (at 0.2 Hz) producing the signal present in Figure 3.40

![Image](image.png)

**Figure 3.40:** Averaged signal in the IC, filtered with a notch filter at 0.2 Hz

Two graphical representations of the signals in the auditory pathway were deemed important to show: The ventral and dorsal parts of the IC associated with higher and lower frequencies respectively and the SOC, LL, and CNC.
Figure 3.41: Averaged data across mice and across cycles for the IC for 5 kHz (Up), 12 kHz (Center) and 20 kHz (Down). Two regions of the IC are shown: Dorsal and Ventral, error bars are in standard error of the mean and the activation is shown in red.
Figure 3.42: Averaged data across mice and across cycles for the CN, SOC, LL and a non task related brain area for 5 kHz (Up), 12 kHz (Center) and 20 kHz (Down). Error bars are in standard error of the mean and the activation is shown in red.

3.2 High Frequency Experiment

The fact that the number of runs in each mouse in the High Frequency experiment was not uniform, made it impossible to build individual SPMt maps since the statistics were not powerful enough. As such,
only global SPMt maps and tonotopy maps were built for this experiment. The spatial normalization of
the 7 mice also produced global maps of only five slices in this case.

**Figure 3.43:** SPMt map for the 35kHz frequency. Colorbar indicates t-values

**Figure 3.44:** SPMt map for the 36kHz frequency. Colorbar indicates t-values
**Figure 3.45:** SPMt map for the 37kHz frequency. Colorbar indicates t-values

**Figure 3.46:** SPMt map for the 38kHz frequency. Colorbar indicates t-values

**Figure 3.47:** SPMt map for the 39kHz frequency. Colorbar indicates t-values
Figure 3.48: SPMt map for all frequencies. Colorbar indicates t-values

Figure 3.49: Global Tonotopy map in the IC for the High Frequency experiment. Colorbars indicate t-values and are ordered by frequency so that Red corresponds to 35 kHz, Green corresponds to 36 kHz and so forth
4 Discussion

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4.1 Auditory Stimulation System

The auditory stimulation system’s performance is briefly summarized in Tables A.1 and A.2. The electric output of the system, described in table A.1 was also measured for total harmonic distortion, but this value was not included since it was always lower than 0.01%. This means that the system is highly capable of producing pure tones with minimal distortion. It should be stated that the amplitude of the signal starts dropping at around 25 kHz and is accentuated after 45 kHz. This happens because the board is sold for “human” applications, meaning its high sample rate is meant for high quality sampling, rather than ultrasonic playback. This difficulty was partially overturned by including an amplification stage in the circuit but since the amplifier used was linear for all frequencies, the roll off still happened, albeit at a slower rate. A reasonable alternative would have been to measure a response function of the board, and used a non linear amplifier to match the inverse of that response function so that the output would be constant across all the frequencies of study (performed posterior to the study). Another alternative, would be to use a waveform generator, which would increase the price of the system greatly.

The use of a soundboard, coupled with an amplifier isn’t usual in such applications (ultrasonic auditory stimulation systems) and also constitutes an innovation. In essence, a low-cost waveform generator was built.

The sound output of the system, described in the table A.2 was very dependent on three key factors: voltage presented to the terminal of the speaker, distance from the speaker to the mouse ear and caliber of the tube guiding the sound. The dependence on the first factor is clearly shown in Table A.2 and one can conclude that Peak SPL values follow the trend of voltage at the terminals. Tests were only made up to 65 kHz since the Kemo speakers are not designed to go over these frequencies although previous studies have made that claim. Other speakers were tested in our setup, namely a pair of commercial ultrasonic speakers widely used in these kinds of studies but did not serve our purpose, since there were minor air gaps in our setup that transformed the system into an open-field like one. Future improvements on the system should focus in closing these air gaps in order to be able to use higher quality speakers. A dependency of the SPL was also observed as a function of distance to the source and caliber of the tube. This was the reason for choosing a short tube (<1m) with a relatively high calibre (14mm). Short caliber tubes interfere more heavily with sound pressure waves, making them disperse energy. As such, the caliber of the tube was only reduced when there was no more alternative, making for smooth transitions in caliber from 31mm at the speaker level to only 1.6 mm at the mouse ear level.

Attention was also given to the magnetic susceptibilities of the circuit. The leads attaching to the speaker were placed at least 20 cm from the bore of the magnet, a point where the manufacturer (Bruker) deemed that the magnetic insulation of the magnet was sufficient to not interfere with electric currents in those leads.

Although reliable and reproducible, the sound output of the system was found to be unstable, with very short variations in frequency, resulting in very large variations in characteristics of the sound measured such as peak SPL and total harmonic distortion (especially for lower frequencies). A sensibility study
should be made, so that future versions of the system have a more complete description of the input-output relationship.

4.2 Auditory Experiments

Two experiments were made to test the capabilities of the Auditory Stimulation System. In both of them, mice between 7-8 weeks old were used since it has been proven that C57BL/6 mice start losing hearing acuity around that age. Additionally, since the Haemodynamic response wildly differs for different anesthesia and brain region, and given the especial susceptibility of mice to isofluorane the choice was made to anaesthetise all mice used with medetomidine. The high frequency experiment used females because the initial idea was to play pup vocalizations as the auditory stimulus, which eventually didn’t happen.

The imaging sequence chosen: FISP was used different parameters because GE-EPI (widely used in previous studies) is not adequate for high field BOLD fMRI for a number of reasons. High susceptibility artifacts, very blurry images due to very very short T2* times at these field strengths, and high influence of big vessels. Additionally, FISP has been shown to have higher SNR for similar temporal resolutions.

The frequencies of choice for the low frequency experiment were picked since they placed in a low threshold well of the mice audiogram, meaning that mice detect frequencies in this range even for very low SPL. For the high frequency experiment, the frequencies used lied in a stable range of frequencies of the auditory stimulation system used, meaning that between these frequencies, harmonic distortion was low and SPL difference was lower than the lowest observed SPL difference in rats that showed difference in BOLD signal. Also, these frequencies lay on the range of ultrasonic sounds which were not found to be emitted by the FISP sequence, therefore minimizing acoustic noise interferences.

4.2.1 fMRI signal analysis

As stated in Section 2.2.6, data was spatially normalized by coregistering every mouse to the same template. Because all acquisitions differ slightly on the exact position of the mouse and also because brain dimensions also differ, the registration made that the global maps only had 5 slices, with the tonotopic being in the first slice. This also happened in mouse 5 of the Low Frequency experiment since it moved between runs and its runs had also to be coregistered. The hemodynamic response function used was based on and was taken as the closest to the thalamus since we were mainly focused in subcortical structures. This biased our analysis by setting a filter of possible signals to be observed and may explain the fact that few mice show AC activation.

4.2.2 Low Frequency Experiment

4.2.2.A Activation Maps

For the low frequency experiment, one claim has to be stated from the start. Looking at Table A.2, it can be observed that the three tones used had very different harmonic distortion values and may
have influenced the final results. This may explain why the SPMt maps are very spread for the lower frequencies (5 kHz and 12 kHz) and localized for the higher frequency (20 kHz) but physiological reasons for this also exist as will be discussed next.

Looking at the activation maps for specific frequencies across mice, we see that activation patterns for specific frequencies have high variability across subjects. This may have happened for several factors: individual susceptibilities to anesthesia, minor differences in tubing placement or even imperfect shimming.

Apart from tonotopy in the IC - present in all mice except Mouse 5 - different structures seem to activate consistently due to differences in frequency.

Looking at figures 3.32, 3.33, 3.34 separately we see that the SOC activated more medially for 5 kHz than for 12 kHz which may confirm the claim that the MSO codes for lower frequencies [47]. The MSO was consistently activated which supports the fact that this region is activated during monoaural stimulation. The SOC activation for lower frequencies also may show the activity of the olivocochlear descending pathway, that tries to adjust the gain of the cochlea to avoid injury. (The sounds we used, and the FISP sounds were very loud , <100 dB SPL) Interestingly, the CNC did not show consistent activation for 20 kHz which might mean that the area of this structure responsive for high-frequency processing isn’t big enough to have sufficient signal to appear in the SPMt maps [46] or, alternatively that the HRF used was not adequate to this kind of study. The NLL seems to have higher activation when the IC also does which is plausible due to the fact that most of the IC’s direct input comes from NLL [42]. A region in the cerebelum also activated for low frequencies and was also observed in another study. [10]

4.2.2.B Tonotopy

The tonotopy map shown in Figure 3.31 shows a very distinct tonotopic pattern with higher frequency specific voxels being located ventromedially and lower frequency voxels being more dorsolaterally. This is excellent agreement with the existing literature for rodents, both with electrophysiological studies and with MRI [71], [16], [49].

4.2.2.C ROI Analysis

In the low frequency experiment, due to the fact that the run number was the same across subjects, an ROI analysis was possible. Some conclusions can be drawn from it. First, there was 0.2 Hz noise component that deeply corrupted the signal and had to be removed using a notch filter for proper analysis. This noise source might have been caused by a downsampled signal either from the heart-beat or respiration or by a bias imposed by the coregistration. Second, there was high variability in the baselines across subjects, but this variability was greatly reduced during stimulation. Further analysis (t-tests) should be made to study the statistical power of the activation vs rest difference in signal.

In the IC two ROI’s were drawn, one more dorsolaterally (dubbed dorsal) and one more ventromedially (dubbed ventral). These ROIs showed the tonotopy in the IC yet again since the medial ROI had higher signal than that the dorsal ROI for high frequencies, while the opposite happened for lower fre-
frequencies. The plateaus observed during activation also showed several peaks, possibly giving insights on the activity of the descending auditory pathway. Further analysis should be done by modeling these several peaks and comparing them to electrophysiological studies [42].

Other structures also showed these several peaks were the NLL and SOC structures also implicated in the descending auditory pathway. A ROI was also drawn in a region not believed to be involved with auditory processing and did not show significant increase in signal during activation. All this analysis will be validated in further studies resorting to t-tests. The CNC showed higher activation for lower frequencies than for higher ones which can be a consequence of its tonotopic distribution or the higher harmonic distortion of lower frequency sounds. But the results agree with the SPMt mapping.

The temporal resolution used (1.307s) didn’t allow for proper separation of rise times for different structures but it is evident that the behaviour pattern across structures is different and rises at stimulation onset, going back to baseline when the stimulation ends.

4.2.3 High Frequency Experiment

The high frequency experiment was designed to try and separate the effects of total harmonic distortion and to map tonotopy in the IC in a very narrow frequency spectra. Both those objectives were fulfilled since the sound stimulation system is very reliable in the interval of 35-39 kHz (<1% total harmonic distortion and very similar SPL) and tonotopy was observed for frequencies separated by 1 kHz.

Looking at figures 3.43, 3.44, 3.45, 3.46 and 3.47 it can be seen that no longer activated regions depend on frequency. The activated regions (CNC, IC, NLL and SOC) are constant which might be due to the no longer existent Total Harmonic distortion or due to physiological reasons such as the lesser separation between frequencies. The CNC in particular shows activation in these range of frequencies while it didn’t in the 20 kHz which might be due to the use of females instead of males (females hear pup vocalization at this range and can have higher gain in the CNC) [52].

Looking at figure 3.48 it can be seen that the higher t-values concentrate in a very specific region of the IC ventromedially with lower t-values existing in the dorsolateral region. When then only using the highest t-values for each frequency to plot a tonotopy map in Figure 3.49 a surprising result emerges with the highes t-values for each frequency occupying slightly different positions in the frequency specific area. This may be only by chance or slight errors on the spacial normalization but similar microtonotopy has been shown in AC using electrophysiology [73] [5].

This experiment suffered from some logistical difficulties as many mice woke up midexperiment. This led to the use of more mice than expected (n=7) and to an unbalance of runs across mice with some mice having 6 times more runs than others which can introduce bias in the results. This also made impossible drawing individual SPMt maps for each mouse since the statistics were not powerful enough. For this reason, ROI analysis should be performed in further analysis by coregistering all animals to the same template so that variability across subjects is also covered and signal from ascending pathways and descending pathways is discerned.
4.2.4 Using different analysis

Different ways of performing the analysis of fMRI data could have been deployed. Recently, a growing number of papers have been proposing that using SPM is no longer a valid way to analyse this kind of data for small species namely due to the multiple comparisons problem. The solution that has been proposed to counteract these adversities has been the use of non parametric tests such as permutation tests. The analysis performed in this study was using SPM due to the fact that for the t-values reported and the number of voxels imaged, only 60 voxels would activate due to chance and our activated cluster had hundreds of them. This means that the multiple comparison problem was not actually a significant cofounder in our data. Furthermore, the use of non parametric tests requires the use of networks with computing power not available at the time of the analysis.
Conclusions and Future Work
This study focused on the creation and validation of an MRI-safe mouse auditory stimulation system. The system was built using open-source software and hardware (Python and Arduino) in conjunction with low-cost equipment (Yamaha Sound Board, Custom-made Amplifiers, Kemo Loudspeakers) and was shown to be very reliable up to 65 kHz. Some limitations of the system were found, namely its high total harmonic distortion for pure tones under 20 kHz and its instability in terms of SPL and future versions of the system should work to mitigate these limitations. The system was then used to perform two fMRI studies aiming at mapping the auditory pathway in mice and, more specifically, the tonotopic patterns in the mouse IC.

The auditory pathway of the mouse was mapped showing activation in the CNC, SOC, NLL, IC and AC. Only MG was not found to be significantly activated for the predicted activated structures. Subsequent ROI analysis showed activation of all these structures starting less than a second after the stimulation started and returning to baseline about 3s after the stimulation ended. The activation patterns, in conjunction with the ROI analysis allow for the conclusion that both the ascending and descending auditory pathways were observed, with future analysis focusing in modeling the the response times of each structure so that a chronological order can be established between structure activations.

The IC showed, as in previous studies, macroscopic tonotopy with very distinct regions activating for different frequencies at high significance levels (p<0.001) despite interference of the harmonic distortion of the sounds used and the FISP acoustic noise. Additionally, more fine and precise tonotopy was observed, with maximally activated voxels for frequencies between 35 and 39 kHz being shown (with spectral resolution of 1 kHz). This was possible due to the spatial resolution of 150 µm employed, together with the multiplicative factor in SNR provided by the use of a Cryoprobe. The present study shows BOLD activations upon auditory stimulation in the mouse auditory pathway. It also demonstrates the first in vivo tonotopic mapping in the mouse IC using pure tones. These in vivo fMRI findings agree well with the previous electrophysiology and immunohistochemistry findings, indicating the feasibility of auditory fMRI in mouse models.
Bibliography


Figure A.1: Frequency profiles of the pure tones used in the Low Frequency Experiment - 5kHz (Up), 12kHz (Center), 20kHz (Down)
<table>
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**Table A.1:** Output of the electrical signal from the Yamaha Board

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**Table A.2:** Output of speaker at the level of the mice ear - measured at the tip of the semi-rigid tube
Figure A.2: Frequency profiles of the pure tones used in the High Frequency Experiment - 35kHz, 36kHz, 37kHz, 38kHz, 39kHz.
Figure A.3: Frequency profile of the sequence used for functional imaging