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Investigating the Role of Auditory Cortex in Decisions about Sound Lateralisation

Ana Mafalda Oliveira Santos Lourenço Valente

Dissertation presented to obtain the **Ph.D degree in Neuroscience**

Oeiras, April, 2025

INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA ANTÓNIO XAVIER | UNIVERSIDADE NOVA DE LISBOA

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To my grandmother,
who wouldn't have approved, but would've still been proud.

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”

“I’ll read my books and I’ll drink my coffee and I’ll listen to music, and I’ll bolt my door.”

— J.D. Salinger

Abstract

Understanding how the auditory cortex (ACx) contributes to sound lateralisation and perceptual decision-making requires precise control over auditory stimulus delivery and the ability to deeply explore the task at hand. In this study, we developed and validated a novel set of miniature headphones, the Ratphones, designed to provide highly controlled auditory stimulation to freely moving rats. The Ratphones allow for independent sound delivery to each ear, enabling precise manipulation of interaural level differences (ILD), the primary cue for sound lateralization. By combining this technology with a well-controlled behavioural task, we investigated the behavioural correlates of auditory decision-making in rats.

Our results demonstrate that rats reliably use ILDs for sound lateralisation, and their behavioural performance obeys the Time-Intensity Equivalence in Discrimination (TIED) principle. This principle posits that increasing overall sound level accelerates decision-making without affecting accuracy, providing a strong mechanistic constraint for models of sensory evidence accumulation. Additionally, we examined the interplay between two distinct decision-making strategies present in our data: stimulus-driven (reactive) and anticipatory (proactive) choices. The latter occurred before sufficient sensory evidence was available, suggesting an interaction between internally driven and stimulus-guided decision processes.

Furthermore, through both ibotenic acid lesions and optogenetic manipulations of the auditory cortex, we provide evidence that challenges conventional understanding of cortical necessity in basic auditory decision-making tasks. While ACx lesions did not impair performance in our sound lateralisation task, optogenetic silencing led to marked declines in accuracy and alterations in reaction times, suggesting a nuanced role for the ACx in optimising auditory decision-making.

This work establishes a robust behavioural paradigm and technological framework for studying auditory perception and its neural underpinnings in freely moving rodents.

Keywords: Auditory Perception, Auditory Cortex, Decision Making, Proactive Process, Reaction Time

Resumo

Compreender como o córtex auditivo (ACx) contribui para a lateralização do som e para a tomada de decisões perceptivas exige um controlo preciso da entrega do estímulo auditivo, assim como a capacidade de explorar profundamente a tarefa comportamental em questão. Neste estudo, desenvolvemos e validámos auscultadores em miniatura, os Ratphones, concebidos para fornecer uma estimulação auditiva altamente controlada em ratos em livre movimento. Os Ratphones permitem a entrega independente de som a cada ouvido, possibilitando a manipulação precisa das diferenças de nível interaural (Interaural Level Differences, ILD), a principal pista utilizada para a lateralização do som. Ao combinar esta tecnologia com uma tarefa comportamental rigorosamente controlada, investigámos as correlações comportamentais da tomada de decisões auditivas em ratos.

Os nossos resultados demonstram que os ratos utilizam de forma fiável os ILDs para a lateralização do som e que o seu desempenho comportamental segue o princípio da Equivalência Tempo-Intensidade na Discriminação (Time-Intensity Equivalence in Discrimination, TIED). Este princípio postula que o aumento do nível geral do som acelera a tomada de decisão sem afetar a precisão, impondo uma forte restrição mecanicista aos modelos de acumulação de evidências sensoriais. Além disso, analisámos a interação entre duas estratégias distintas de tomada de decisão presentes nos nossos dados: escolhas reativas, guiadas pelo

estímulo, e escolhas antecipatórias (proativas), que ocorrem antes de evidências sensoriais suficientes estarem disponíveis, sugerindo uma interação entre processos de decisão internos e guiados pelo estímulo.

Adicionalmente, através de lesões com ácido iboténico e manipulações optogenéticas do córtex auditivo, apresentamos evidências que desafiam a visão convencional sobre a necessidade cortical em tarefas básicas de tomada de decisão auditiva. Enquanto as lesões no ACx não comprometeram o desempenho na nossa tarefa de lateralização do som, a inibição optogenética resultou numa queda significativa da precisão e em alterações nos tempos de reação, sugerindo um papel mais subtil do ACx na otimização da tomada de decisão auditiva.

Este trabalho estabelece um paradigma comportamental robusto e um enquadramento tecnológico para o estudo da perceção auditiva e dos seus fundamentos neurais em roedores em livre movimento.

Palavras-chave: Percepção auditiva, Córtex Auditivo, Tomada de Decisão, Processo Proactivo, Tempo de Reação

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Acronyms

A1	primary auditory cortex
AAF	anterior auditory field
ABL	Average Binaural Level
ACx	Auditory Cortex
CF	Characteristic Frequency
CN	cochlear nucleus
DDM	Drift Diffusion Model
DDMs	Drift Diffusion Models
DV	Decision Variable
FN	frozen noise
FT	Fixation Time
HRTF	head-related transfer function
IC	Inferior Colliculus
ILD	Interaural level differences
ITD	Interaural time differences
ITI	Inter Trial Interval

JND Just Noticeable Difference

LL Lateral Lemniscus
LSO lateral superior olive

MGN Medial Geniculate Nucleus
MNTB Medial Nucleus of the Trapezoid Body

PAF posterior auditory field
PSIAM Parallel Sensory Integration and Action Model

RT Reaction Time
RTD Reaction Time Distribution
RTDs Reaction Time Distributions
RW reward

SAT Speed Accuracy Tradeoff
SC Superior Colliculus
SDmax Maximum Sampling Duration
SDs Sampling Durations
SDT Signal Detection Theory
SL Sound Level
SOC superior olivary complex
SPL Sound Pressure Level
SRAF suprarhinal auditory field

TIED Time-Intensity Equivalence in Discrimination

VAF ventral auditory field

WF Weber Fraction
WL Weber's Law

1

General Introduction

Our perception of the world shapes who we are – it allows us to interact with others and the rest of our environment, interpreting it. With the information we gather we can adapt our behaviour to enact changes on the world around us. This requires us to gather meaningful information, extracting it from complex and potentially ambiguous sensory inputs. And, importantly, these processes that underlie perception can be shaped by both the external environment and internal states.

With a fundamental goal of neuroscience being the explanation of behaviour and how it is generated in the brain, linking perception to decision-making allows the probing of the fundamental processes by which sensory evidence is selected, integrated, and interpreted in order to inform a decision; and, ultimately, how the brain turns external stimuli into sensory evidence into action.

1.1 Perceptual Decision-Making

Scenarios in which animals must interpret noisy sensory information from their environment, and make appropriate decisions that are translated into actions are very common in nature and often crucial for survival. A decision can be defined as the process that results in the commitment to a categorical preposition (Gold & Shadlen, 2007), and perceptual

decision-making is the process by which one makes use of sensory signals to make decisions based on the state of the external world. For instance, an animal must take an ambiguous sensory stimulus, identify it, and integrate it with prior knowledge in order to perform basic actions that are often essential for survival. Processes such as running away from predators, tending to the young, or finding food, all require the gathering of, and acting upon sensory information. The information gathered by the sensory system is, however, incomplete, leading to uncertainty in decisions. This differs from value based decisions, where the choice is between several options of uncertain value but with no sensory uncertainty. Therefore, how we perceive the world dictates how we relate to it, and together with what we have previously learned, can be combined into a decision on what action to take upon our environment, and, in the end, how to live. Taking this into consideration, it is clear why the nervous system allows us to so efficiently extract information from our surroundings and quickly convert it into the evidence we need to choose the most appropriate courses of action. It is also clear why it is so important to study the intricacies of perceptual decision-making, both at the level of behaviour and of its neural underpinnings. For this, a common framework employed by neuroscience is the sensory motor arc – subjects are asked to make decisions based on the sensory stimuli, inferring the state of the outside world, and respond through a motor action. Several features have been used for the sensory stimuli, such as the direction of moving dots (Newsome et al., 1989), frequency of a sound (Jaramillo & Zador, 2014) or its lateral location (Pardo-Vazquez et al., 2019).

For these behavioural tasks, the experimenter has a high degree of control over the conditions in which the decisions occur, minimising external factors that can affect the choices, and controlling the amount of evidence available.

For this reason, the study of perceptual decision making, and this particular work, focuses on binary choices (Laming, 1986) (yes/no, left/right), which are simple enough to study with precision, but whose mechanisms can be generalised to more complex choices.

1.1.1 Psychophysics

Perceptually guided decisions are affected by sensory uncertainty. This uncertainty means that even when confronted with the same stimulus several times, the subject might respond in different ways. The behaviour being stochastic in this way suggests the presence of noise at the level of the underlying neural processes that guide the decision, and reveals that the behaviour is not purely reactive to the stimulus, but there are other factors that influence choices as well. Psychophysics, the field of study that relates physical events to the sensation and perception they elicit (Laming, 1986), can help understand these latent causes for decisions to be made.

In psychophysical studies, subjects are usually presented with several stimuli of the same or different modalities, with different magnitudes of the studied feature. A commonly reported parameter is the proportion of reports to one of the two categories for each stimulus fraction, which can be well describe through a sigmoidal curve – the psychometric function. In this curve, the probability of reporting either response category changes with the evidence presented. This is especially clear for harder stimuli, with lower magnitude of the sensory evidence – at this point of the psychometric, the slope is a measure of sensitivity (Carandini & Churchland, 2013). In opposition, stimuli that provide stronger sensory evidence will be easier, not limited by the sensory uncertainty, and will not vary strongly. These correspond to the tails of the psychometric function if these differ from $0/1$, with this difference making up the lapse rate (Carandini & Churchland, 2013; Clark & Merfeld, 2021). In the context of the psychometric function, the lapse rate refers to the probability of errors that occur independently of the stimulus itself, leading to incorrect responses even at the more extreme stimulus magnitudes (Clark & Merfeld, 2021; Pisupati et al., 2019). This is an important consideration when analysing perceptual tasks, as ignoring these parameters might lead to misestimation of other psychometric parameters such as slope and threshold (Clark & Merfeld, 2021; Pisupati et al., 2019). These parameters account for the fact that the subject might make mistakes that

are stimulus independent, due to inattention or exploratory behaviour (Pisupati et al., 2019). These errors constitute one of the challenges faced in the study of psychophysics.

As a research field, psychophysics was pioneered by Fechner in the 1800s (Fechner, 1860), inspired by previous work by Weber (Weber, 1834), naming after him one of the earliest established regularities of psychophysics: Weber's Law (WL) (Fechner, 1860; Laming, 1986). Weber's Law states that when comparing two stimuli, the just noticeable difference will depend on the ratio between the two intensities and not on their overall magnitude. For instance, a subject should distinguish between a weight of 100 g and another of 110 g with the same reliability as they would distinguish between a weight of 1 Kg and one of 1.1 kg. WL has been studied for many decades now, in numerous discriminations and across different species and sensory modalities (Deco & Rolls, 2006; Fechner, 1860; Laming, 1986; Link, 1992; Simen et al., 2016; Teodorescu et al., 2016; Treisman, 1964), however, a mechanistic explanation for how it takes place has only recently been proposed (Pardo-Vazquez et al., 2019), by studying both performance in the task and its temporal dynamics together.

Through appropriate task design, researchers can assess the effect of internal and external factors on behaviour through analysis of the changes in the psychometric curve (Carandini & Churchland, 2013). One can also condition the analysis on several parameters to ascertain sequential dependencies or biases on behaviour. But more than assessing the psychometric and focusing on performance, one can also focus on other parameters of behaviour, such as its temporal dynamics.

The Mechanistic Foundation of Weber's Law

As previously mentioned, Weber's Law is a fundamental principle of sensory perception which states that when comparing two stimuli, the Just Noticeable Difference (JND) will depend on the ratio between the two intensities and not on their overall magnitude. Although this psychophysical law has been extensively studied, it was only recently that a

mechanistic explanation has been proposed (Pardo-Vazquez et al., 2019). In previous work, the focus had been mostly on the effect of WL on accuracy (Laming, 1986), even though explanations based on bounded accumulation of evidence (Link, 1992), or already relating WL and Reaction Time (RT) had previously been proposed (Simen et al., 2016; Teodorescu et al., 2016).

A key finding in understanding the mechanistic basis of Weber's law comes from the study by Pardo-Vazquez et al., 2019, where we notice that besides choice data being compatible with WL, there is a psychophysical regularity at the level of the reaction times (the period of time the subject chooses to experience the stimulus). This regularity, Time-Intensity Equivalence in Discrimination (TIED), describes how reaction times change as a function of absolute stimulus level – for a given intensity ratio, the reaction time distributions for different absolute levels of the sound were rescaled versions of each other. Thus, changing the absolute intensity of the stimulus is completely equivalent to changing the units of time of the discrimination. This conclusion enabled the mathematical specification of a model to describe the TIED. Four necessary conditions were identified: (1) a linear relationship between the variance and the mean of the sensory evidence, (2) a power-law relationship between physical stimulus intensity and its internal representation, (3) a constant decision bound, and (4) perfect accumulation of evidence (that is, no leak or intrinsic decay of the Decision Variable (DV) across time); and these enabled us to find the model that best describes the TIED.

These findings can pave the way for new research into decision-making and its underlying mechanisms, ultimately contributing to a deeper understanding of perceptual dynamics and decision-making processes. In particular, Pardo-Vazquez et al. focused on the auditory system, but showed the results extend to other modalities as well.

1.2 The Sensory Pathway

Indeed, several modalities are used in the study of perceptual decision-making and psychophysics, relying on the fact that the sensorimotor

pathway's components are common to the different sensory systems. The senses can be divided into chemical senses – olfaction and taste –, the somatosensory system – including, for instance, nociception, thermoreception, and itch –, vision, and audition (Squire et al., 2008). In both humans and other model organisms such as rodents, the sensory stimuli are first detected by sensory receptors and transmitted through the sensory system of its corresponding modality. These sensory systems also perform a series of common functions that translate external stimuli into neural signals (Squire et al., 2008, Chapter 23). At late stages in the sensory pathways, the target regions of the sensory signals are capable of making associations and comparisons with other signals, from past time points or different modalities, forming the basis of perception. This information is eventually reflected onto an intended response, usually a motor action, which will be initiated in the motor regions of the brain and eventually transmitted onto motor efferents, allowing it to reflect back on the external world (B. Katz, 1950; Squire et al., 2008). Although both the sensory and the motor portions of this pathway are essential for a perceptual decision-making task, this thesis is more focused on the sensory portion of the task, and thus the following will focus on the sensory pathways.

The function of each sensory system is to provide a representation of the state of the external world and the changes it suffers, so that our internal representation is constantly up-to-date. This is accomplished by an interplay of parallel ascending (stimulus driven) and descending (goal directed) mechanisms of the same pathway (Squire et al., 2008, Chapter 23). The ascending pathways start at the level of peripheral receptors and their information, a representation of the outside world, is transmitted to higher order sensory areas; and it is at the level of these higher order areas that the descending pathway starts. Descending mechanisms have an effect on the ascending ones, altering perception according to context, motivation, attention, etc (Malmierca, 2015). This contributes to the difference between sensing a stimulus – detecting an event – and perceiving a stimulus – interpreting the event in its full context. Perception may change, even the stimulus remains unchanged

(Squire et al., 2008).

Sensory receptors, the most peripheral cells of the sensory systems, respond to specific forms of energy and transduce it to neural signals. These are specific for each sensory modality, differing in type, number, density, location/position in the body, and, within a modality, the specific stimulus to which they are sensitive. Depending on this stimulus, they will also perform their main function, the transduction of external signals into neural signals, in different ways. Because their physical placement on the body is not random, the location of a receptor can provide information on the stimulus, just as its firing rate and temporal structure of their activity (Squire et al., 2008).

Although different nuclei are part of different pathways, after the transduction at the level of the peripheral receptors, the signals will make their way to one of the nuclei in the thalamus. Some modalities will decussate, although how complete is this decussation or where exactly it takes place depends on the modality and the animal. These pathways include multiple parallel ascending streams that allow for different types of processing of their signals that need to be combined again into a coherent percept, which might occur at the level of the cerebral cortex (Singer, 1994; Squire et al., 2008).

The thalamus relays information to a confined portion of the cerebral cortex, which might be composed of several neighbouring areas, providing them with information about the sensory periphery. These areas are referred to as primary sensory areas and the areas they, in turn, project to, are referred to as associative areas. Areas of the sensory cortex (as well as other areas in the cerebral cortex) are organized in 6 different layers (I through VI) – in particular, the middle layers, III and IV are the main site of termination for thalamic fibres (Squire et al., 2008). Other layers are responsible for projecting to the thalamus (layer VI); to subcortical structures such as the striatum, pons, and spinal cord (layer V); to other regions of cortex in the same hemisphere (layer II); and to the contralateral cortex (layer I) (Squire et al., 2008, Chapter 23). At this point, information collected within the context of a behavioural task will be transmitted to motor centres and eventually culminate in a motor

response.

1.2.1 Sensory Cortices

The primary sensory cortices, including the visual, auditory, and somatosensory cortices, constitute the final stage for their specific sensory pathways, where sensory information is relayed after passing through several subcortical structures. These cortices are thought to play a crucial role in the initial processing and interpretation of sensory information, and are responsible for the parsing of key stimulus features (Andermann et al., 2011; Bosking et al., 1997) and other contextual features, such as temporal expectation (Jaramillo & Zador, 2010; Rabinovich et al., 2022). Accumulating evidence suggests that these areas engage in multisensory integration, interacting with each other and subcortical structures to encode information regarding different sensory modalities and how they relate to the preferred one (Brosch et al., 2005; Laramée & Boire, 2015; Mazo et al., 2024; Werner-Reiss et al., 2003). For instance, visual cortex not only processes visual information originating from subcortical structures of the visual pathway, but also integrates inputs from the auditory system, contributing to a multidimensional representation of the external environment (Mazo et al., 2024). This integration is facilitated by cortico-cortical connections between primary sensory cortices, as demonstrated in both primates and rodents (Stehberg et al., 2014; Xu et al., 2022).

One key difference between rodents and primates lies in the relative importance of cortical versus subcortical pathways. Primates, like humans and monkeys, rely heavily on cortical signals to perceive the world. Damage to the primary sensory cortices in primates can lead to a significant loss of sensory awareness, even with intact subcortical structures. Rodents, on the other hand, exhibit greater reliance on subcortical pathways. This suggests that rodents may retain some level of sensory responsiveness even with cortical damage, as subcortical structures can compensate for the loss of cortical input. Furthermore, cortical layer IV is thicker in rodents (Charvet et al., 2015; Scala et al., 2019), while primates'

brains have layers III and V thicker than the remaining ones (Charvet et al., 2015). This suggests that rodent sensory cortex is primed for subcortical input, while primates favour intracortical and corticospinal connections.

Besides differences between species, there might also be important differences between the several sensory cortices even within the rat model organism. Lesions to visual cortex are known to disrupt even simple visual roles, such as visual acuity, pattern discrimination, orientation discrimination, motion detection and parallax, and running toward and orienting to both stationary and moving visual stimuli (Dean, 1981; Ellard et al., 1986; Glickfeld et al., 2013; Ingle et al., 1979; Palagina et al., 2017; Petruno et al., 2013; Rhoades & Chalupa, 1978). Deficits at the level of the visual cortex may also have an effect on subcortical structures (Gil et al., 2024; Glickfeld et al., 2013; Rhoades & Chalupa, 1978), showing the importance of visual cortex for even simple visual behaviours. Lesioning somatosensory cortex affects even simple texture discrimination (Finger & Simons, 1976; Park et al., 2020; Simons et al., 1975), but may not have an effect on detection (Ryan et al., 2022).

1.2.2 The Ascending Auditory Pathway

The work detailed in this thesis will focus on the auditory modality and processing of auditory stimuli for decision-making, so I will give a brief overview of these concepts, particularly the ascending auditory pathway and how it processes sound for source localisation. Sound refers to pressure waves generated by the vibration of air molecules and is the sensory stimulus underlying auditory perception (Purves et al., 2012).The auditory pathway is highly conserved throughout mammalian species (Holt et al., 2019; Schnupp, 2011; Wilde et al., 2022), sharing a fundamental organisation and main mechanisms. Besides, the auditory modality is very salient for these animals (Carandini & Churchland, 2013; Gleiss & Kayser, 2012; McIntosh & Gonzalez-Lima, 1998; Raposo et al., 2012), making both quite useful to the study of the decision-making process.

Sound is detected by mechano-receptors localised in the inner ear. Different features in the sound will correspond to distinct aspects of the resulting auditory perception – the amplitude of a sound wave will impact the perceived loudness and its periodicity will influence the perceived pitch of the sound (Schnupp, 2011). These pressure waves are guided by the outer ear into the inner ear, where they are transduced into neural signals. Pressure waves travelling through the inner ear reach the cochlea and there cause vibrations of the basilar membrane. These vibrations are then transformed into neural activity by the hair cells, the receptor cells of the auditory system (Purves et al., 2012). The basilar membrane, through differences in flexibility and width, can decompose sounds into their frequency components by having distinct locations for maximum vibrations for different frequencies, generating frequency-specific neural patterns in the hair cells (Purves et al., 2012; L. Squire et al., 2012). The hair cells release neurotransmitters that, in turn, activate neurons of the spiral ganglion, still in the inner ear, propagating the resulting activity to the central nervous system (Purves et al., 2012). These neurons receive input from very few hair cells, sometimes only one, so the frequency decomposition formed in the basilar membrane can be maintained. This continued frequency-specific organisation – tonotopy – represents a hallmark organising principle in the ascending auditory pathway, as neighbouring neurons, typically, have similar characteristic frequencies (L. Squire et al., 2012).

In the central nervous system, sound-triggered neural activity is propagated through the brainstem. The early stages of sound processing in the brain take place in the brainstem, at the cochlear nucleus (CN) and at the superior olivary complex (SOC). The CN is the first stage of auditory processing in the brain; it inherits the temporal and spectral characteristics of the sound from the cochlea and processes this information before relaying it further up the pathway. It can be divided into a dorsal portion, responsible for the processing of spectral cues, such as the ones generated by the pinnae of the ear (Malmierca, 2015; Shepherd & Grillner, 2018), and a ventral portion, responsible for encoding timing and intensity cues (Malmierca, 2015; Oertel, 1997), the precursors of the cues rodents will

use for sound source localisation.

From the CN, the information passes through the Medial Nucleus of the Trapezoid Body (MNTB) and then reaches the SOC. The SOC, comprised, in the rat, of four distinct nuclei and also a periolivary region (Malmierca, 2003, 2015), is the first structure in the auditory pathway where the information from both ears combine, making it a crucial structure for localising sound (Grothe & Pecka, 2014; Grothe et al., 2010; Malmierca, 2003, 2015). Of its sections, the medial superior olive is responsible for encoding signals related to timing differences of the sounds reaching both ears; and the lateral superior olive (LSO) responsible for encoding signals related to intensity differences between the sounds that reach both ears (Grothe et al., 2010; Malmierca, 2015; Schnupp, 2011; L. Squire et al., 2012).

These structures in the brainstem then relay the information to the midbrain, through the Lateral Lemniscus (LL) and converging at the Inferior Colliculus (IC) (L. Squire et al., 2012). The LL is a fibre bundle that connects the brainstem to the midbrain, converging in the IC (Malmierca, 2015; L. Squire et al., 2012).

The IC is a central hub for the processing of both binaural and monaural cues, integrating this information with input from lower and higher auditory structures, as well as other sensory inputs (Ito et al., 2020; Malmierca, 2015; Schnupp, 2011; Xiong et al., 2013). Neurons from the IC have receptive fields that are sensitive to particular spatial locations, with each IC being particularly responsive to sounds presented to the contralateral ear; and also communicate with each other, providing further binaural interactions (Malmierca, 2015; Schnupp, 2011). The central nucleus of the IC, with its tonotopic organization, projects to the Medial Geniculate Nucleus (MGN) of the thalamus. The thalamus serves as the final relay station of auditory processing before reaching the Auditory Cortex (ACx), transmitting precise frequency and timing information about sound to the ACx (Malmierca, 2003; L. Squire et al., 2012).

1.2.2.1 The Auditory Cortex – Divisions and role

The ACx corresponds to the final stage of the ascending auditory pathway, it receives input from MGN, and is located in the temporal cortex for most mammalian species, including the rat (Fig. 1.1 A.). Although its presence and location is highly conserved, this is the structure of the ascending auditory pathway that presents the most species-specific differences (Paxinos, 2014). These differences include the number of accepted areas, their relative position and arrangement in the cortex, the terminology used to refer to these areas, their cell density, the connections to and from the areas, and their tonotopy (Paxinos, 2014; Schnupp, 2011).

Several maps have been proposed for the rat auditory cortex, a consensus being difficult to reach as most of the research into auditory cortex comes from work done in cats and, contrary to these, rats are lissencephalic and thus some of the previously found anatomical landmarks can no longer be used, although some remain useful (Malmierca, 2015) (Fig. 1.1 B.). It is, however, consensual, that the ACx is composed of a "core" region of granular cortex that includes one or more ordered representations of the full tonotopic space of the audible frequencies, and a "belt" area surrounding it, where tonotopy is coarser.

Based on immunostaining techniques, the ACx has been divided into a primary auditory cortex, surrounded dorsally, caudally, and ventrally by a belt area, further divided into ventral and dorsal secondary auditory cortex (Fig. 1.1 C. and D.). Currently, one of the most commonly used maps for the ACx, and the one we followed for this work, is the one proposed by Polley and colleagues (Polley et al., 2006). They divided ACx into five different areas: A1; PAF; AAF; VAF; SRAF. This division was based on the spatial orientation of the tonotopic maps, spectral and intensity tuning characteristics, response thresholds and latencies and thalamic inputs (Paxinos, 2014).

Besides the organization into different cortical areas, auditory cortex shows, like other areas of the cortex, a layered neural architecture. This is an important feature of mammalian neocortex and the six layers that usually comprise the cortical structure are numbered according to depth.

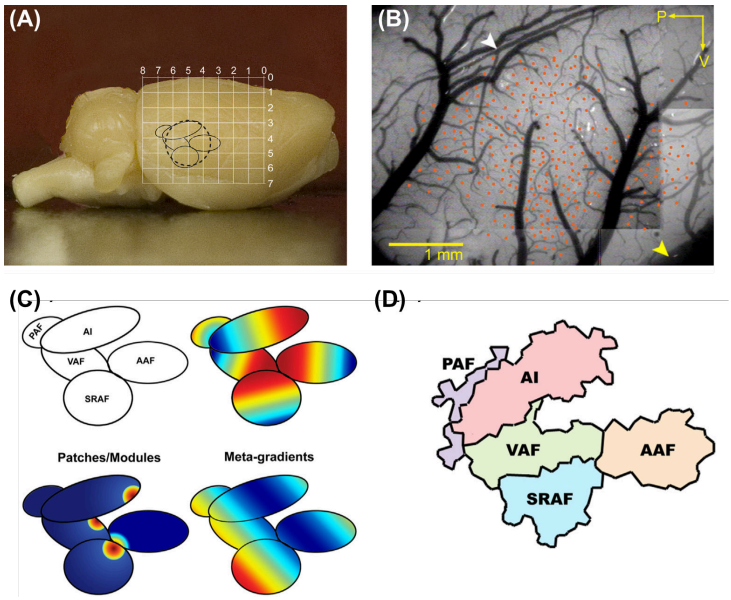

Figure 1.1: **Auditory Cortex Organization**: **(A)** 8 × 7-mm grid is overlayed onto a lateral view of the right hemisphere of the rat brain. Numeric values denote distance with respect to bregma (0, 0). Five, solid line black ellipses are superimposed onto the temporal cortex to represent the typical position and shape of the five cortical auditory fields described in Polley et al (Polley et al., 2006) detailed and illustrated in **(D)**. A circular dashed line region is also superimposed, delineating the area covered by a 3 mm cranial window. **(B)** Representation of microelectrodes used to map the cortex (orange dots) in reference to the surface vasculature of the right temporal cortex that may help localize ACx. Yellow arrowhead identifies the rhinal vein. White arrowhead identifies the middle cerebral artery. P and V indicate posterior and ventral, respectively. **(C)** Schematic diagram depicting three distinct representational schemes in the rat ACx. The relative position of primary auditory cortex (A1), ventral auditory field (VAF), posterior auditory field (PAF), suprarhinal auditory field (SRAF), and anterior auditory field (AAF) are shown schematically (top left). Dark lines forming each ellipse represent the functional boundary for each field.

Figure 1.1: (Continued caption) Rainbow gradient used in each section represents the spatial distribution of different response parameters that are organised into intrafield gradients (e.g., Characteristic Frequency (CF)), patches or modules (e.g., sharpness of tuning), or perifield "meta" gradients (e.g., response latency or monotonicity). **(D)** schematic drawing of the relative position of five tonotopically organised auditory cortical fields. Borders between fields were defined by reversals or shifts in the CF gradients. Adapted from (Malmierca, 2015).

The principal neurons of the cortex, pyramidal cells, make up about 80% of the neural population release the neurotransmitter glutamate and excite their postsynaptic targets (Harris & Mrsic-Flogel, 2013). While pyramidal cells have both local and distant targets, the remaining cortical neurons inhibit firing in their local postsynaptic targets through the release of the neurotransmitter GABA (Harris & Mrsic-Flogel, 2013). ACx has a thickness of about 1.1 to 1.2 mm (with SRAF corresponding to the thinnest part of the cortex) (Malmierca, 2003; Paxinos, 2014) and includes:

- **Layer I** is the more superficial layer and is about 140μm thick, with few neurons.
- **Layer II** is comprised of densely packed small neurons and about 125μm thick.
- **Layer III** is comprised by both pyramidal and non-pyramidal neurons, and approximately 190μm thick.
- **Layer IV** contains smaller, more densely packed neurons, when compared with layer III and its more common cell are described as small stellate, with pyramidal cells the most abundant excitatory cell type.
- **Layer V** is the thickest layer, comprising about 270μm of the cortex and localised about midway through the total cortical thickness. Again the main cell type is the pyramidal cell, and cell density is lower than in layer IV, their size and number increase with depth.

- **Layer VI** contains closely packed pyramidal and non-pyramidal cells and represents about 245μm thick.

The most superficial layer, layer I, is very sparsely populated with neuronal cell bodies and mainly receives input through projections from higher order cortical areas, and forms connections with layers II/III and layer V (Harris & Mrsic-Flogel, 2013). Thalamic projections target layers III and IV (Barbour & Callaway, 2008; P. H. Smith et al., 2012; Winer, 1984; Winer & Schreiner, 2010), although some projections have also been observed in the border of cortical layers V and VI (Harris & Mrsic-Flogel, 2013; Sakata & Harris, 2009). Layer IV then provides excitatory input to layers II/III pyramidal neurons, especially the more superficial pyramidal cells, and also recurrently to itself, while projecting to all other layers (Harris & Mrsic-Flogel, 2013; Sakata & Harris, 2009). Layers II/III provide local excitatory input, to the deep layers of the cortex, layers V and VI, and connect with higher order cortical areas (Harris & Mrsic-Flogel, 2013; Sakata & Harris, 2009). Layer V can be divided into two sub layers, one that projects into the striatum, other cortical areas or layers II/III (Harris & Mrsic-Flogel, 2013), and also provides excitatory input to layer IV, specifically to the star pyramidal neurons on this layer (Barbour & Callaway, 2008). The second sub layer targets the ipsilateral striatum, as well as the thalamus and other subcortical areas (Harris & Mrsic-Flogel, 2013; Sherman, 2012). Neurons in layer VI receive mostly descending projections from higher order cortical areas or thalamus, while in turn projecting to the thalamus or layers I and VI of other cortical areas (Harris & Mrsic-Flogel, 2013; Sakata & Harris, 2009).

The intricate organization of cortical layers, each with specific connectivity patterns and inputs, underpins a wide range of sensory processing tasks, including auditory perception. This laminar structure allows the integration of cortical and subcortical input to enable complex auditory functions (Steinfeld et al., 2024). In rats, as in other rodents, the ACx plays a crucial role in sound processing and localisation. Both A1 and other areas of ACx are tonotopically organised, allowing for the differentiation between the different frequencies (Malmierca, 2003, 2015). While A1

receives direct input from subcortical structures such as the thalamus (Malmierca, 2003, 2015), secondary auditory regions receive input from A1 and contribute to more complex auditory processing, such as the processing of more complex sounds, like speech and music for humans.

Overall, the auditory cortex serves as a central hub for processing spatial auditory information, enabling organisms to effectively interpret and respond to their acoustic surroundings. One of the most critical spatial auditory tasks is sound source localisation, a function that depends on the precise encoding and integration of spatial cues. This is a very well characterised function of the auditory pathway and easy to manipulate, making it a very interesting alternative to be used in the study of perceptual decision-making.

1.3 Sound source Localisation

The ability to localise the origin of a sound is a crucial auditory function for many species. Being able to pinpoint the source of a sound is often critical for survival in nature, be it to localise predators and other dangers, prey, food, or water. Sound localisation may be along the vertical direction – referring to elevation –, or along the horizontal plane – referring to azimuth (Grothe et al., 2010; Koka et al., 2008).

1.3.1 Mechanisms of Sound Localisation in the rat

The auditory systems of mammals have a lot in common, both in terms of structures and computations. This is very apparent for mechanisms of sound source localisation as they are highly conserved throughout different species. However, the preferred mechanism for the localisation of a sound source will differ from species to species and will depend on body shape, position relative to the sound source, and on the frequency of the sound being located.

The size, shape and posture of the whole body are relevant for sound source localisation, originating differences in the time the sound travels, in the magnitude of the sound, and in the phase with which it reaches the

ears. Depending on the frequency of a sound, the head of the animal may reflect or diffract it while it travels, making the sound that reaches one ear different from the sound that reaches the other (Grothe & Pecka, 2014; Grothe et al., 2010). While humans are tuned for low frequency sounds (20 Hz to 20 kHz) (Purves et al., 2012; Squire et al., 2008), favouring speech and spatial hearing; rodents, however, are able to detect a wide range of frequencies, including ultrasonic frequencies for social interaction, with rats being able to detect sound ranging from about 200 Hz to over 80 kHz (Kelly & Masterton, 1977) and showing particular sensitivity for sounds in the 6 kHz to 48 kHz range (Escabi et al., 2019; Heffner & Heffner, 2007; Kelly & Masterton, 1977). This is important for sound localisation in rats, as the size of their head is of the same order of magnitude as the frequencies they are most sensitive to, originating a shadow effect for sound (Koka et al., 2008). This greatly influences how rats localise sound and which mechanisms are used.

Rodents may use monaural or binaural cues in order to locate a sound source. Monaural cues are pertaining to information from a single ear and are essential for elevation assessment, or localisation in the vertical plane, and for situations where binaural cues are limited, for instance when the source is directly in front of the rat (Grothe & Pecka, 2014). One of the main monaural cues a rat may use is the spectral shape of the sound. As the sound travels through the animal's surroundings and it passes through the animal's pinnae and ear canal in order to reach the inner ear, the spectrum of the sound suffers alterations that are dependent on its spatial origin in relation to the ear and the shape of the pinnae. Different shapes of the pinnae and ear canal can create a specific pattern of effects (attenuation, amplification and cancellation) on the sound – head-related transfer function (HRTF). And these will be modulated by different locations for the sound source, especially different elevations in relation to the animal, creating specific spectral patterns for the sound (Blauert, 1997; Grothe & Pecka, 2014).

These cues, while very useful (Grothe & Pecka, 2014; Ito et al., 2020), are not used in isolation, and the rat also makes use of binaural cues, that depend on frequency and head features (Erulkar, 1972; Grothe &

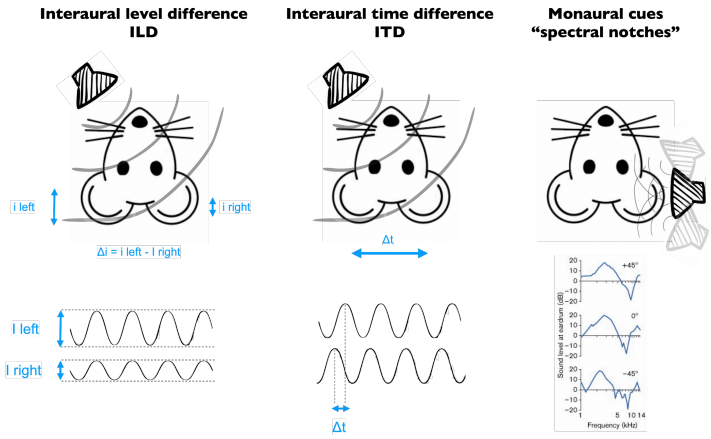

Figure 1.2: **Sound localisation strategies** Illustration of the path of sound waves when encountering the head and the strategy enabled for each example. In the case of Interaural level differences (ILD)s – first column –, the sound is more intense when reaching the ear ipsilateral to the source (intensity denoted by "i") due to the shadow effect of the head. As noted before, there is an extra length of path for the sound to reach one of the ears, accounting for the Interaural time differences (ITD) – second column –, where the sound reaches the ear farthest from the sound source some time later than it reached the ear ipsilateral to the source. Monaural cues or spectral cues – third column – depend very much on the position of the source relative to the body and even the posture of the ear at the time of localisation, as all these changes after the spectrum (third column, bottom) *Illustration by: Frederico Severo*

Pecka, 2014) especially for sound azimuth localisation (Koka et al., 2008; Kyweriga et al., 2014; Wesolek et al., 2010). The two ears are necessary for these computations because the sensory information is tonotopically and not topographically organized (Grothe et al., 2010; Koka et al., 2008).These binaural cues are ITD or ILD for sound source localisation.

Interaural time differences (ITD): These refer to the differences in the time of arrival of sound waves to the two ears. When a sound is emitted from a source located at a certain angle in relation to the animal, the source of the sound is not at the same distance from both ears, and

the corresponding sound waves will reach the closest ear to the source first (Fig. 1.2). The maximal ITD will correspond to 90 degrees to either right or left.
ITDs are small differences, usually less than 1 millisecond, and can be detected by the auditory system and used as spatial information (Grothe & Pecka, 2014; Grothe et al., 2010; Schnupp, 2011). However, because rats have relatively small heads and focus on relatively high frequencies, these differences are – although still detectable – small, and not the main mechanism used (Grothe & Pecka, 2014; Koka et al., 2008; Rayleigh, 1907). Even still, low-frequency sounds, with large enough wavelengths, produce phase differences that can be detected by several structures along the auditory pathway (Schnupp, 2011).

Interaural level differences (ILD): These refer to the differences in sound pressure level of the waves that reach each of the ears. For instance, if the sound source is located 90 degrees to one side on the sagittal plane, the sound that reaches the contralateral ear has been attenuated by the head. This occurs because the width of a rat's head is larger than the wavelength of the sounds in the preferred hearing range of the rat, which is especially relevant above 5kHz (Grothe & Pecka, 2014; Koka et al., 2008; Rayleigh, 1907). Thus, the head acts as a physical barrier, creating a "shadow" (Fig. 1.2). Due to this shadow, the sound reaches the farthest ear with lower intensity than sensed from the proximal ear. This level difference between the two ears is what the auditory system interprets as spatial information. Evolutionarily, ILDs seem to be the oldest form of sound localisation for mammals (Grothe & Pecka, 2014; Rayleigh, 1907). More recent mammals, such as rats and mice, have even expanded their hearing range into higher frequencies, when compared to their ancestors, allowing them to fully explore the ILD range. While other mammals, such as humans, have instead expanded to use ITDs.

Rats may be able to use of all types of cues for sound source localisation (Schnupp, 2011), depending on the frequency of the sound, but they show a clear preference for ILDs (Grothe & Pecka, 2014; Koka et al., 2008; Wesolek et al., 2010), and so, in this work, we will be focusing on ILDs.

The neural coding of ILDs occurs at multiple levels along the auditory pathway, including the brainstem, midbrain, and cortex (Grothe & Pecka, 2014; Malmierca, 2015). ILD signals are particularly noticeable on the IC, which integrates input from several brainstem nuclei besides the LSO and, through a push-pull mechanism, is also capable of encoding ILDs (McAlpine et al., 2001; Severo et al., 2024). The IC then transmits information to the Superior Colliculus (SC) (Hu & Dan, 2022; Schnupp, 2011), motor centres and, within the auditory pathway, to the MGN, the last relay station before the ACx. In the ACx, neurons represent ILDs as broad tuning curves with maximum slopes at ILDs close to zero – corresponding to sounds arriving from the front of the animal (Campbell et al., 2006; Kobak et al., 2019; Stecker et al., 2005; Yao et al., 2013).

The representation of ILDs across multiple auditory structures — from the brainstem to the auditory cortex — illustrates how sensory information is progressively transformed and integrated turning ILDs into very attractive stimuli, potentiating the study of the decision arc at several stages. However, encoding these spatial cues alone does not determine behaviour, and it is important to study the computational principles that govern the transformation of percept to behaviour.

1.4 The Study of Auditory Decision-Making

Auditory stimuli may be used to specifically probe the auditory system or as a highly controllable stimulus for the study of universal decision-making mechanisms. Regardless, the study of auditory decision-making in laboratory settings, particularly using rodent models, has significantly advanced our understanding of how sensory information is processed and transformed into behavioural responses.

Studying decision-making employing auditory stimuli offers several unique benefits compared to other stimulus modalities. Auditory stimuli are easily manipulated and statistically well-defined. This precise control allows researchers to systematically vary auditory cues and measure their impact on decision-making processes, providing insights into

how different acoustic parameters influence choices. Furthermore, auditory stimuli can be presented sequentially, offering a useful temporal dimension, particularly useful for studying how the brain integrates information over time.

Several task paradigms have been employed with auditory stimuli, such as Go-No Go tasks (Bagur et al., 2018; Noda & Takahashi, 2019; Yu et al., 2021), or two alternative forced choice (2AFC) (Brunton et al., 2013; Hermoso-Mendizabal et al., 2020; Jaramillo & Zador, 2010; Sanders & Kepecs, 2012; Steinfeld et al., 2024). The 2AFC paradigm is particularly useful as it allows to disentangle between errors and disengagement, and working with rodents allows for the simultaneous focus on computational, electrophysiological, and imaging techniques to explore how sounds are represented and how these representations influence actions. Such tasks allow for the examination of how auditory stimuli are integrated with cognitive factors such as attention and motivation, providing insights into the neural mechanisms that govern perceptual decision-making. In these auditory decision-making tasks, rodents exhibit a complex range of behavioural strategies that reflect their processing of auditory information. The speed and accuracy of decisions can be influenced by the duration and intensity of auditory stimuli.

Overall, the exploration of auditory decision-making in rodents not only enhances our understanding of sensory processing but also provides a framework for investigating the neural basis of decision-making across modalities. By examining how various factors such as stimulus duration and intensity affect behaviour, researchers can elucidate the underlying mechanisms that govern perceptual choices. This line of inquiry is crucial for developing a comprehensive model of decision-making that encompasses both sensory and cognitive dimensions, paving the way for future studies aimed at unravelling the intricacies of auditory perception and its implications for broader cognitive functions.

1.4.1 Brain Manipulations

In order to ascertain whether a particular brain region plays a role in a certain process, researchers have developed and employed several techniques that allow for the direct manipulation of neural activity. Manipulating the activity of an area is an important tool in testing its functional role in perceptual and cognitive tasks, especially when used in conjunction with correlational studies, such as neural recordings. In particular, inactivating brain areas allows testing for their causal involvement in behavioural and cognitive tasks. There are several possible inactivation methods that differ in terms of mechanism, pervasiveness, and outcomes, all of these affecting the temporal scale of the inactivation.

During this work, we aimed at manipulating the auditory cortex in order to ascertain its function during our behavioural task, and we employed two distinct methods for this purpose.

1.4.1.1 Lesions

Lesions studies have been a cornerstone of neuroscience and have been credited as major drivers of cognitive neuroscience, especially for the study of the human brain (Adolphs, 2016). Lesions are irreversible inactivations of the brain, in which the targeted region is permanently damaged or even fully removed (Slonina et al., 2022). They offer a unique advantage over correlational methods like electrophysiology, as they allow for the confirmation of direct causal relations of necessity between brain regions and their associated functions (Adolphs, 2016). Additionally, lesion studies can provide insights into the recovery mechanisms of the brain, as following a lesion the brain tries to adapt in order to compensate for the lost area and function through plasticity mechanisms (Depner et al., 2014; Lomber, 1999). In this sense, the lesion of a particular area is informative about the essential contribution of that area to the task at hand, the contribution that cannot be taken over by other brain areas through plasticity mechanisms. However, lesions have important limitations that complicate the interpretation of their results, as it is difficult to have them be fully confined to the area in study, so they might affect

it in an incomplete manner or affect neighbouring areas; lesions might also affect connections between areas (Koo et al., 2004); and even the previously mentioned plasticity might have unforeseen effects(Depner et al., 2014; Pai et al., 2011). Finally, even if a brain area does not play an essential, irreplaceable role in a specific behaviour, it might still, under normal conditions, be necessary for performing this behaviour. This degree of normal involvement cannot be inferred from a lesion. In this work we made use of excitotoxic lesions to study the necessity of the auditory cortex for the process of sound source lateralisation. As we will show, our results suggest that ACx might not be essential for sound lateralisation, but it appears to nevertheless be playing a role for perfomance in a sound lateralisation task.

1.4.1.2 Optogenetics

Optogenetics refers to the combination of genetic and optical methods for the manipulation of cell function. By introducing light-sensitive proteins into specific neurons through genetic promoters, one can control neuronal activity with precise light pulses. This allows for the activation or inhibition of targeted cells in real-time, enabling detailed studies of neural circuits and their roles in behaviour and cognition (Deisseroth, 2011). These manipulations may be used to potentiate or suppress cell activity and allow for the manipulation of specific cells within living tissue with millisecond precision, while the remaining cell populations are left largely undisturbed (Deisseroth, 2011; Fenno et al., 2011).

Optogenetics allows for cell type specificity, either through genetic promoters included in the viral vectors used to enable light-sensitivity on cells, or through the transgenic line of animals used (Fenno et al., 2011; Zhang et al., 2010). It also allows for the manipulation to occur at very specific moments, which enables the study of decision-making dynamics is a more accurate manner as with optogenetics one is able to test not only if a brain region is involved in a task or behaviour, but in what specific moment this involvement takes place (Wiegert et al., 2017).

This technique, however, is not without its pitfalls. Expression is a lot

more controllable in the mouse model, as the genetic toolbox that had been developed for mice is still unparalleled (Fenno et al., 2011; Zeng & Madisen, 2012; Zhang et al., 2010). Opsin expression might also not be necessarily completely specific to one cell type, with off-target effects a possible occurrence (Mahn et al., 2018). It is also possible that shining light on the brain contributes to an increase in temperature of the tissues, which in itself might cause a change in neural activity (Monteiro et al., 2023) both to the intended cell population and neighbouring ones (Fenno et al., 2011).

In addition to purely technical issues, a more subtle, but arguably more important problem, is to how to infer the function of a brain area from the effects of its perturbation in a behavioural task. Our work in this Thesis analysing the effect of optogenetic manipulations of the rat auditory cortex offers a good example of the difficulty of this problem, as well as of the way in which a strong theoretical grounding of the behaviour can be critical for elucidating the precise functional alterations associated to a given optogenetic perturbation.

1.5 Theories of Decision Making

How do neural systems convert sensory representations into categorical choices? What computational principles govern this transformation? Understanding decision-making models provides a framework for addressing these questions by describing the mechanisms through which sensory evidence is accumulated, a decision is reached, and acted upon. Different theoretical frameworks have been applied to attempt to explain different tasks and behaviours, and we will briefly mention some.

1.5.1 Signal Detection Theory

Signal Detection Theory (SDT) is a foundational framework in the study of perceptual decision-making. Established in the 50's (Green, Swets, et al., 1966), it provides a quantitative model for how decisions arise under conditions of uncertainty. SDT posits that when detecting the presence

of a stimulus, there are two underlying probability distributions – one corresponding to the stimulus, the signal to be detected; and another corresponding to the background noise –. In order to decide, the subject must rely on a DV whose value is sampled from one of the distributions, the same principle also being applicable to discrimination between two stimuli (Meyniel et al., 2015). A decision criterion (c) separates the two possible responses and the discriminability between these is quantified by the d' (d prime), a measure of the signal's detectability relative to noise. Uncertainty in this model comes from the overlap between the two distributions, meaning that some decisions will be made under perceptual uncertainty, and similar DVs can originate different responses. The criterion is dependent on many factors, such as engagement, motivation, and other internal states, but may also reflect task demands and it allows the subject to regulate the tradeoff between responsiveness and accuracy (Green, Swets, et al., 1966; Meyniel et al., 2015).

SDT remains a powerful tool for describing the static relationship between stimuli, noise, and decisions. But not all stimuli are static and some tasks rely on sequential sampling of the available information over time (Bogacz et al., 2006; Forstmann et al., 2016)

1.5.2 Sequential Sampling

Temporal dynamics of perceptual decisions are meaningful because sensory information is not static, it arrives to the subject over time. This means perceptual tasks have important temporal dynamics. Thus, sequential sampling models have emerged as a powerful framework for understanding the cognitive processes underlying perceptual decision-making. These models take into account that individuals accumulate noisy sensory evidence over time until a decision threshold is reached, triggering a response (Bogacz et al., 2006; Forstmann et al., 2016; Ratcliff & McKoon, 2008). This process explains the well-established speed-accuracy tradeoff observed in perceptual tasks, where faster decisions tend to be less accurate and vice versa. A classic example of sequential

sampling in perceptual decision-making is seen in the random dot motion task (Britten et al., 1992, 1993; Forstmann et al., 2016; Kiani, Corthell, & Shadlen, 2014; Newsome & Pare, 1988; Roitman & Shadlen, 2002; Shadlen & Newsome, 2001), where subjects must determine the overall direction of motion in a display of moving dots.

1.5.3 Drift Diffusion models

A prominent sequential sampling model is the Drift Diffusion Model (DDM). The DDM has been particularly successful in accounting for both choice probabilities and response times in such tasks (Bogacz et al., 2006; Forstmann et al., 2016; Ratcliff & McKoon, 2008), making it a useful tool for the study of decision-making.

The DDM can reproduce qualitative features of behaviour, such as right skewed Reaction Time Distributions (RTDs); the speed accuracy tradeoff for fixed bound– higher values of drift allow for faster and more accurate choices as the DV hits the correct bound more often; emphasis on speed vs accuracy – increasing the bound separation leads to slower and more accurate choices (Ratcliff & McKoon, 2008). Certain parameters can also be changed or added to better recapitulate behaviour, for instance, the bounds may be made to collapse over time, or the integration may be leaky – evidence is lost during integration (Ratcliff et al., 2016).

By linking these model parameters to neural activity, it is also possible to study the relationship between neural function and perceptual decision-making. DDMs have been shown to provide a quantitative framework for understanding how the brain transforms sensory input into decisions and actions through their application to several behavioural tasks (Brunton et al., 2013; Nikbakht et al., 2018; Pardo-Vazquez et al., 2019; Roitman & Shadlen, 2002; Uchida & Mainen, 2003).

In this Thesis, we pushed the state of the art in terms of the expressivity of sequential sampling models to describe behaviour in perceptual decision-making tasks. Using high-quality psychophysical data from rats, we use this theoretical framework to uncover novel rules for decision commitment when the duration of sensory stimuli is not under the

control of the subject, and we also made precise inferences on the functional consequences of optogenetic perturbations of the auditory cortex in terms of the computational processes underlaying this behaviour.

2

Ratphones: An Affordable Tool for Highly Controlled Sound Presentation in Freely Moving Rats

2.1 Summary

Encoding and processing sensory information is key to understanding the environment and to guiding behaviour accordingly. Characterizing the behavioural and neural correlates of these processes requires the experimenter to have a high degree of control over stimuli presentation. For auditory stimulation in animals with relatively large heads, this can be accomplished by using headphones. However, it has proven more challenging in smaller species, such as rats and mice, and has been only partially solved using closed-field speakers in anaesthetized or head-restrained preparations. To overcome the limitations of such preparations and to deliver sound with high precision to freely moving animals, we have developed a set of miniature headphones for rats. The headphones consist of a small, skull-implantable base attached with magnets to a fully adjustable structure that holds the speakers and keeps them in the same position with respect to the ears.

2.2 Introduction

Processing sensory inputs is crucial for understanding the environment and adjusting behaviour accordingly. In addition to psychophysics, in which sensory evidence is critical, sensory stimuli are used in many paradigms within behavioural neuroscience. For these experiments to be valid and reliable, it is key to ensure a high degree of control over the physical properties of the stimulation that reaches the sensory organs, so that they can be accurately replicated. Moreover, precise stimulus control is critical for the experimenter to be able to interpret neural variability and its relationship with behaviour; only if one can accurately repeat the same stimulus, can a distinction between internal and external neural variability be made.

In visual experiments, this requirement has been fulfilled mostly by using eye-tracking systems in head-fixed (or head-restrained) subjects (Britten et al., 1993), but also by using head-mounted eye-trackers (Cognolato et al., 2018). Recently, a magnetic eye-tracking system that can be used in both head-fixed and freely moving mice has been developed (Payne & Raymond, 2017). For olfactory and tactile stimulation, researchers have developed high-precision devices for this purpose (Kepecs et al., 2006; Romo et al., 2002). In the auditory modality, there have been two main strategies. On the one hand, in species with relatively large heads, such as monkeys (Fishman & Steinschneider, 2009; Schroeder et al., 2001) or ferrets (Keating et al., 2013; Nodal, Keating, & King, 2010), sound presentation can be controlled by using headphones. On the other hand, for smaller animals, such as rats and mice, the sound can be reliably delivered by using head-fixed (Joachimsthaler et al., 2014) or anaesthetised (Yao et al., 2013) preparations. However, to our knowledge, delivering sound under strictly controlled conditions to freely moving rodents is a challenge that has been only partially solved so far in rats by chronically implanting a plastic structure into which the speakers were screwed before each behavioural session (Otazu et al., 2009).

We have designed the Ratphones, an affordable set of miniature headphones that consists of a small, skull-implantable base attached

with magnets to a fully adjustable structure that holds the speakers and keeps them in the same position with respect to the ears. With the Ratphones, the experimenter only needs to implant a small base, which is less disruptive for the animals than implanting the whole structure except for the speakers, and the headphones are attached to this base with magnets, thus avoiding the need to screw (and unscrew) the speakers before (and after) every behavioural session.

These headphones allow the experimenter to control independently the sound delivered to the two ears, which is especially important for studying sound localization, where the Interaural level differences (ILD) of the sound is the main cue used by the auditory system to extract azimuth in the horizontal plane (Wesolek et al., 2010). The speakers we chose are adequate for high frequencies (up to 40 kHz) and can deliver pure tones and narrowband noise. Thus, the Ratphones can be used in most experiments requiring highly controlled sound presentation. However, the speakers are limited in terms of sound intensity [maximum, 79 dB Sound Pressure Level (SPL) (measured in 0.1 m distance)] and may not be the best option for experiments demanding very high sound intensities.

2.3 Design Requirements

The main functional requirements behind this design were to have a precise, reliable, and robust relative positioning between the speaker and the pinnae, while at the same time allowing flexibility to adjust this positioning to the variations between base positioning and pinnae location on each individual animal. To achieve this, (1) the design contains movable pieces that can be adjusted on the anteroposterior, mediolateral, and dorsoventral axes; and (2) the procedure to configure the Ratphones consists of a first step in which the base is implanted, and a second step in which the pieces are adjusted for each individual animal under anaesthesia and glued in their final configuration for each rat.

Another critical design requirement for the Ratphones was to use them in behaving animals, as opposed to anaesthetised or head-fixed

preparations. Mostly because of this requirement, we decided to use external headphones instead of placing them in the ear. In-ear headphones in principle afford a higher degree of control, as sounds not coming from the speakers are blocked. They also allow pure monaural stimulation (i.e., one is sure that each ear only hears the sound from its corresponding speaker), which, as previously mentioned, is important for controlling ILD. They have, however, the important drawback that they are much more invasive and uncomfortable for the subject, which, especially in a behavioural context where one has to fit them on every behavioural session, is critical. If the animal starts every session stressed and uncomfortable, it will interfere with the behavioural readouts of the sensory measurements the experimenter is trying to perform. A second important drawback for internal headphones, based on human subjective experience, is the sensitivity of this configuration to slight adjustments in the positioning of the earphone (and supporting sound-isolating material) relative to the inner pinnae. A bad seal can completely compromise accurate sound delivery, and the rats cannot report on a bad seal. For all these reasons, we decided to use an external design with close placement. Regarding the downsides, we tested explicitly that monaural contamination is small compared with behavioural ILD sensitivity, but we would in general recommend performing experiments in a sound-isolation box, where the possibility of interference because of a lack of the seal provided by an internal design is minimised.

In this work, we provide all necessary information for building, adjusting, and using the Ratphones to reliably present auditory stimulation to freely moving rats. Empirical data obtained with the Ratphones can be found in the study by Pardo-Vazquez et al. (2019) (Pardo-Vazquez et al., 2019).

2.4 Methods

The Ratphones consist of a small, chronically implantable base and a set of movable parts (Fig. 2.1 A, B) that can be put together to form a structure that holds the headphones in the desired position with respect

to the ears (Fig. 2.1 C,D). The structure is attached to the base using magnets. All parts, except for the magnets and speakers (Table 2.1), can be 3D printed using the stereolithography files (stls) we provide in https://github.com/JosePardoVazquez/RatHeadphones.

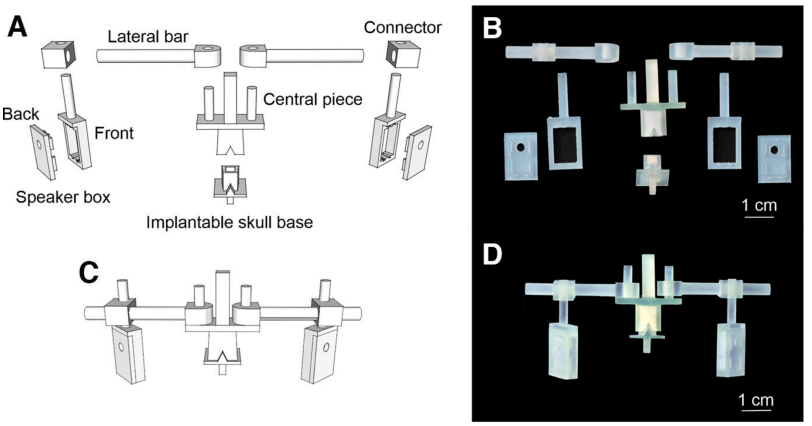

Figure 2.1: **Ratphones 3D design and 3D-printed resin pieces. A**, Set of 3D-printable parts. **B**, Set of resin-printed parts. **C, D,** Front view of all the parts assembled to form the final structure that holds the speakers, in the 3D model and printed, respectively.

These parts have been designed to be printed using stereolithography (Fig. 2.1 B,D), but can be easily adapted to other 3D-printing methods. In its current form, the speaker box is designed to be used with a specific receiver model (Table 2.1), but it can be easily redesigned to fit other models without modifying the other parts. Below, we describe the procedure we used for implanting the base and adjusting the headphones. All procedures were reviewed and approved by the animal welfare committee of the Champalimaud Centre for the Unknown and approved by the Portuguese Direcção Geral de Veterinária (reference no. 0421/000/000/2019).

Base Implant Surgery

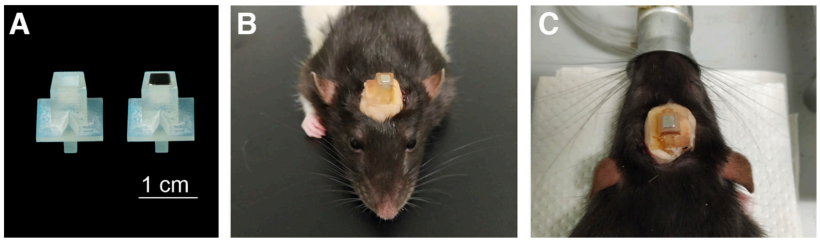

Figure 2.2: **Base and Implanted base. A,** Skull base 3D printed in resin, without and with the magnet in place. The base is chronically implanted to the skull of the animals with this strong miniature magnet which both holds the speakers in place during task performance and also allows easy attachment/detachment. **B, C,** Front and rear view of a skull base chronically implanted.

Anaesthesia was induced by inhalation of isoflurane at a concentration of 5% (oxygen at 2 L per min) and maintained by an injection of ketamine/xylazine (0.1 ml/100 g, i.p.). More isoflurane was occasionally administered for longer surgeries if the animal exhibited signals of pain or discomfort. The animal was shaved and fixed to the stereotaxic frame, and eye ointment was applied to the eyes. Lidocaine (0.2 ml) was injected subcutaneously at the incision site before the incision was made, for local anaesthesia. The skin was cleaned using iodine, an incision (2 cm in length) was practised along the midline, and the skin was displaced laterally, exposing the surface of the skull. After cleaning the top region of the skull by blunt dissection, four drilling holes were made and titanium screws (length, 3 mm; thread diameter, 1 mm) were attached to the skull,

allowing for most of their length to remain outside. Cement was poured on top of these screws, ensuring it reached the space between the screws and the skull for a secure attachment [using a strong dental adhesive, such as Super-Bond (Sun Medical), it might be possible to firmly implant the base without screws; this cement has shown high tolerance, resistance, and durability for chronic implants in different species, including mice (Lohse et al., 2021) and ferrets (Nodal, Keating, & King, 2010).

A small cube-shaped magnet (Table 2.1) was placed inside the resin base (Fig. 2.2 A), which was then placed on top of the cement layer, and more cement was added around the lower part of the base until it was covered. The displaced skin was then stitched around the base, only allowing the necessary structure for the attachment of the headphones to remain visible (Fig. 2.2 B,C). Antibiotics (8 mg/kg, s.c.; cefovecin, Convenia) and analgesics (5 mg/kg, s.c.; carprofen, Rimadyl) were administered after the surgery. The base (with the magnet) weighs 0.9 g, but together with the cement it weighs 2.4 g.

Individual adjustment of the Ratphones

This procedure was performed 1 week after the base implant surgery, during which the animal was allowed to recover with free access to water and food. Under anaesthesia (induced and maintained with isoflurane at 4% and 2.5%, respectively), the structure with all pieces temporarily assembled, including the speakers and their connections, was placed on the implanted base and the different angles between the pieces were adjusted so that the speakers were placed at 5mm from the opening of the ear of the animal. The pieces were then fixed with cyanoacrylate, removed from the animal, and covered with flexible silicone rubber (Sugru, tesa SE), providing extra fixation to the resin pieces and protection to the electrical cables that connect the speakers. Initially, we covered most of the structure of the headphones with flexible silicon rubber (Fig. 2.4) (Pardo-Vazquez et al., 2019), but lately we have been covering only the central piece (Fig. 2.3), reducing the weight of the Ratphones while keeping a strong attachment in the part that supports more tension

when attaching/detaching them. A magnet (Table 2.1) was glued in the bottom of the structure to attach it to the implanted base during the behavioural sessions. A three-pin male connector (Table 2.1) was affixed to the part of the 3D-printed structure. Before each behavioural session, a standard three-wire sound cable—soldered to a matching three-pin female connector— was plugged to the male connector. A passive commutator, attached to the ceiling of the behavioural box, was used to avoid tangling. The headphones weigh 7.22 g without flexible silicone rubber and 12.8 g with a full coverage of this material.

We think that it should be possible to scale the Ratphones down to be used in freely moving mice. The 3D parts can be easily scaled; there are miniature neodymium magnets that are strong enough to keep the headphones attached to the base (e.g., a 1.6x1.6x1.6 mm neodymium cube weighs 0.06 g and can hold 90 g); and there are speakers that are light (0.6 g) and small (diameter, 10 mm) enough to be used in mice.

Table 2.1: **List of parts needed to build a set of Ratphones**

Parts	**Description**
3D-printed pieces	Implantable skull base, Central piece, Lateral bar (2), Speaker box front (2), Speaker box back (2), Lateral bar to speaker box connector (2)
Magnets	**B222** (K&J Magnets, Inc.): 1/8 x 1/8 x 1/8 in., grade N42, nickel-plated, magnetized through thickness **B3301** (K&J Magnets, Inc.): 3/16 x 3/16 x 1/32 in., grade N42, nickel-plated, magnetized through thickness
Speakers	Knowles active receiver (MOD 2403260 00031; "Petra")
Connector	Connector header, through-hole, 3 position, 0.100 in.

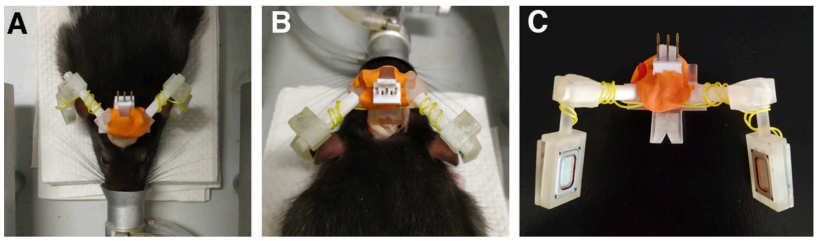

Figure 2.3: **A, B,** Front and rear view of a set of Ratphones adjusted under anaesthesia. **C,** Set of Ratphones assembled and glued together, including the speakers, cables, and connector.

Behavioural and sound attenuation tests

The Ratphones were used in freely moving rats performing a sound lateralisation task (Pardo-Vazquez et al., 2019). The arena consisted in a standard Coulbourn Instruments modular box (30x25x30 cm) equipped with three nose-pokes, one of them with a water delivery system. Before each behavioural session, the rat was placed in the box and a set of individualised Ratphones was brought near to the implanted base, until the magnets were attached, without restraining the movement of the animal. The Ratphones were plugged, through a standard sound cable, to a real time processor (RP2 by Tucker-Davis Technologies) that controlled the behavioural task, including presenting the sound and recording the responses of the animal. To avoid tangling, the cable was attached to a passive commutator.

Since we decided to minimise any physical contact between the speakers and the pinnae, it is expected that some residual sound from one speaker will reach the contralateral ear. We addressed this empirically,

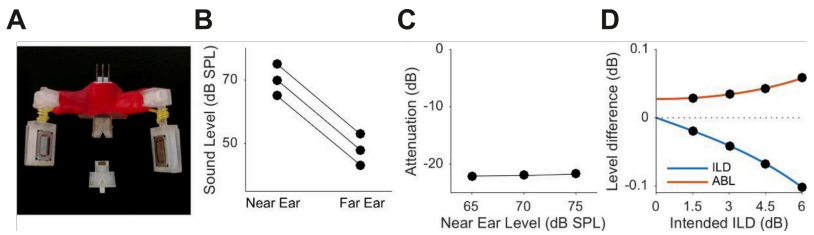

Figure 2.4: **Headphones and acoustic shadow of the head.(A)** Example headphones used for stimulus delivery (with more Sugru coverage), and base (at the bottom). **(B)** Measurements of the acoustic shadow generated by the head. Broadband noise stimuli were played at 65, 70 and 75 dB SPL and sound level was measured placing the microphone by the ear canal of the 'far' ear. **(C)** The head plus near field positioning of the speakers causes an attenuation of 22 dB. **(D)** Because the head attenuation is not infinite, the sound at each ear contains a contribution from the intensity of both speakers. Thus, the intended (using the sound intensity only from the near speaker) and actual experienced intensity at each ear are not identical. We calculated the difference between the actual and experienced intensity assuming additivity of the squared pressure RMS from each speaker, and we used this to compute the difference between actual and intended ILD and Average Binaural Level (ABL), which are shown in panel (D) as a function of intended ILD. Since these differences are always less than 0.1 dB (which is less than an order of magnitude smaller than the Just Noticeable Difference (JND) for ILD of our animals) we have, for simplicity, ignored the difference between actual and intended levels throughout this study.

by playing cosine-ramped (10 ms) broadband noise (5–20 kHz) at 65, 70, and 75 dB SPL from one speaker and recording the sound with the microphone placed by the contralateral ear canal (Fig. 2.4 (B)). The noise was independently generated for each presentation using a RP2 module at a sample rate of 50 kHz. The speakers were calibrated using a Brüel & Kjaer Free-field one-quarter inch microphone placed in front of the speaker, 5 mm apart. We found that the head plus near-field positioning of the speaker attenuates the sound by 22 dB (Fig. 2.4 (C)) (Pardo-Vazquez et al., 2019). Since the just noticeable difference for lateralisation of sound in this task is 2.2 dB (Pardo-Vazquez et al., 2019), this suggests that level

differences played through the Ratphones are an accurate approximation of actually experienced level differences (relative to the behavioural accuracy for sound lateralisation in rats), which validates the use of the Ratphones for psychophysical testing of sound lateralisation (Fig. 2.4 (D)).

2.5 Discussion

Presenting sensory stimulation in a reliable manner is critical in many experimental paradigms. Different methods have been used to accomplish this goal in different sensory modalities, but it has proven difficult to control sound presentation in small species, such as rats and mice. The Ratphones, a set of miniature headphones, can be used to reliably present auditory stimulation to freely moving animals. The Ratphones allow for precise sound presentation to rodents without the limitations of head-fixed preparations or the need for anaesthetized preparations, addressing a longstanding challenge in the field, striking a balance between stimulus control and allowing for natural behaviour. Our findings demonstrate that the Ratphones provide a reliable solution for auditory experiments in rats (Pardo-Vazquez et al., 2019), with potential applications for other model animals and implications for a wide range of research questions.

During an auditory task, especially for a sound source localisation task, it is important that the speakers maintain a stable position with relation to the ears of the animal, as changes in this parameter might significantly alter the perception of the animal (Koka et al., 2008; Wesolek et al., 2010). However, it is easy for a freely moving animal to alter its position with relation to fixed speakers in the behavioural setup, thus jeopardising the stimulus delivery and the behavioural readout. One of the strengths of the Ratphones is then the possibility to deliver very precise auditory stimuli while the animals are still freely moving. Their adjustable structure ensures that the positioning of the speakers with relation to the ears is consistent from animal to animal, allowing for

precise manipulation of ILDs. Which had previously only been achievable in head-fixed or anaesthetised preparations, limiting the study of more naturalistic behaviours. Our results show that Ratphones maintain stimulus precision while allowing the animals to move freely, ensuring the ability to investigate the relationship between auditory processing and complex behaviours.

Previous work focusing on precise delivery of sound stimulus had used a chronically implanted plastic structure into which the speakers were screwed before each behavioural session (Otazu et al., 2009). This mechanisms still requires a surgical intervention and is more time consuming and potentially stressful for the animals at the beginning and end of each behavioural session. Conversely, the magnetic attachment system of the Ratphones (Fig. 2.2) offers significant advantages. With a small footprint after implantation, it allows for easy application and removal of the Ratphones structure, making its use very easy over several behavioural sessions. This feature is particularly valuable for longitudinal studies and experiments requiring multiple training sessions over long periods of time. Moreover, the minimally invasive nature of the Ratphones daily placement reduces potential confounds associated with more invasive procedures, enhancing the ecological validity of the experimental setup.

We have also demonstrated the sounds that reach the opposite ear to a certain Ratphones speaker are greatly attenuated – by 22 dB (Fig. 2.4 (C)) – indicating one can accurately present animals with sound differences that are perceptible in the context of sound source localisation, as an accurate approximation of the sound level differences that would happen in nature.

The fact the Ratphones allow to precisely control ILDs (Fig. 2.4) in freely moving animals might facilitate the exploration of how rats process spatial auditory information in more naturalistic contexts, especially when involving sensorimotor integration and complex motor behaviours, such as navigation.

In spite of their significant advantages, the Ratphones still have some limitations. Their weight could be decreased, to lower its impact on behaviour and more easily allow its use with smaller model animals such

as mice. Additionally, eliminating the connecting cable to the computer through wireless technology would further enhance the animals' freedom of movement and decrease the need for experimenter surveillance, allowing the amount of animals working simultaneously to escalate. Additionally, integrating Ratphones with other sensing technologies, such as scopes or accelerometers, could provide even richer datasets linking auditory stimuli to behaviour.

2.6 Acknowledgements

Acknowledgment: We thank M. Bayonas for help with Ratphones prototyping.

2.7 Author Contributions

Alfonso Renart (A.R.) and José Luís Pardo-Vazquez (J.L.P.-V.) designed research; Mafalda Valente (M.V.), Juan Ramón Castiñeiras de Saa (J.R.C.-d.S.), and J.L.P.-V. performed research.

3

Sound Lateralisation as a Perceptual Decision-Making Task

3.1 Summary

We have developed an auditory discrimination task that leverages naturalistic mechanisms of sound source lateralisation and the accumulation of sensory evidence over time. Using a computational Drift Diffusion Model (DDM), we provide a comprehensive framework to study the mechanisms underlying perceptual decision-making.

Our behavioural task is grounded in the rodent strategy for sound source localisation, which relies on comparing Interaural level differences (ILD). To achieve this, we present broadband sounds of varying intensities to each ear through individually fitted Ratphones. The rat compares these auditory cues and selects the appropriate side to receive a water reward. By analysing the accuracy and reaction times (RT) of the animals, we identified a mathematical regularity, which we termed Time-Intensity Equivalence in Discrimination (TIED). This principle states that altering the overall sound level presented to both ears is functionally equivalent to changing the timescale of the accumulation process. This discovery provides a strong mechanistic explanation for the animals' decision-making behaviour.

To deepen our understanding of both task performance under different contingencies and the neural mechanisms supporting it, we conducted further manipulations, aiming to reduce and account for stimulus-independent responses. Our results confirm that the TIED regularity holds, preserving our ability to accurately and quantitatively describe the computations underlying this task.

Ultimately, our findings establish our auditory task as a robust paradigm for investigating perception, sensory integration, decision-making, and their computational and neural basis.

3.2 Introduction

The study of perceptual decision-making is key to understanding the relationship between sensory processing and behaviour. Perceptual decision-making is a fundamental cognitive process in which organisms interpret sensory input and adapt their behaviour accordingly. Rigorous experimental paradigms based on sensory psychophysics are essential for increasing the understanding of the neural basis of decision-making, or how sensory information is accumulated, evaluated, and ultimately translated into behaviour (Okazawa & Kiani, 2023; Waskom et al., 2019). These perceptual tasks allow researchers to systematically control and manipulate sensory inputs while observing the behavioural outputs to then be able to build a model that can explain the different aspects of behaviour (Carandini & Churchland, 2013; Gold & Shadlen, 2007; Hanks & Summerfield, 2017; Okazawa & Kiani, 2023; Roy et al., 2021). Many of the tasks used for this purpose (e.g. judging the direction of moving dots, judging the side with larger number of pulses, and frequency categorisation) are believed to rely on accumulation of sensory evidence towards a bound (Britten et al., 1992; Brunton et al., 2013; Gimenez et al., 2015; Jaramillo & Zador, 2010; Jaramillo & Zador, 2014; Palmer et al., 2005; Parker & Newsome, 1998; Shadlen & Newsome, 2001), although note that rigorously establishing that the subject uses accumulation of evidence is not a trivial matter (Hyafil et al., 2023; Kopec et al., 2024; Stine et al., 2020). These tasks have been described, based on this computation,

through the Drift Diffusion Model (DDM), which provides an explanation that includes both the choice and reaction time (Bogacz et al., 2006; Forstmann et al., 2016; Ratcliff & McKoon, 2008; Shadlen & Kiani, 2013). Even though, historically, perceptual decision-making tasks were mainly developed for primates (Britten et al., 1992; Gold & Shadlen, 2007; Parker & Newsome, 1998), rodents have become an increasingly popular model organism (Carandini & Churchland, 2013). Besides cost-effectiveness and ethical considerations, mice and rats are especially attractive given the genetic tools available, facilitating neural manipulations, such as optogenetics and chemogenetics, for the probing of brain circuits and their involvement in behaviour (Carandini & Churchland, 2013; Juavinett et al., 2018). Rodents also have faster reproductive cycles and larger litter sizes, allowing for high-throughput that enables studies with multiple conditions and variations in a shorter time frame when compared to primates (Juavinett et al., 2018; Laboratory et al., 2021). Moreover, rodents are capable of a wide range of behaviours, and are able to perform tasks that require complex neural computations, such as perceptual discrimination, evidence accumulation, and choice under uncertainty, much like primates (Brunton et al., 2013; Juavinett et al., 2018; Posner et al., 2022). Interestingly, they can also be trained in tasks that mimic ethologically relevant behaviours, allowing for the study of decision-making in natural contexts or using naturalistic strategies, more closely recapitulating how decisions are made outside the highly controlled environment of the lab.

We focused on a previously established auditory decision-making task with Interaural level differences (ILD) as a stimulus (Pardo-Vazquez et al., 2019), achieving a high degree of control over these stimuli, as discussed in the previous chapter, with the Ratphones (Valente et al., 2023). This task focuses on sound, a highly controllable and manipulable phenomenon, and differs from other auditory tasks in that it makes use of a naturalistic stimulus feature (lateralisation, for which the categorisation boundary is hardwired), instead of an experimenter-determined categorisation bound (Gimenez et al., 2015; Guo et al., 2017). The use of a naturalistic behaviour allows for fast training paradigms, for a high fraction of animals to become high performers, and eliminates

decision lapses (see below, Figure 3.2). Overall, we believe that our rats understand the latent variable (sound lateralisation) that they need to categorise, and this makes a big difference to their psychophysical performance. Our animals respond to the different ILDs, which effectively allow for manipulation of the amount of evidence available to the rat. But the difficulty of the task can be manipulated using a second characteristic of the sound, the Average Binaural Level (ABL) (the average sound level between the two ears, that can be varied independently of the ILDs). Indeed, we have previously tested ILD discrimination at different overall sound levels, and accuracy remained invariant, depending on ILD alone, demonstrating compliance with Weber's Law. At the same time, Reaction Time (RT) scaled systematically with absolute intensity, leading to the formulation of the Time-Intensity Equivalence in Discrimination (TIED) principle (Pardo-Vazquez et al., 2019). Leveraging the TIED and its necessity for Weber's Law (WL) to be verified, we also managed to develop a mechanistic explanation of the animals' behaviour through a DDM. Understanding the computational mechanisms underlying task performance provides a strong foundation for investigating its neural basis. However, in our previous work, we focused only on responses that were triggered by the auditory stimulus. In other words, we ignored anticipatory responses where the animals leave the central port before, or immediately after sound starts based on their estimation of stimulus onset. As we will show in this thesis, understanding the interaction between anticipatory and stimulus triggered choices is important for providing a complete understanding of the behaviour of the rats in our task.

3.2.1 Reactive and Proactive Choices

Stimulus dependent choices are explained by computational models, like the DDM, for humans, non-human primates, and rodents (Bogacz et al., 2006; Gold & Shadlen, 2007; Hanks & Summerfield, 2017; Ratcliff & McKoon, 2008; Ratcliff et al., 2016). Based on the work of Hernández-Navarro et al., 2021, we refer to these as "reactive choices", defined

as responses triggered by the accumulation of sensory evidence until a predefined decision threshold is reached (Ratcliff & McKoon, 2008; Ratcliff & Smith, 2004). In this framework, the timing of this reactive response is inherently linked to the processing of the sensory stimulus – RTs are directly influenced by the characteristics of the stimulus and the efficiency of evidence accumulation. Thus, reactive responses, dependent on external stimuli, can be influenced by factors such as stimulus intensity and duration, which affect the speed and accuracy of decisions (Gold & Shadlen, 2007).

However, actions are not always prompted by a stimulus or the external state of the world. These self-initiated actions may be referred to as "proactive responses" (Hernández-Navarro et al., 2021; Murakami et al., 2014; Romo & Schultz, 1992), and can take place in a number of situations, such as when the stimulus can be anticipated (Green et al., 1983; Haith et al., 2016; Hardwick et al., 2019; Hernández-Navarro et al., 2021; Jung et al., 2014; Stanford et al., 2010). In fact, it may even be advantageous to proactively respond if subjects can adjust their responses after initiation to integrate new sensory information (Hernández-Navarro et al., 2021), as sometimes, accumulating evidence to a certain criterion might take too long (Churchland et al., 2008; Drugowitsch et al., 2012; Hernández-Navarro et al., 2021). Thus, these proactive processes give rise to more impulsive responses, that can correspond to premature, or aborted decisions. This has been modelled before in different ways: such as adding an endogenous process to the stimulus-motivated one, generating random guesses as opposed to reactions to the stimulus. Although able to account for short RTs, endogenously motivated decisions were not influenced by the stimulus and always corresponded to random guesses, and reactive responses always had their RT decided by the stimulus (Trappenberg et al., 2001). For other tasks, where speed is emphasised, the process that decides when the decision is made is fully separated from the decision itself, and as such the RT was never stimulus-dependent (Haith et al., 2016; Hardwick et al., 2019); or evidence is responsible for accelerating or decelerating a single process of action initiation, which is unable to account for trials where choice is stimulus dependent but not RT (Hawkins

& Heathcote, 2021; Salinas et al., 2010; Stanford et al., 2010). The model we will be focusing on throughout this work to attempt to model our proactive choices relies on adding a second accumulation process to a DDM, encompassing the two competing processes, one time dependent and the other evidence dependent. The Parallel Sensory Integration and Action Model (PSIAM), includes a parallel process to the usual evidence accumulation to bound (Hernández-Navarro et al., 2021). This process also increases a decision variable, but to a single bound that corresponds to action initiation, competing with the usual evidence accumulation process.

The coexistence of these two processes suggests a more complex interplay in perceptual decision-making than previously understood, indicating that both proactive and reactive mechanisms may simultaneously inform choices depending on contextual demands and providing evidence in favour of two decisions within a response – a 'when' decision, determining the timing of the choice, and a 'what' decision, regarding what response to execute -(Hawkins & Heathcote, 2021; Hernández-Navarro et al., 2021).

In this chapter, we reintroduce the auditory task described in Pardo-Vazquez et al., 2019 and explore it in further detail. We aim to reproduce and consolidate the main behavioural results shown in the previous work, showing its robustness, while looking more deeply into anticipation and stimulus-independent choices displayed by the animals that have not been explored. Ultimately, this chapter aims to motivate the use of this behavioural task for studies regarding decision-making and its neural basis, as well as exploring in depth the different responses it encompasses.

3.3 Methods

All procedures were reviewed and approved by the animal welfare committee of the Champalimaud Centre for the Unknown and approved by the Portuguese Direcção Geral de Veterinária (reference no. 0421/000/000/2019).

Experimental Animals

Experiments were performed on a total of 42 adult female Long–Evans hooded rats. Animals were 12–13 weeks old and weighed between 250 and 300 g at the beginning of the experiments. They were kept above 85% of the initial weight throughout. All animals were naive to any behavioural tests. Rats had free access to food, but water was restricted to the behavioural sessions, which were conducted during five consecutive days per week. Animals had access to water during the sixth day and were deprived of water for 24 h before each round of five sessions. No statistical methods were used to predetermine the number of animals or the number of trials per animal, but our sample sizes were similar to those reported in previous publications (Uchida & Mainen, 2003). We used a 'within subject' design in which the animals were tested in all experimental conditions, so there was no need to apply blinding or randomization.

Auditory Stimuli

A percept of lateralization was created by presenting cosine-ramped (10 ms) broadband (5–20 kHz) noise bursts with different intensities to each ear (ILD) (Fig. 3.1 C). The waveforms for the noise were generated prior to training using a computer running Matlab with Psychtoolbox-3 (http://www.psychtoolbox.org), and equipped with an Asus Xonar DX/XD PCI-E Low Profile Sound Card. For initial training, stimuli was delivered by speakers placed on the side walls of the behavioural box; and for the latter stages of training, through speakers placed in headphones as described in chapter 2. All speakers were magnetic rectangular speakers – earpiece speakers, compatible with Nokia phones, Coopart, through Aliexpress®. Headphones were calibrated before first use, using a Brüel & Kjaer Free-field one-quarter-inch microphone placed in front of the speaker, 5-mm apart to mimic the position of the speaker relative to the ear. The sounds were previously generated using Matlab 2016a (https://www.mathworks.com) and four waveforms saved and were pseudo-randomly alternated for every trial.

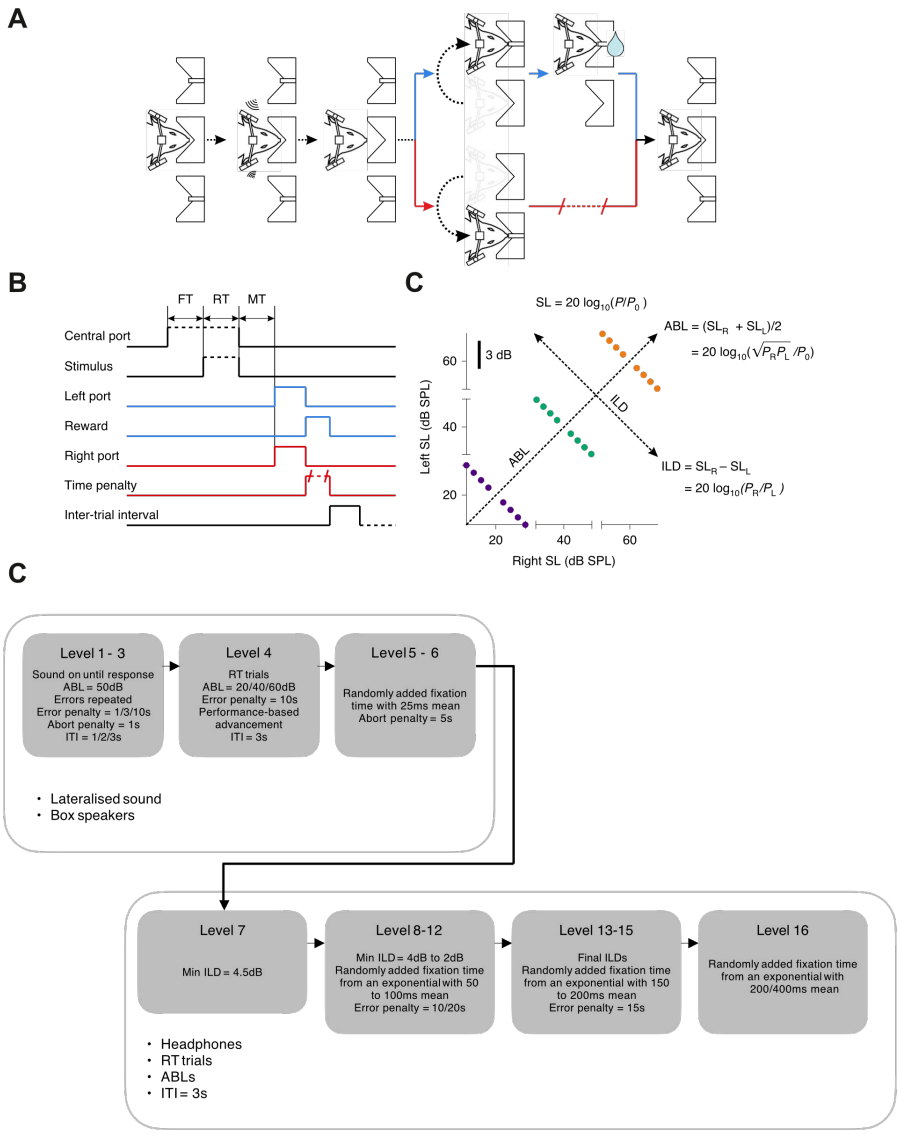

Figure 3.1: **Task structure and stimulus set. A** Schematic depiction of the different task events. Rats were rewarded with water for making the correct choice (blue) and were punished with a time delay for making an error (red). **B** Timeline of the relevant task events. FT, fixation time; MT, movement time. **C** Stimulus set. The ABL (ILD) of a particular stimulus is given by the average (difference, by convention, right minus left) of the intensity of the sound in dB Sound Pressure Level (SPL) (Sound Level (SL)) across both speakers.

Figure 3.1: (Continued caption) Constant ILD (ABL) implies constant intensity ratios (intensity products). P0 = 20 μPa is the reference pressure of the dB SPL scale. Purple, green and orange circles indicate ABLs of 20, 40 and 60 dB SPL, respectively. **D** scheme of the training progression for the animals in the studied task. The initial levels make use of the lateral sound speakers placed in the box, and the animals are exposed to fully lateralised sound only. After being fit with headphones, ILDs are introduced, as well as the different ABLs.

Behavioural Apparatus and Headphone design

Rats were trained and tested on the sound lateralization task (Fig. 3.1 A, B) using an acrylic custom-made behavioural box (X x Y x Z) with three ports (X cm apart) placed on the back wall, and two speakers, each placed on one lateral wall. All components of the behavioural setup were connected to an in-house developed IO board and accessed by a computer running Matlab 2016a (https://www.mathworks.com) with Psychtoolbox-3. The behavioural setup was placed inside a soundproof box that was illuminated by infrared lights and equipped with an infrared camera to observe the animals during the sessions. Both the base to be implanted in the skull and the structure of the headphones were designed in accordance with the protocol described in chapter 2. The headphones were attached to the base at the beginning of each behavioural session and detached before taking the animal back to the holding cage. They connected to the controlling computer through a stereo audio jack cable.

3.3.1 Base Implant Surgery

Similar to what is described in section 2.2.1. Anaesthesia was induced by inhalation of isoflurane at a concentration of 5% (oxygen at 2 L per min) and maintained at 3/2.5% during surgery. The percentage of isoflurane could be increased if the animal exhibited signals of pain or discomfort. The animal's head was shaved, and the rat fixed to the stereotaxic frame through a mouthpiece and earbars. Eye ointment (Bepanthen® Augen und Nasensalbe, Bayer, Germany) was applied to the eyes. Lidocaine (0.2

ml) was injected subcutaneously at the incision site before the incision was made, for local anaesthesia. The skin was cleaned using iodine, an incision was made along the midline of the skull from just behind the eyes until between the ears, and the skin was displaced laterally, exposing the surface of the skull. After cleaning the top region of the skull by blunt dissection, four drilling holes were made and stainless steel screws (length, 3 mm; thread diameter, 1.6 mm) were attached to the skull, allowing for most of their length to remain outside. Cement was poured on top of these screws, ensuring it reached the space between the screws and the skull for a secure attachment. A 3D printed oval well was placed on top of the cement layer and filled with more cement, the resin base, already fitted with the cubic magnet (Fig. 2.2 A), which was then placed inside the well, and more cement was added around the lower part of the base until it was covered. The displaced skin was then stitched around the base, only allowing the necessary structure for the attachment of the headphones to remain visible (Fig. 2.2 B, C). Antibiotics (8 mg/kg, s.c.; cefovecin, Convenia) and analgesics (5 mg/kg, s.c.; carprofen, Rimadyl) were administered after the surgery and the animal was housed alone after this procedure.

3.3.2 Behavioural Task

Rats started a trial by poking in the central port (Fig. 3.1 A) within a 6 s time window (start trial waiting time) triggered by the end of the Inter Trial Interval (ITI) – 3 s –, signalled by the overhead light in the box turning off. After a short, variable Fixation Time (FT) (uniform distribution, 300-350 ms) the sound was played binaurally, through the headphones, until the rat left the central port or until the maximum presentation time (6 s) was reached. Rats had to communicate, within a 2 s time window (maximum response time), whether the sound was louder at the left or right ear by poking with the snout in either the left or right ports, respectively. Correct choices were rewarded with a drop of water (28 μl) and incorrect responses were penalized with a 10-s timeout, during which the rat was not able to start a new trial (Fig. 3.1 A, B).

Trials in which the rat failed to start a new trial within the start trial waiting time, broke fixation during the FT or failed to poke in either lateral port within the response waiting time were considered aborts. Aborts were repeated after a 1-s time penalty except for the fixation aborts, which had a 5 s time penalty.

Each session was divided into blocks of 80, 96 or 100 trials (depending on the amount of ILDs being presented to the animals). For some sessions, the animals were experiencing the trials in blocks, within each block, the ABL was kept constant, while the ILD changed pseudo-randomly from trial to trial; for no-block sessions, both ABL and ILD were changed pseudo-randomly. Typically, sessions lasted for 2 h and rats performed between 600 and 1,000 trials.

3.3.2.1 Initial Training

To easily track the animals' progression, the task was divided into training levels, as shown in Figure 3.1 D. Levels 1–6: these training levels had the goal of teaching the rats the general contingencies of the task. During these levels, sound was presented through speakers placed on the side walls of the behavioural box, and it consisted of fully lateralized sound. Levels 7–16: these training levels made use of the previously mentioned headphones for sound delivery and had the goal of increasing the difficulty of the task until the animals reached their psychophysical threshold. This was accomplished through progressively decreasing the minimum ILD the animal experienced.

Animals were initially trained in a simplified version of the task, in which fully lateralized sounds (50 dB SPL broadband noise) were presented from either of the arena speakers. Rats quickly understand the basic contingency of the task, within a few hundred trials. The sound was played until the animal entered one of the lateral ports, and errors were repeated immediately. Short fixation times and long waiting times were used to increase the chances of the rat to complete the trial while exploring the box. Every time the rat completed a trial, the fixation time was increased by 1 ms. Once performance with ABL of 50 dB and fully

lateralised sound was stable, the magnetic base for the headphones was implanted, and the animals were allowed to recover for at least 1 week, during which they had free access to food and water. After surgery, the ABL was kept at 50 dB and the ILD step was set to 4 dB with 8 ILDs; depending on the performance of the animals – level progression happened when the animal accomplished 80 trials with a minimum performance of 80%, the step was gradually decreased until the final 1 dB step. By the final level, once performance for the final set of ILDs at an ABL of 50 dB was stable, the final set of ABLs was introduced – 20, 40 and 60 dB. For the remainder of this chapter, we will focus on this level.

3.3.2.2 Final Training Level and Task variants

This chapter focuses on the Final Training level for all cohorts analysed. This usually corresponds to the level of the final ILD set and different ABLs, but it was modified for the different cohorts in order to test various hypotheses. We modified the basic task in several ways:

- **Batch A:** Rats from Batch A (N = 5 rats) were tested in five types of blocks:
  - (A1) “standard” blocks, in which four ILDs (of each sign) linearly spaced from 1.5 dB to 6 dB steps were presented. Usually referred to as the WL dataset.
  - (A2) “hard”, in which only the four ILDs closer to the midline (±1.5 and ±3 dB) were presented (Fig. 3.3 D, E). We did not present only the hardest condition because when we attempted this, the rats “gave up” and became biased.
  - (A3) “uneven reward (RW)", in which we increased (decreased) the amount of water delivered after correct discriminations for the two hardest (easiest) ILD conditions by 20% (Fig. 3.3 C).
  - (A4) “log noise”, in which five ILDs (of each sign), logarithmically spaced between 1 and 8 dB, were used (Fig. 3.3 A).

  - (A5) “log pure tones”, identical to the previous one but using 10 kHz tones instead of broadband noise (Fig. 3.3 A). In all types of blocks except for A5, three ABLs = 20, 40 and 60 dB SPL were used. For blocks of type A5, only ABL = 60 dB SPL was used.

- **Batch B:** Rats from Batch B (N = 6 rats), and subsequent ones, were tested with different FT (200 ms minimum time and an added variable time sampled from an exponential distribution of mean 200 ms and at least 4*mean limit) in five types of blocks with ABL = 50 dB SPL:

  - (B1) “standard", in which four ILDs (of each sign) logarithmically spaced between 1 dB and 8 dB were used (Fig. 3.3 E).
  - (B2) “hard", in which only the two most difficult conditions (1 and 2 dB) were used (Fig. 3.3 D).
  - (B3) “frozen noise (FN) coherent", in which the exact broadband sound (out of four different examples) was presented in the two headphones appropriately scaled to produce a given ILD (ILDs were the same as in the standard blocks).
  - (B4) “FN non-coherent", in which a different one of the four examples was played in each headphone (same ILDs). Blocks B3 and B4 were used in (Fig. 3.3 B).
  - (B5) “easy sessions", in which only ILDs = ± 4.5, 6, 9, 15 dB were used (Fig. 3.3 E).
  - (B6) four remaining rats from this batch were trained in a variant of the standard RT task with a different set of conditions (ILD = 0, 0.5, 1.25, 2.25, 4 and 8 dB and ABL = 10, 25, 40, 55, 70 dB SPL) designed to sample effect of difficulty and intensity more densely, and without blocks (Fig. 3.3 F).
  - (B7) capped sound duration experiment, and we then switched conditions to ILD = 1, 2, 4, 8 dB (of each sign) and ABL = 20

and 40 dB SPL. The range of maximum sound durations (Maximum Sampling Duration (SDmax)) tested was 50, 75, 100, 150, 250 ms.

- **Batch C:** Rats from Batch C (N = 6 rats) were tested in two different block types:
  - (C1) “standard", in which four ILDs (of each sign) logarithmically spaced between 1 dB and 8 dB were used.
  - (C2) capped sound duration experiment with ABL = 20, 40 and 60 dB SPL. The range of maximum sound durations (SDmax) tested was 15, 30, 60, 120, 240, 480 ms and RT (analysed in Chapter 4).
- **Batch D:** Rats from Batch D (N = 20 rats) were tested in two different block types:
  - (D1) “standard", in which five ILDs (of each sign) logarithmically spaced between 1 dB and 16 dB were used with ABL = 20, 40 and 60 dB SPL.
  - (D2) LED manipulation blocks, in which a maximum of 30% of the trials corresponded to LED ON trials. The remaining trials were as (D1).
- **Batch E:** Rats from Batch E (N = 5 rats) were tested in twelve different block types in order to manipulate the stakes of each trial. For this chapter, we will only focus on one block type:
  - (E1) “standard", in which five ILDs (of each sign) logarithmically spaced between 1 dB and 16 dB were used with ABL = 50 dB SPL.

Task variants: Discrimination of the ILD of pure tones

Three of the rats were tested with blocks of type A4 and A5 mixed pseudo-randomly.

Task variants: Manipulations of motivation

For rats in Batch A, blocks of type A2 were randomly included among standard blocks within a set of sessions, in such a way that every time 'hard' conditions were tested, at least three consecutive blocks (one for each ABL) were used. Results from this manipulation are in Figure 3.3 D. These same rats also performed a series of sessions where all blocks were of type A3 (Fig. 3.3 C). Longer-lasting and bidirectional manipulations of motivation were tested with rats of Batch B. These rats were tested in a series of sessions, where an initial standard block was followed by blocks of type B2 until the end of the session (Fig. 3.3 E).

Task variants: External vs Internal noise

We selected four broad band noise samples and either used the same or different samples at the two ears. Rats of Batch B were tested in a series of sessions, with blocks of type B3 and B4 alternating pseudo-randomly within the same session (Fig. 3.3 B). Note that even with frozen-noise stimuli, waveforms at the two ears will not be perfectly coherent because the headphones are not inserted in the ear canal.

Task variants: Maximum sound duration

The task was still in reaction time configuration but, for a given SDmax, the sound was stopped at that SDmax if the rat had not left the central port at that time. For choices with RT < SDmax, the sound offset was triggered by the central port exit. Rats performed the task in mini-blocks of 16 trials. Each mini-block contained two permutations of all the ILDs at fixed ABL and SDmax, and ABLand SDmax were chosen randomly from their possible values in each mini-block.

3.3.3 Statistics

To determine the minimum RT to be analysed, we used a two-sample Kolmogorov–Smirnoff test. For assessing the ABL dependence of ILD

discrimination accuracy, we used Fisher's exact test, two-tailed, Bonferroni– corrected. Specific details on the employed testing procedures are given in Pardo-Vazquez et al., 2019.

3.3.4 Data Analysis

Data analysis methods employed in this chapter are described in detailed in Pardo-Vazquez et al., 2019.

3.3.4.1 Isolating stimulus-dependent RTs

To exclude those trials in which behaviour was not driven by the stimulus, we looked for the minimum RT for which there was evidence of condition dependence. To this end, we used two-sample Kolmogorov–Smirnoff tests to compare the distribution of RTs corresponding to the two conditions with the shortest (ILD = 6 dB, ABL = 60 dB SPL) and the longest (ILD = 1.5 dB, ABL = 20 dB SPL) mean RT. Starting at 50 ms, we systematically included longer and longer RTs. As evident in Supplementary Fig. 2a,b, if the maximum RT is sufficiently short, the two distributions are not significantly different, but for RTs > RTmin = 90 ms, they become different. For all analyses, we excluded trials with RT < RTmin. In addition, since the shape of the RT distributions is very well-behaved and understood in our study, we also excluded trials that had exceedingly large RTs, which presumably reflect disengagement. For all analyses except model fitting, we chose a conservative value of RTmax = 1,000 ms (the fraction of trials with RT > 1,000 ms was always very small: 0.39 ± 0.39%, mean ± s.d., across rats). For model fitting, trials with RTs above the 97% percentile in the RT distribution of each rat were excluded. Empirical estimates of the RT distribution (Fig. 3.2) were made – the methods described in detail in Pardo-Vazquez et al., 2019.

3.3.4.2 Accuracy of temporal rescaling

We assessed how accurately the RTDs for different experimental conditions resembled a uniform scaling of time (Figs. 3.2 C. D. and 3.9) using

the fact that when two distributions are related by a uniform scaling, their quantiles are proportional. For fixed ILD (ABL), we regressed the percentiles of each condition on those of the fastest, that is, 60 dB SPL (6 dB or 4 and 8 dB). The method is described in detail in Pardo-Vazquez et al., 2019.

3.4 Results

3.4.1 Review of Previous Behavioural Results

We initially focused on reproducing key behavioural results from Pardo-Vazquez et al., 2019. With the purpose of introducing these experiments reproducing previous work, we first review the main behavioural results from the paper, the rats' usage of ILDs for task performance and the TIED.

The time-intensity equivalence in discrimination

We trained rats to discriminate the lateralization of binaural broadband (5-20 KHz) noise bursts, played through headphones, in order to minimise uncontrolled stimulus variations (Chapter 2). Rodents use ILDs (caused by the acoustic shadow of the head) as the main binaural cue to localise sound sources in the horizontal plane, (Grothe et al., 2010; Wesolek et al., 2010) which means they are accomplishing a comparison of sound intensities, making this a useful task with which to study WL (Stellmack et al., 2004). We manipulated discrimination difficulty by varying ILDs pseudo-randomly across trials at different ABLs, (Methods, block type A1, Fig. 3.3 A.). To probe the effect of overall sound level on ILD discrimination, ABLs varied pseudo-randomly in blocks of 80 trials. We used a RT paradigm with sound termination triggered by the rats' exit from a central port (Fig. 3.1 B.; Methods). Because WL states that discrimination accuracy should only depend on intensity ratios, and since pressure RMS ratios and ILDs (in dB) are equivalent (Fig. 3.1 C.), WL predicts that the accuracy of a given ILD discrimination should not

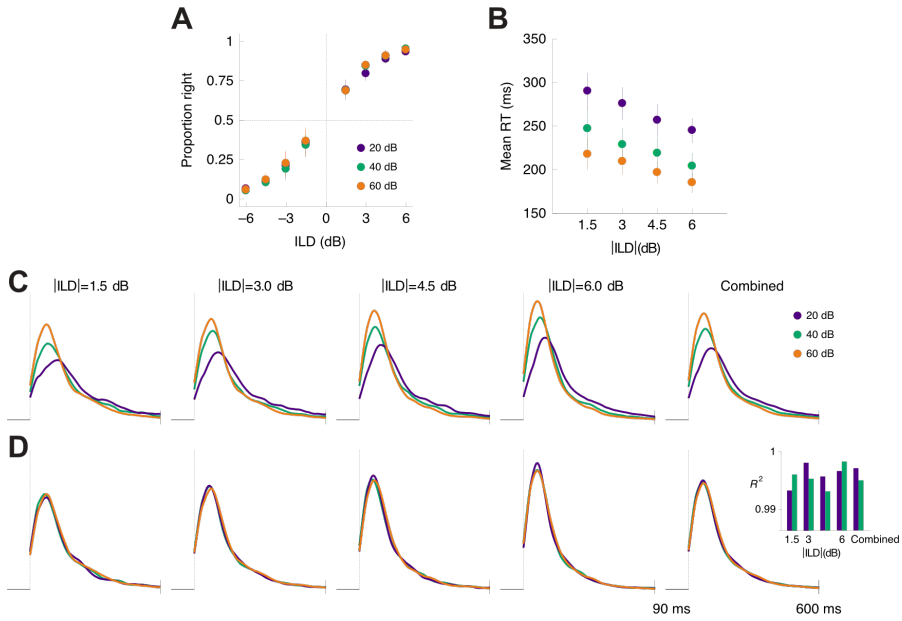

Figure 3.2: **Time-intensity equivalence in sensory discrimination A.** Choose-right probabilities as a function of ILDfor each ABL separately (mean ± SD across rats, N = 5 rats. **B.** Reaction time (mean ± SEM across rats) as a function of difficulty for each ABL separately. **C.** Reaction Time Distributions (RTDs) for the three ABLs are shown separately for each difficulty, and combined across difficulties (right). For all RTDs, the dashed line indicates the time at which RTs become condition-dependent (90 ms, scale bar in all plots. Each Reaction Time Distribution (RTD) contains all data for that condition from all rats. **D.** For each difficulty, we have rescaled time uniformly to maximize the overlap of each RTD with that for ABL = 60 dB SPL. Inset, Fraction of variance (R^2) that the rescaled RTD at ABL = 60 dB explains about the RTDs at 20 and 40 dB SPL. The slope of this fit is the temporal rescaling factor

depend on ABL. Consistent with this prediction, psychometric functions for different ABLs overlapped (Fig. 3.2 A.).

Harder discriminations were characterised by longer RTs (Forstmann et al., 2016; Gold & Shadlen, 2002) (Fig. 3.2 B.), but discriminations of the same difficulty between sounds of lower ABL also took longer on average (Recanzone & Beckerman, 2004; Simen et al., 2016; Teodorescu et al., 2016) (Fig. 3.2 C.). Both ILD and ABL had a significant impact on mean RT for each individual rat, as well as at the group level (two-way RM-ANOVA; significant effect of ILD: $F(3, 12) = 17.54$, p=0.0001, and of ABL: $F(2, 8) = 77.12$, p <0.0001). We found that changes in ABL appeared to rigidly stretch the RTDs without changing their shape (Fig. 3.2 D.). Indeed, an appropriate uniform scaling of time (Pardo-Vazquez et al., 2019) revealed that the RTDs are almost perfectly scale-invariant as a function of ABL, for each difficulty and for all difficulties combined (Fig. 3.2 D.). In each case, more than 99% of the variance in the shape of the RTD for one ABL could be explained by a uniform stretching of the RTD for a different ABL (Fig. 3.2 D. inset; mean R^2=0.996) (see Methods section in Pardo-Vazquez et al., 2019). Thus, for any given intensity-ratio, discriminations appear to run faster when the absolute stimulus intensities are larger. This suggests the following regularity, which we named Time Intensity Equivalence in Discrimination (TIED): Changes in the absolute intensity of two stimuli being discriminated under a fixed intensity-ratio are completely equivalent to a change in the effective unit of time with which the discrimination duration is measured (Pardo-Vazquez et al., 2019).

The TIED is an important result as it is a striking demonstration that accuracy and Reaction Time can be completely decoupled during perceptual decisions, and it is also a very restrictive regularity that provides — together with Weber's Law — a strict quantitative regularity of behaviour that can be used to infer the mechanisms at play during sound lateralisation, as we did in Pardo-Vazquez et al., 2019. In this way, overall sound level can be considered a 'nuisance parameter' for the identification of the lateralisation of the stimulus. ABL changes the gain of ILD on the average sensory evidence (equation (40), Supplementary

Note of Pardo-Vazquez et al., 2019). Link (Link, 1992) predicted that a DDM would respect WL under Poisson variability (observed in the auditory localization pathway (Jones et al., 2015; Tollin et al., 2008)). In such a case, drift rates will increase with ABL, but so will the noise in the trajectories, resulting in exactly the same probabilities of hitting either bound (Link, 1992). This ensures level-invariant accuracy (Fig. 3.2 A.). While Link was the first to emphasise a connection between WL and sequential sampling, only more recently was the relevance of RTs for understanding the effect of absolute intensity (ABL in our case) in sensory discrimination addressed (Simen et al., 2016; Teodorescu et al., 2016). Simen and colleagues realised that Link's model predicts that WL should be associated with scale invariance of the RTD, thus highlighting the connection with temporal production that we have also observed (Fechner, 1860; Simen et al., 2016).

3.4.1.1 Rats rely on Interaural level Differences for Task Performance

We have also previously characterised certain aspects of the behaviour in order to demonstrate that it is consistent with our implicit modelling assumptions, namely that the rats rely on ILD and not on other characteristics of the sound, that their threshold is hard-wired and that noise has internal origin. To further prove these assumptions, we devised several control experiments to isolate sources of uncertainty other than the stimulus and rule them out.

Rats could discriminate the ILD of 10 KHz pure tones at 60 dB SPL and broadband noise with the same accuracy (Methods, block type A5, Fig. 3.3 A.), showing that they exclusively use the sound's ILD to perform the task and not other specific parameters of the sound that might be present in a broadband noise burst. Discrimination accuracy for 'frozen-noise' bursts was identical to control conditions (Methods, block type B3, Fig. 3.3 B.), showing that performance-limiting noise has an internal origin and is not due to interactions between the different waveforms randomly created for the trials. These findings, together with the virtual absence of history effects (Pardo-Vazquez et al., 2019), confirm that the

ILD discrimination task we used is an appropriate paradigm to read out the subjects' percepts from their behavioural choices with minimal confounding factors.

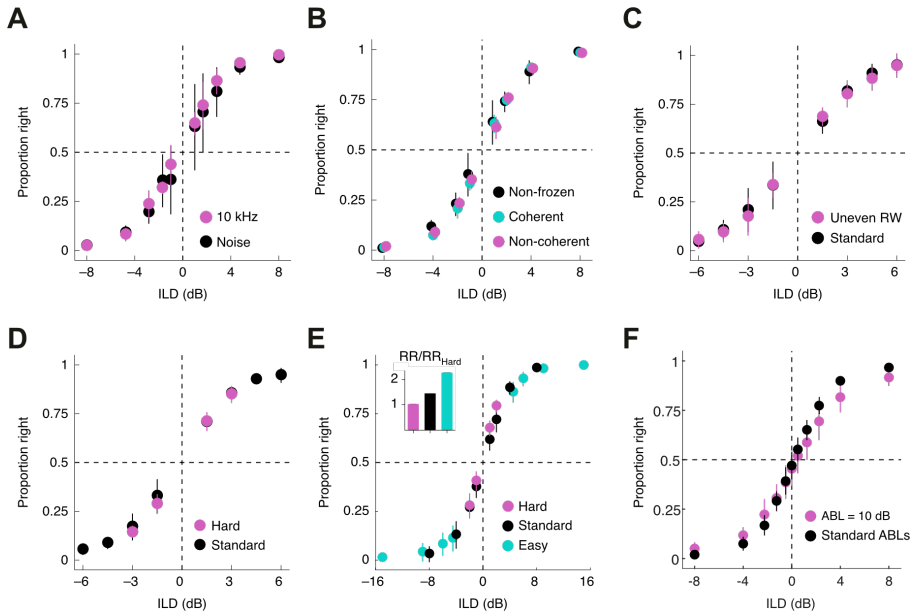

Figure 3.3: **Stimulus manipulations A.** Choose-right probability (mean ± SD across rats, N = 3 rats) for broad band noise and 10 KHz pure tones of ABL = 60 dB SPL. **B.** Same for the standard non-frozen condition and for frozen noise, either coherent or incoherent across ears (mean ± SD across rats, N = 6; Methods). **C.** Choose-right probabilities (mean±SD across rats, N = 4 rats) for standard blocks and for 'uneven reward' blocks where rewards for correct choices in the two hardest (easiest) conditions are 20% larger (smaller). **D.** Same (N = 3 rats) for hard versus control blocks. **E.** Same for more extreme, bidirectional and longer lasting manipulations of motivation (N = 5 rats) **Inset**, Reward rate (RR) in each condition of motivation relative to the reward rate in the hard condition (RRHard). **F.** Choose-right probability (mean ± SD across rats, N = 5 rats) for comparison of standard ABLs (grouped, ABLs between 20 dB and 60 dB) and a more extreme ABL of 10 dB. This dataset also includes the ILD of 0 dB.

The Weber Fraction (WF) is typically used to quantify the minimal discriminable stimulus increment at psychophysical threshold, i.e., when

accuracy is not limited by motivational factors. In order to investigate what determines the WF in ILD discrimination, we performed a series of behavioural manipulations with the goals, first, of establishing whether our rats are indeed operating at psychophysical threshold, and also of clarifying other potential factors, beyond motivation, limiting choice accuracy in our task. First, we attempted to increase discrimination accuracy by increasing the reward magnitude for correct discriminations in the hardest conditions relative to the easiest ones (Methods, block type A3), but this failed to induce changes in performance (Methods, block type A3, Fig. 3.3 C.). Next, we presented blocks with only the two most difficult conditions (Methods, block type A2), a manipulation expected to improve discriminability both due to a motivation-enhancing decrease in reward rate, a narrower prior on stimulus difficulty (Drugowitsch et al., 2012), and a potential removal of uncertainty about the location of the categorization boundary (Mendonça et al., 2020). Nevertheless, accuracy again did not change (Fig. 3.3 D.).

To confirm this result, we performed manipulations of stimulus difficulty throughout a larger number of sessions, and we also presented only-easy (in addition to only-hard) blocks of stimuli (inducing changes in experienced reward rate of more than 100%, Methods, block types B2 and B4 Fig. 3.3 E. (inset)), but we observed the same negative results (Fig.3.3 E.). We further tested a subset of rats in the same ILD task but with a broader range of both ILDs and ABLs (Methods, block type B6, Fig. 3.3 F.). We included ABLs in the range of the previously established level-invariance (Standard ABLs), as well as the more extreme one of 10 dB. The animals were still able to perform the task, although their accuracy worsened for the ABL of 10 dB, possibly due to it being harder to detect considering it corresponds to a very faint sound, close to the dB SPL threshold for detection in the frequency range of the broadband noise we are using (Heffner et al., 1994). As for ILD, we probed the animals' accuracy at smaller ILDs and also introduced the ILD of 0 dB to observe a measure of bias. Animals reached similar performances for the previously probed ILDs in this block configuration, and the performance for the smaller ILDs follows the normal ones, showing a

continuous progression of performance with interaural level difference. At ILD of 0 dB, animals had responses close to chance level as expected (Fig. 3.3, F.). These findings show that accuracy in our task is not significantly limited by the rats' uncertainty about the indifference point, nor by stimulus priors. They also show that accuracy is not limited by motivation, which provides further evidence that rats are performing the task at psychophysical threshold.

3.4.2 Anticipatory Choices are Present in the Behaviour

From Figure 3.2 C. and D. it is apparent that we only started to plot the RTDs for RTs longer than 90 ms. The reason for this is that, as we now show, below this value, RTs are not stimulus dependent and seem to reflect anticipation. In order to show this, we looked for the minimum RT for which there was evidence of condition dependence. To this end, we compared the distribution of RTs corresponding to the two conditions with the shortest (ILD= 6 dB, ABL = 60 dB SPL) and the longest (ILD = 1.5 dB, ABL = 20 dB SPL) mean RT (see Methods section in Pardo-Vazquez et al., 2019). Starting at 50 ms, we systematically included longer and longer RTs. As evident in Figure 3.4 A. and B., if the maximum RT is sufficiently short, the two distributions are not significantly different, but for RTs > RTmin = 90 ms, they become different. Additionally, this value for the RTmin does not seem to change if determined based on the ABL instead of ILD (Fig. 3.4 C.).

These anticipatory trials would have been aborts but due to processing delays (both sensory delays, reflecting the time between stimulus onset and the beginning of evidence accumulation, and motor delays, reflecting the time between decision commitment and our ability to register the onset of movement, i.e., the moment where the infrared bean in the central port signals port exit) can have positive Reaction Times, and thus still be valid trials. Therefore, in trials with RT< 90 ms, the decision was made before the sound could influence the 'when' decision.

Previously, for all analyses, we excluded trials with RT < RTmin, and since the shape of the RT distributions is very well-behaved and

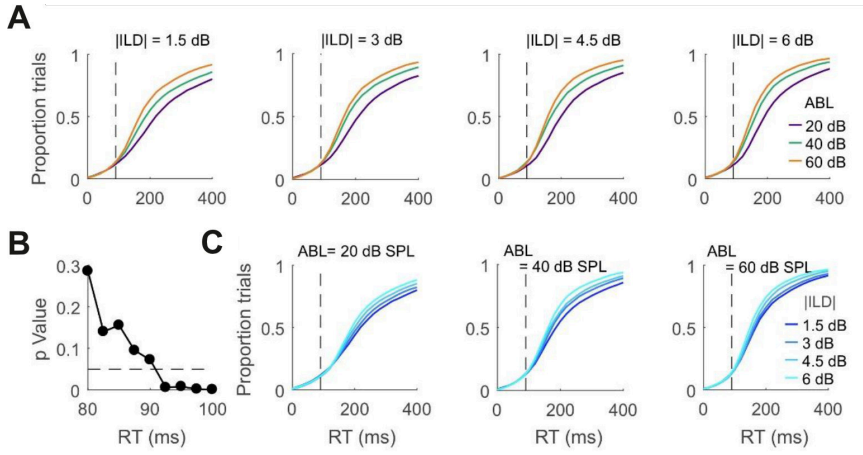

Figure 3.4: **Short RT and processing delays. A.** Each plot shows the cumulative distribution of RT for a given difficulty (absolute value of ILD) for the three ABLs (RTs merged across rats). The shape of the distribution for the shorter RTs is the same across all conditions. This is presumably due to the fact that sound onset after central-port entry was to some extent predictable (fixation period was drawn from a uniform distribution between 300 and 350 ms) and to the fact that we did not impose a minimum RT. These short, condition-independent RTs are thus the result of anticipation. **B.** To detect the earliest time where RTs become condition-dependent, we run a two tailed Kolmogorov-Smirnoff test comparing the RTs for the fastest and slowest conditions ($|\text{ILD}|$ = 6 dB and ABL = 60 dB SPL versus ($|\text{ILD}|$= 1.5 dB and ABL = 20 dB SPL) as a function of the maximum RT included in the comparison. This panel shows the p-value of the test. We defined RTmin as the value at which this comparison becomes significant (90 ms, $p<0.05$). This value is shown as a dashed vertical line in (A) and (C). Since we are interested in the stimulus-dependence of RTs, we excluded from all analyses RTs smaller than RTmin. **C.** Same as A, but comparing the cumulative RTDs across ILDs for each ABL separately. As expected, the distributions start to diverge later for fainter sounds. However, it is obvious that this unspecific intensity-dependent delay cannot account for the changes in the RTD as a function of ABL shown in (A). None of our results changes qualitatively if we define a separate RTmin for each ABL.

understood, we also excluded trials that had exceedingly large RTs, which presumably reflect disengagement. For all analyses except model fitting, we chose a conservative value of RTmax = 1,000 ms (the fraction of trials with RT > 1,000 ms was always very small: 0.39 ± 0.39%, mean ± s.d., across rats) (Pardo-Vazquez et al., 2019).

Removing these choices allowed us to focus our analysis on trials that were stimulus-dependent. However, rats continue to exhibit anticipatory behaviour, and our study aims to investigate the underlying mechanisms driving this phenomenon.

3.4.2.1 Increasing Fixation-Time Variability Decreases Stimulus-independent Responses

The same process that gives rise to stimulus-independent but valid trials will also cause a number of aborted fixations – trials in which the animals left the central port before the sound started and were considered invalid. We plot Reaction Time Distributions such as the ones in Figure 3.2 C. but not removing trials for RT < 90 ms (Figure 3.5, panel A. left column, with uniform fixation) and it is noticeable that at RT = 0 ms, responses start already above 0 and ramping up, indicating that the animals performed anticipatory trials before sound onset that we were not prepared to analyse at the time. In order to further study these anticipatory trials, we manipulated the Fixation Time for subsequent cohorts trained in this task. We trained a number of control animals with Uniform Fixation time in order to verify the results were robust for this setting, but to decrease the animals' ability to predict sound onset and thus anticipate, we modified the Fixation Time statistics turning them exponential (Figure 3.5, panel A. centre and right columns). Exponential fixation periods correspond to a constant hazard rate for the stimulus to take place. Thus, these are the conditions where stimulus onset is the least predictable given a certain rate of occurrence. For these cohorts, we kept data for aborted trials, which correspond to the negative portion of the RTD).

The WL RT distribution was very accurately recapitulated for the new cohort, with uniform FT (200 ms base Fixation Time and added

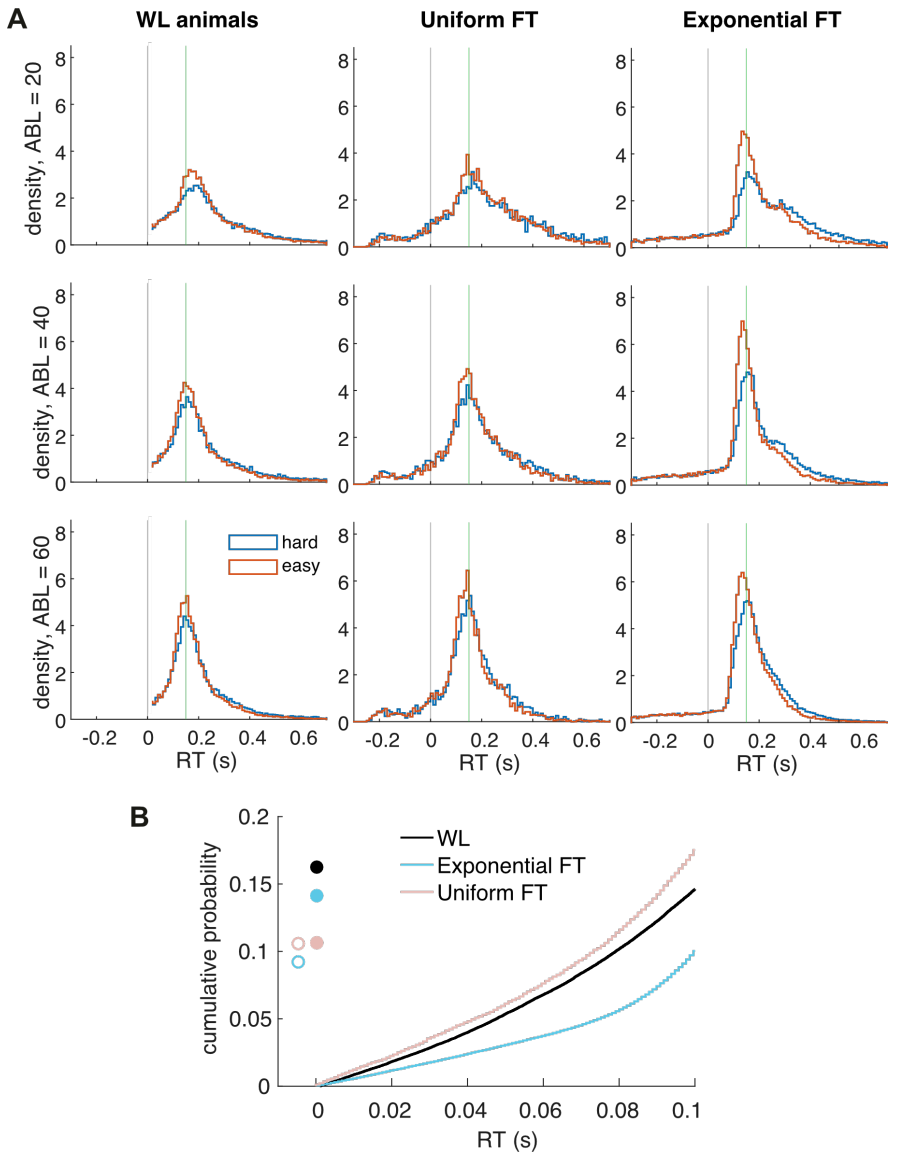

Figure 3.5: **Effect of Fixation Time on Aborts and Reaction Time Distributions A.** RTDs for the different cohorts according to fixation time statistics, WL (N = 5 rats), uniform FTs (N = 4 rats), and exponential FT (N = 19 rats). Trials are divided by ABL (rows) and into hard (ILDs of 1 to 3 dB) and easy (ILDs of 4 to 8 dB) and each row corresponds to one ABL.

Figure 3.5: (Continued caption) The green vertical line marks the 150 ms mark and the black vertical line marks the onset of RT or sound and so, negative RTs correspond to aborts. **B** Cumulative distribution function (CDF) for RTs of the different cohorts as in A. The markers indicate cumulative distribution of aborts, RT<0, also for the three cohorts. The filled circles show the cumulative distribution throughout the entire fixation time for all cohorts, and the open circles focus on the time period both uniform and exponential fixations have in common (250 ms before stimulus onset).

period sampled from a uniform distribution from 0 to 50 ms – Fig. 3.5 A. central column). The shape is very similar and for these animals we can verify that the distribution does indeed start rising before sound onset, showing a small bump at the very beginning of fixation and eventually a steady increase, with sound onset seemingly not eliciting a large ramp up.

Conversely, when we increased FT variability (randomly added time was sampled from an exponential distribution with mean of 200 ms and cut-off at 1 s, see Methods) the distributions suffer a clear change. Although aborts are still present before sound onset (Fig. 3.5 A. right column), they decrease in these conditions (Fig. 3.5 B. circle markers show fewer aborts when time-matched to the uniform cohort in terms of fixation time – open circles). Furthermore, aborts are no longer continuously rising through sound onset, and instead they have a much shallower ramp until after sound onset, at which point there is a steep increase in the density of the distribution. This can also be observed in Figure 3.5 B., where the CDFs for the different cohorts show how the curve is much less steep for the one experiencing exponential fixation (blue). The steep increase of the RTD for more variable Fixation Times indicates a clear moment at which the stimulus information begins to motivate responses, the temporal difference between stimulus onset and this steep increase of the RTD consistent with the presence of sensory and motor delays. RTDs for exponential Fixation Times also show a larger difference between hard and easy trials than for less variable Fixations,

which might indicate a larger dependency on the stimulus.

Although short Reaction Times are clearly decreased, they are still present, as evidenced by the RTDs for exponential FT (Fig. 3.5 A. right column), these were not abolished with a more variable FT. Furthermore, Tachometric functions, showing the relationship between accuracy and RT, show a clear difference in the probability of a correct response from very early RTs (Fig. 3.6) – at which point they are still not stimulus dependent (Fig. 3.5 A.) This aligns with what was noticed by Hernández-Navarro et al., 2021 – for very early RTs (express responses) the tachometric rises until a maximum and then plateaus for the remaining responses (nonexpress). Much like what we see, their responses follow two modes: express responses, where the timing was independent of evidence accumulation, but the choice did depend on the stimulus; and slower responses, where both the timing and the choice accuracy depend on the stimulus. As mentioned before, these very early responses are incompatible with standard DDMs and extensions because these models inherently rely on the Decision Variable (DV) reaching a bound to trigger the response. This predicts that if the response is stimulus-dependent, so is the RT (Bowman et al., 2012; Drugowitsch et al., 2012; Hernández-Navarro et al., 2021; Thura et al., 2012); and also that a response may only be stimulus-dependent after the sensory and motor delays have elapsed (non-decision time), at which point the animal can make use of the evidence and act (Hernández-Navarro et al., 2021). Slower responses, on the contrary, are compatible with the standard DDMs as they can be triggered by the evidence accumulation process. This indicates there might be more processes at play – other than the perfect accumulation of evidence the TIED requires – ruling the animals' performance in our task. In fact, the TIED predicts constant accuracy throughout RT, but that does not seem to be the case, as tachometrics do not always plateau in our data, but seem to decrease for longer RTs (Fig. 3.6).

This agrees with the hypothesis that proactive and reactive choices might coexist in our behaviour. If this is so, these proactive choices are not only present in the form of aborted decisions or short RTs and will take place throughout the RTDs. The interplay between reactive

and proactive processes might be the cause for the difference in the shape of the RTD that can be noticed for the different fixation statistics (Fig. 3.5 A.). While for Uniform Fixation the RTD is unimodal, for Exponential Fixations the distribution presents as bimodal, especially for fainter stimuli. This might indicate a second process taking place besides the expected choice process. In fact, changes in the fixation statistics and the corresponding stimulus predictability might unveil different neural pathways connecting sound to action, similarly to the differences in saccadic RT distributions observed between the standard and GAP conditions (Carpenter, 2001; Carpenter & Williams, 1995), or might alternatively unveil the effect of time-varying sensory evidence on RTs (see Discussion).

3.4.3 Changes in Fixation do not Jeopardise the Behavioural Phenotype

In order to further explore these processes using this task, we required it to remain robust. After changing the fixation time statistics, the behaviour of the animals suffered little change. In order to further characterise this behavioural task and continue to study decision-making, we have trained several more rat cohorts following the two sets of animals mentioned in Pardo-Vazquez et al., 2019 (cohorts A and B in Methods). In Figure 3.7, some of these cohorts are represented. In order to do away with fixation time aborts due to the animals anticipating the sound onset, we have changed the statistics of the FT and made it more variable. This decreased aborts and early reaction times. We have also compared animals experiencing uniform and exponential FTs in terms of their accuracy and RT. In Figure 3.7, panels C. and D. we compare the WL cohort with a cohort from which some of the animals experienced uniform fixation time (minimum of 200 ms and a randomly added period from a uniform distribution from 0 to 50 ms) and others experienced exponential fixation time (minimum of 200 ms and a randomly added period from an exponential distribution of mean 200 ms and cut at 1 s). We can see that while fixation being uniform or exponential, on its own,

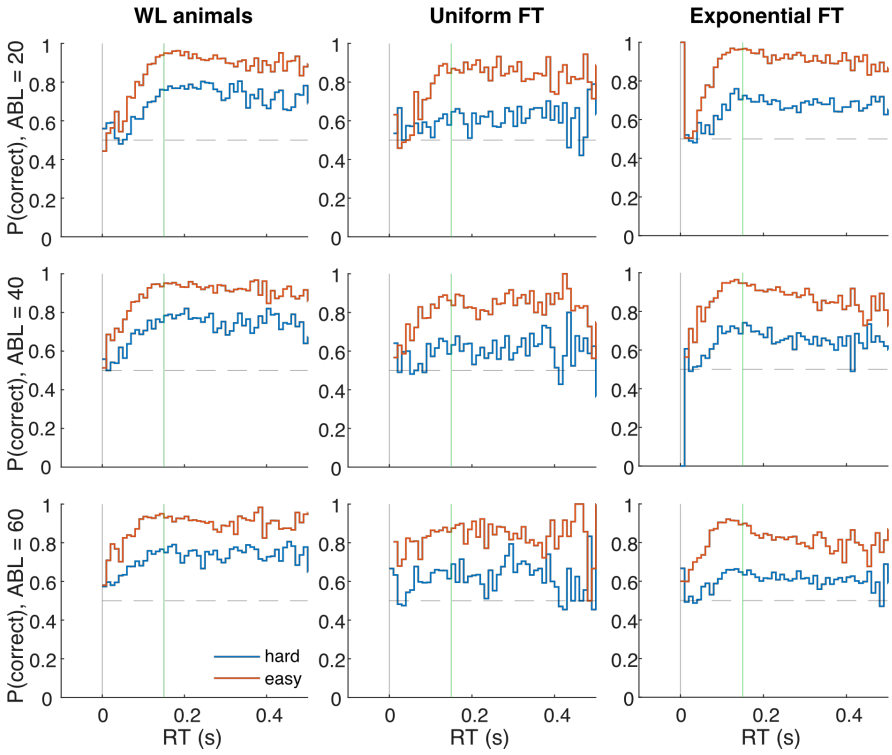

Figure 3.6: **Tachometric Functions for the different Fixation Time statistics** Probability correct over Reaction time for the different cohorts according to Fixation Time (columns): WL (N = 5 rats), uniform FTs (N = 4 rats), and exponential FT (N = 19 rats). Trials are divided by ABL (rows) and into hard (ILDs of 1.5 and 3 dB for WL; and 1 and 2 dB for remaining two cohorts) and easy (ILDs of 4.5 and 6 dB for WL; and 4 and 8 dB for remaining two cohorts). Although accuracy is lower for harder stimuli in the two new cohorts when compared to WL, these were performing with harder stimuli. Green lines mark the peak of the corresponding RTD.

does not seem to affect accuracy (fig. 3.7 C.) it is possible the minimum duration has an impact, seen as the animals in pink show slightly less accurate performance. This is also visible at the level of RT, as animals experiencing, on average, the shorter amount of fixation time seem to show a different trend with regard to their RT.

However, overall, FT does not seem to be a jeopardising factor for accuracy in this behavioural task. Animals still maintain high levels of accuracy and the speed-accuracy trade-off that characterised the initial behavioural cohort.

3.4.4 Different Cohorts Reach Similar Performance in the Task

In order for a behavioural task to be useful in the study of decision-making and its neural underpinnings, it is required to be robust and highly reproducible. Training must be repeated with several animal cohorts in order to test a multitude of parameters and manipulations. However, in our case, the behavioural phenotype that allowed for the initial mechanistic explanation of the processes underlying the responses needs to be replicated for each new cohort in order to explain the potential differences that originate from the task manipulations. To this effect, we employ a consistent training protocol to all animal cohorts, regardless of the manipulations to the original task they might afterwards experience. This protocol is detailed in the Methods section, but briefly, all cohorts experience fully lateralised sound until they learn the basic contingencies of the task. Afterwards they undergo surgical implantation of the headphone base and after recovery experience ILDs that are progressively decreased until reaching a minimum. This process is common to all groups, and as such, we believe all batches are comparable at this level, but we have also tested several changes such as introducing both smaller and larger ILDs, new ABLs and modifying the Fixation Time.

We show that different animal groups reach similar levels of performance (fig. 3.7 A. and B.) focusing on the final training level, when we believe the rats to be at psychophysical threshold, and before any other manipulations. Batch B would undergo changes in the statistics of trial

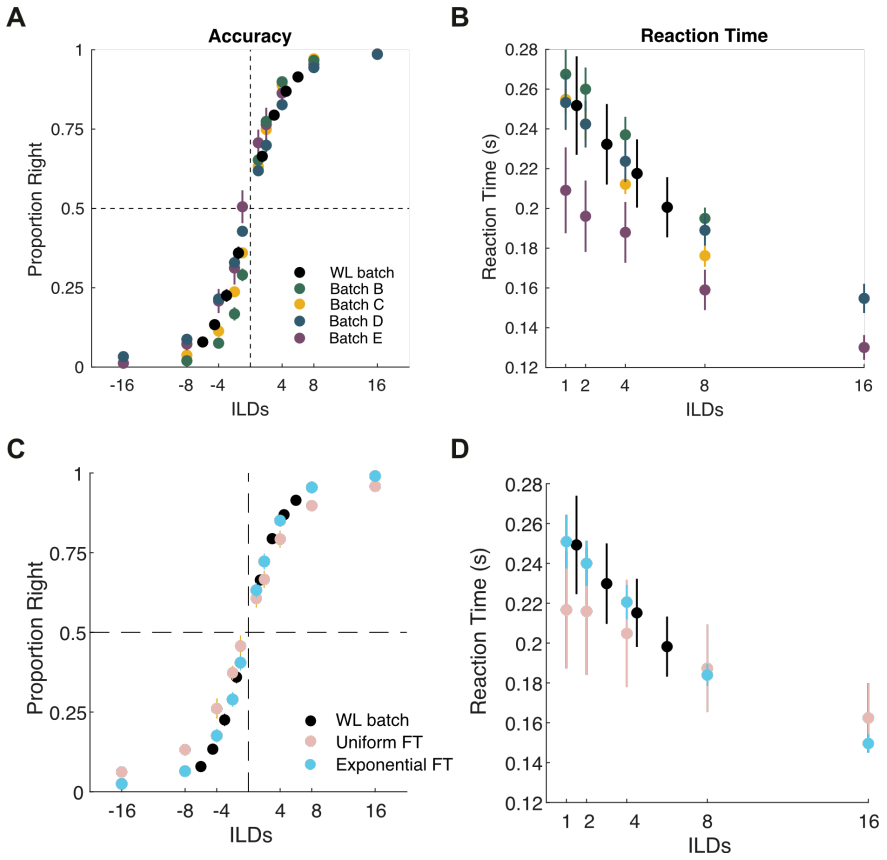

Figure 3.7: **Performance comparison between batches trained in sound lateralisation A.** Choose-right probability (mean ± SEM across rats) for five different batches of animals trained in the sound lateralisation task previously described. WL denotes the batch utilised for the study of WL (A1 in methods, black markers, N = 5 rats); batch B, block type B6 (green markers, N = 5 rats); batch C, block type C1 (yellow markers, N = 6 rats); batch D, block type D1 (blue markers, N = 15 rats); and batch E, block type E1 (purple markers, N = 5 rats). Error bars correspond to the standard error of the mean across animals. Data corresponds to all ABLs the animals experienced between 20 and 60 dB, which corresponds to 20, 40 and 60 dB for all batches except batch E, which experienced only 50 dB and batch B, which experienced 25, 40 and 55 dB ABLs.

Figure 3.7: (Continued caption) **B.** Reaction time (mean ± SEM across rats) as a function of difficulty separately for each batch from A.. **C.** Choose-right probability for the same animals as A. but divided according to their fixation times, compared once more to WL animals. Uniform fixation animals, in pink, had an FT of 200-250 ms (N = 4 rats). Exponential fixation animals, in blue, had a FT with a 200 ms base and randomly added period from an exponential distribution of mean 200 and maximum of 1 s (N = 19). **D.** Reaction time (mean ± SEM across rats) as a function of difficulty separately for each group from C.

difficulty, the noise shown for each trial and experience more extreme ILDs and ABLs after the sessions we focus on for this section; batch C would be tested with capped sound durations; batch D would undergo optogenetic manipulations and batch E would undergo manipulations of the stakes of each trial. But for these manipulations to allow the application of our model and analysis as previously shown, we had to ensure they are able to recapitulate the basic behavioural phenotype.

Animals reach similar levels of accuracy in the task regardless of cohort (fig 3.7 A.). These different animal groups have been trained with different ILDs, ABLs, and also in a different behavioural setup than the initial five rats from the WL batch, and yet have comparable accuracy, indicating that this is a very robust task. Latter batches (D and E) have had ILD = 16 dB added to their stimulus list. This more extreme ILD allows us to saturate the accuracy curve and to notice that our animals show an absence of lapses. The speed accuracy trade-off that was clear in the WL batch is still present for the remaining cohorts (fig. 3.2 B.) and indeed RTs are very similar between the different groups. We theorise batch E (batch that experienced manipulations to alter the stakes for each trial, see Methods) might have shorter RT simply because they only experienced an ABL of 50 dB, while for other batches we are showing the average over several ABLs, the smaller of which will correspond to longer RTs.

Dividing animals not according to cohort but according to their Fixation Time statistics also shows that the performance for this task is

robust and can be replicated (Fig. 3.7, panels C. and D)

3.4.4.1 Level Invariance is Maintained

Besides maintaining a comparable level of performance, it was important for our analysis and modelling that animals maintained the level invariance that characterised the WL group and allowed us to formulate the TIED. Under the PSIAM framework, there are two processes that make up the RTDs, the proactive and reactive process. Because the proactive process is not stimulus dependent, it will resemble a non re-scalable portion added to the RTD. Therefore, we do not expect the TIED to be verified as precisely as it was before in Pardo-Vazquez et al., 2019. However, since we hypothesise that there is a much larger number of reactive trials than proactive trials, the reactive component should dominate the RTD, ensuring an approximate rescaling

We continued to divide the animals according to the statistics of their FT for this analysis, and we show that both these cohorts show similar performance for the three studied ABLs (Figure 3.8). Both groups show overlapping levels of accuracy for all three ABLs over all the difficulty levels (fig. 3.8 A.) and show both the speed-accuracy trade-off and the ABL dependency as seen in the WL cohort (fig. 3.8 B.). The newer cohorts could, despite their level invariant performance, show differing performances when compared to each other, and it seems to be the case that Exponential Fixation Time animals are more comparable with the WL cohort (fig. 3.8 C. and D.). This difference to the Uniform Fixation Time group could be related to the fact their fixation is overall the shortest (200-250 ms), which might promote a larger preponderance of anticipatory trials, causing lower performance and shorter RTs. In line with this, we found that although these new RTDs are not rigidly stretched versions of one another like the WL data, we manage to approximate a rescaling that works well across conditions.

Contrary to what we did in Pardo-Vazquez et al., 2019, for these data we rescaled the RTDs for easy and hard ILDs separately, as shown in Figure 3.9. We first applied this new method to the WL data (Fig. 3.9 A.)

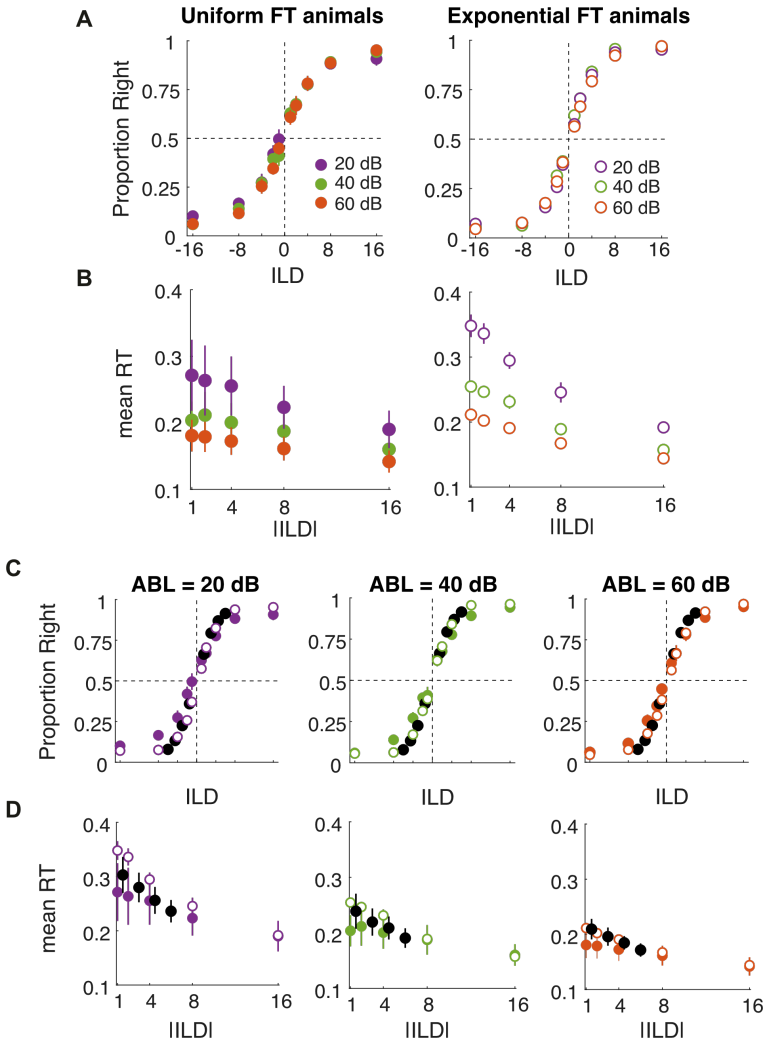

Figure 3.8: **New animal cohorts maintain performance across ABLs A.** Choose-right probability (mean ± SEM across rats) for animals experiencing uniform and exponential fixation (N = 6 rats and N = 15 rats, respectively) divided by ABL. Accuracy for the different ABLs tends to overlap regardless of the difficulty of the stimulus, much like for the WL animals. **B.** Reaction time (mean ± SEM across rats) as a function of difficulty for each ABL separately, like A..

Figure 3.8: (Continued caption) **C.** Choose-right probability (mean ± SEM across rats) comparing the different cohorts' performance from A. and B. for each ABL with the performance from the WL cohort. Black circles corresponding to the WL batch, open circles to exponential fixation animals and filled circles to uniform fixation animals**D.** Same as C. but for Reaction Times.

in order to verify that it would still show perfect scaling. We then applied it to the newer cohorts, Uniform FT cohort (Fig. 3.9 B.) and Exponential FT cohort (Fig. 3.9 C.). For neither of these groups of animals is the rescaling as perfect as the one we showed before for the WL animals, but it is still a good approximation of what seems to be taking place. For the Uniform FT cohort (Fig. 3.9 B.), rescaling seems to resemble the WL level, probably due to the fact that the shape of the distributions is also more similar between these two groups. For the Exponential FT animals though, because the shape of the RTDs is visibly distinct –the bimodality is a deviation from the "standard RTD shape" and only present for these animals – a rigid stretch of the distributions does not manage to perfectly rescale them (Fig. 3.9 C.). It seems this bimodality does not allow for the RTD of an ABL to be rescaled by a constant value into the RTD of another, and there seems to also be some scaling of the ILD as a function of the ABL which was not the case for the WL animals. However, we believe this constitutes a good approximation for the moment, and we will continue to analyse this issue.

This suggests the TIED (changes in the absolute intensity of two stimuli being discriminated under a fixed intensity ratio are completely equivalent to a change in the effective unit of time with which the discrimination duration is measured) is also a very robust feature of the animals' behaviour and is generally verified when performing in this task.

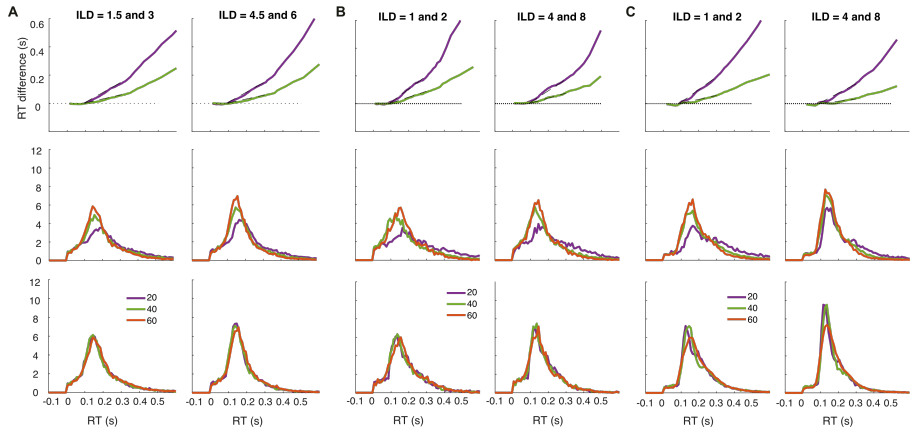

Figure 3.9: **Quantile-Quantile plots and RTD rescaling for Uniform and Exponential Fixation cohorts A.** Quantile-Quantile plots and RTD rescaling for WL cohort (N = 5 rats). First row: Quantile-Quantile plots of the difference of the quantiles of the lower ABLs (20 and 40 dB respectively) minus the highest ABL (60 dB) as a function of this same higher ABL quantiles. The dash lines represent the linear fit on the range of 90 to 300 ms, which was used to estimate the slope for rescaling. Middle row: RTDs for the different ABLs. Bottom row: Rescaled RTDs. The RTDs for 20 and 40 ABL were rescaled using the above linear fits to match the RTD for ABL 60. The two columns represent hard stimuli (absolute ILDs of 1 and 2 joined) and easy stimuli (absolute ILDs of 4 and 8 joined). **B. and C.** Same as A. but for the Uniform Fixation cohort (N = 4 rats) and Exponential Fixation rats (N = 15 rats) respectively. While this rescaling is not perfect, it is a good approximation, even for the Exponential FT cohort, especially at the level of the tail of the RTD.

3.5 Discussion

We have demonstrated that our sound lateralisation behavioural task is a powerful paradigm in the study of decision-making and its neural basis due to the possibility to systematically manipulate the sensory evidence presented while maintaining precise control over the different parameters of the experiment. Our task, like other widely used perceptual behaviour tasks for the study of decision-making (Brunton et al., 2013; Jaramillo & Zador, 2010; Palmer et al., 2005), allows for controlled manipulation of the sensory information being presented to the subject, in our case,

by manipulating two independent axes of the stimulus, the difference between the sounds presented to the two ears (ILD) and the overall level of the sound (ABL). We have a high degree of control over the level of perceptual difficulty for each trial, and so we can attempt to accurately describe how sensory evidence is detected, accumulated, and processed until a decision in made. This deep understanding of the process can then be used to inform computational models to further describe the mechanisms behind the decision process and formulate regularities such as the TIED (Pardo-Vazquez et al., 2019).

We focus on the interplay between overall stimulus intensity and stimulus discriminability, which has been a key question in psychophysics, and forms the foundation for Weber's Law. This connection motivated us to analyse our results in relation to classical psychophysical principles, highlighting how perceptual decision-making is shaped by the relationship between stimulus strength and discriminability. Modern studies of decision-making have largely overlooked this issue. Some notable exceptions include studies by Scott et al., 2015, Palmer et al., 2005, and Koay et al., 2020. Our study aims to bridge this gap, bringing psychophysical principles into the contemporary understanding of perceptual decision-making.

Similarly to other tasks, the quantitative nature of our lateralisation task provides rich datasets capturing parameters of both accuracy and response times across all the difficulty levels. These data may then be used for the fitting of computational models, such as Drift Diffusion Models (DDMs), to describe how sensory information is dynamically accumulated to reach a decision threshold (Bogacz et al., 2006; Forstmann et al., 2016; Ratcliff et al., 2016).

We first summarised the main behavioural results in Pardo-Vazquez et al., 2019 and then we have shown that the rats' performance in this task is very robust, as the characteristics of accuracy and Reaction Time were conserved through multiple animal groups, changes and manipulations of both task contingencies and stimuli. We have managed to train multiple animal cohorts in this task, and of all the animals less than 3% were unable to perform above chance level in the task. It is also quick to

train, as it takes the animals usually less than two 2-hour sessions to understand the contingencies of the task and about three weeks to reach the final level of training (one week is spent in surgical recovery after the headphone base implantation). This is significantly faster than some similar tasks (Brunton et al., 2013), but in line with others (Jaramillo et al., 2014; Kane et al., 2024; Laboratory et al., 2021), and it might be related to the simplicity of the task. Furthermore, multiple batches, although trained in different behavioural setups, at different times and in different contingencies of the task (be it regarding timings, the type of stimuli used, or reward rate) have managed to reach similar levels of performance and perform hundreds of trials per session, in line with similar tasks (Jaramillo & Zador, 2014). This degree of reproducibility and high throughput is essential, as the use of a behavioural task for the study of decision-making benefits from a high amount of data in order to form a complete picture of all the strategies the subjects might be using and how these computations might be taking place. Only with high amounts of data are we able to accurately fit computational models of decision-making that allow us to better understand the mechanisms that underpin this behaviour.

Rats manage to consistently reach high accuracies and show an absence of lapses in the task, as well as a clear speed accuracy tradeoff in their reaction time, both at the level of ILD and ABL. This has been verified in all the cohorts we have trained, as well as at the level of the individual animals. With the initial cohort, we realised the animals were predicting the time of sound onset and exiting the central port as fast as possible. For some trials this culminated in an abort, as they would leave the port even before the sound had started, but for others, the trial was valid even though the response was not sound dependent. To minimise these trials, we made the fixation time longer and more variable and managed to decrease the amount of such aborts and stimulus-independent trials.

Higher uncertainty on the timing of stimulus onset does not fully eliminate stimulus-independent responses, and we hypothesise these might correspond to an inherent part of the behaviour that was previously

excluded from our framework. The framework, detailed in Hernández-Navarro et al., 2021, deems these responses as proactive and proposes their coexistence with reactive responses (Hawkins & Heathcote, 2021; Hernández-Navarro et al., 2021). In future chapters we will examine them in more detail and attempt to integrate them in our modelling of the task as opposed to excluding them from the analysis as before. Our hypothesis is also that these proactive responses are the explanation for the most observable difference between the WL animals and the subsequent Exponential FT ones – the differences in the shape of the RTD. For the WL cohort, potentially due to the Fixation Time statistics, fixation aborts and early RTs were significant and might have corresponded to proactive trials due to a predictable stimulus onset (reproduced for a new cohort with Uniform Fixation). Conversely, the animals that experienced Exponential Fixation Time showed a smaller number of fixation aborts and a sharper RTD, suggesting a decrease of the amount of proactive responses in the early portion of the RTD. However, the entire shape of the distribution changed for these animals and a bimodality arose. We cannot, at this time, fully explain or reproduce this bimodality through modelling, even including the proactive process. We do, however, have some hypothesis on what might be causing it. On one hand, it is possible that the early proactive responses are abolished by the highly variable Fixation Time, sharpening the peak of the RTD, but leaving later proactive responses to create a second peak that would have been otherwise occluded within the rest of the distribution. On the other hand, these responses might also correspond to different neural processes and parallel pathways that require different computing times. These different types of responses would originate due to the more variable Fixation time the more recent cohorts experienced, this variability making it, so some trials would have their RT stimulus dependent, while others would be stimulus independent. Variability in the temporal predictability of a stimulus has been shown to cause bimodality at the level of the distributions before (Carpenter, 2001; Carpenter & Williams, 1995), where express saccades occur soon after stimulus onset, before the standard saccades, and, contrary to our scenario, in conditions where subjects

can accurately predict the onset of the stimulus (e.g., the gap paradigm) (Carpenter, 2001). It is, however, accepted these are reactive responses dependent on the stimulus (Fischer & Weber, 1993), which is probably not the case for our early responses due to the sensory and motor delays that are part of a sensorimotor response that limit just how fast an animal can actually react to a sound, and which might in fact be exacerbated by the high variability of stimulus onset time (Nobre et al., 2007; Vangkilde et al., 2012).

Furthermore, it is possible that the second peak is due to the sensory evidence not being constant: Even though our stimulus has no temporal structure, sensory neurons have time-varying activity profiles even when the sensory stimulus is constant. In fact, preliminary evidence (data not shown) shows that the shape we observe for the RTD naturally and robustly emerges if the sensory evidence has an initial peak and a subsequent trough, before reaching steady state, a very reasonable assumption (Carland et al., 2015; Urai et al., 2019).

Towards our goal of establishing this task as one of the gold standards for the study of decision-making and leveraging the fact that our animals are freely moving while behaving, we have also started exploring capturing video from behavioural sessions to track the animals' movements and quantify their responses, not just in terms of movement time and choice, but also regarding trajectories and the dynamics of the movement and how it is affected by the stimulus. Studying the movement dynamics and trajectories of the rats' responses might help us understand what distinguishes proactive and reactive responses. Currently, we are able to know when the animal leaves the central port; but we have no access to the movement preparation before this choice to leave. It is possible that proactive and reactive choices already show key differences even before our detection of the animals' movement, and access to this data will show it. It is also possible proactive choices involve a type of provisional choice that the animal then updates with the sensory evidence acquired, which might cause updates to the trajectories during movement already, culminating in changes of mind (Boyd-Meredith et al., 2022; Kaufman et al., 2015; Kiani, Cueva, et al., 2014; Molano-Mazón et al., 2024; Peixoto

et al., 2021; Resulaj et al., 2009; van Den Berg et al., 2016; Visser et al., 2023), and which we are currently unable to access.

Crucially, the cohorts trained in these distinct conditions show similar performance between themselves and to the original batch. Differences registered between the WL batch and the Uniform Fixation batch are thought to be due to the overall duration of Fixation, as WL has longer base fixation and due to low N. Importantly, even with these differences in fixation time and the corresponding differences in RTDs, the animals' performance still shows level invariance and the RTDs are still re-scalings of one another, providing further evidence of the robustness of this task.

The ability to observe a mathematical regularity in animal behaviour in response to a task, and to fit a simple model to explain this behaviour, allows us to infer latent cognitive processes underlying decision-making and how they adapt to changing parameters and conditions. A good understanding of the computations underlying the decision process will allow us to more confidently interpret the role of specific brain regions and the meaning of neural correlates we might record. This makes the simultaneous measurement of physiological and behavioural information a logical follow up in order to understand the neural underpinnings of this task.

3.6 Acknowledgements

We thank the vivarium staff for their help maintaining the animals and all the members of the lab for their input, especially Gökçe Dogu for the help establishing video tracking for future work.

3.7 Author Contributions

Alfonso Renart (A.R.), José Pardo-Vazquez (J.L.P.-V.), Juan Castiñeiras (J.R.C.-d.S.) and Mafalda Valente (M.V.) designed the experiment, M.V. performed the experiments, and together with Tiago Costa (T.C.), all analysed the data.

4

Effect of Reaction Time manipulations on a sound source lateralisation task

4.1 Summary

Understanding how temporal dynamics influence decision-making is crucial for building mechanistic models of perceptual processes. In the context of the Drift Diffusion Model (DDM), Reaction Time (RT) reflects the time taken for a Decision Variable (DV) to reach a decision bound, and for our sound lateralisation task, the Time-Intensity Equivalence in Discrimination (TIED) posits that louder sounds accelerate the accumulation of evidence, allowing faster decisions. According to this, prematurely stopping auditory stimuli should disrupt the performance invariance observed across different Absolute Binaural Levels (ABLs), leading to reduced discrimination accuracy for short stimuli. To test these predictions, we conducted experiments with imposed maximum sound durations, some shorter than typical RTs. Our results demonstrate that discrimination performance decreases significantly for shorter stimulus durations in an Average Binaural Level (ABL)-dependent manner, confirming the predicted breakdown of Weber's Law (WL). However, we had not explored these results in detail yet. In spite of the breakdown

of WL, animals were still able to reach surprisingly high accuracy levels, even for very short stimuli. Furthermore, the animals remained in the central port for longer in these conditions, suggesting a shift in the decision-making process when sensory evidence is limited.

This chapter explores how limiting the stimulus duration affects perceptual accuracy and RT, drawing upon the framework proposed by Pardo-Vazquez et al., 2019, which emphasizes the role of time in shaping choices. Furthermore, we succeeded in adapting our previous model to account not only for these short duration stimulus trials, but also for the short Reaction Times we were previously discarding, highlighting the presence, and importance, of anticipatory behaviours in our task.

4.2 Introduction

Perceptual tasks in which the subject can be in control of the stimulus duration can be referred to as Reaction Time (RT) tasks and differ from those with fixed-duration stimuli as they allow subjects to control the timing of their responses, thus providing a clear readout of the moment of decision commitment. In a task where accuracy is emphasised, subjects might choose to gather evidence for longer periods of time in order to increase their chances of a correct response (Brunton et al., 2013; Funamizu, 2021; Kiani et al., 2008; Recanzone & Beckerman, 2004; Simen et al., 2016; Teodorescu et al., 2016). In natural environments, however, stimuli are rarely presented for extended periods nor is an animal required to withhold its decision until the entire stimulus has elapsed. Similarly, it is also uncommon for animals to have complete control over the duration of the stimulus presentation. However, for the study of decision-making and when focus is placed on performance, it is common to impose a certain period of stimulus exposure (for instance, Erlich et al., 2015; Jaramillo and Zador, 2014; Jaramillo et al., 2014; Nashaat et al., 2024; Parker and Newsome, 1998).

Within the framework of Drift Diffusion Model (DDM), the RT is defined as the time elapsed until the Decision Variable (DV) reaches a predetermined bound, leading to a decision (Bogacz et al., 2006; Forstmann

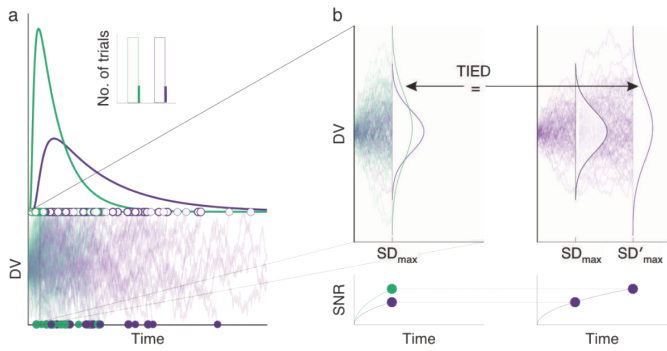

Figure 4.1: **Breakdown of WL for controlled short sound durations. a,** Schematic illustration of the TIED. Sample paths of the DV for two stimuli with a high (green) and low (purple) Average Binaural Level (ABL) but the same Interaural level differences (ILD) are shown. Horizontal lines are the decision bounds. Open circles mark the RT for correct trials, while filled circles mark incorrect trials. The RTDs for the two stimuli are plotted on the top. Because the green stimulus is stronger, the green sample paths rise faster, but if the four conditions we identify are met, the proportion of trials hitting either bound is ABL-independent (inset) and the two RTDs are related to each other by a rigid stretching of time. **b,** Schematic illustration of prediction. For times that are short compared with the typical RTs (rectangle outlined with a broken line in **a)**, the likelihood of hitting either bound is still low and one can focus on the dynamics of the DV ignoring the bound. Left: sample paths and distribution of the DV at the putative offset SDmax of the same two sounds in **a**. Lower left: temporal evolution of the signal-to-noise ratio (SNR) of the DV for the two stimuli up to that time. Right: the TIED implies that the distribution of the DV for the green (loud) stimulus at SDmax should be identical to the distribution of the DV for the purple (quiet) stimulus at a later time SDmax. Lower right: since the SNR of the sub threshold DV always increases with time under evidence accumulation, performance for the weaker (purple) stimulus at SDmax is expected to be worse.

et al., 2016; Mazurek et al., 2003; Ratcliff, 1980). By imposing a duration for evidence sampling (either shorter, or longer, than average RTs), one can gain insight into how the decision-making process progresses and how a response still takes place even when evidence accumulation is interrupted prior to the DV reaching one of the bounds. However, in these situations, it becomes unclear how, and when, choice is determined. Signal Detection Theory (SDT) (Green, Swets, et al., 1966) proposes decision bounds are not needed in this situation – the DV representing the evidence can just be readout at stimulus end –. This boundless accumulation has also been seen in other works that essentially assumed subjects were able to accumulate evidence as long as it was being presented (Britten et al., 1993; Brunton et al., 2013). However, certain evidence also supports the theory that animals are actually making use of bounds (Ratcliff, 1980) to decide in situations where the stimulus duration was controlled (Kiani et al., 2008). The relationship between choice and Reaction Time in these conditions remains poorly understood, and shedding light onto it is one of the main objectives of this chapter.

Our initial motivation for exploring this issue comes from experiments in our previous study (Pardo-Vazquez et al., 2019) designed to test whether Weber's Law (WL) depends on bounded accumulation of evidence. In particular, in our task, presented in Chapter 3, if the DV reflects the temporal accumulation of evidence and evolves more rapidly for louder sounds, then stopping the stimuli prematurely should result in the break-down of the level invariance typically observed for accuracy across different ABL (Pardo-Vazquez et al., 2019). This is because, according to the Time-Intensity Equivalence in Discrimination (TIED), DV distributions at the offset of two short fixed-duration sounds of different ABL should mirror those of two sounds at identical ABLs but differing durations (Fig. 4.1). Since longer fixed duration stimuli are expected to lead to better performance – higher discrimination accuracy – under evidence accumulation, the TIED predicts that WL should break down for short sounds. In particular, performance for the smaller ABLs should decrease, as fewer instances of the DV manage to reach the bound. This breakdown should also become less pronounced as the fixed durations

become longer, because the likelihood that the DV crosses one of the bounds starts approaching that of RT trials, and from that WL ensues. To test this hypothesis, we conducted experiments using the ABLs previously employed (20, 40, and 60 dB) and imposed several maximum durations for the sound, Maximum Sampling Duration (SDmax). Although animals retained freedom in deciding when to exit the central port, they lost control over sound duration, as it was predetermined to terminate after specific intervals.

Beyond Weber's Law, this dataset provides a unique opportunity to understand decision-making dynamics under conditions that reflect scenarios where sensory information may be incomplete or temporally constrained. To further explore how Reaction Time manipulations affect decisions in this task, one may also explore how decisions are made when evidence is constrained in the opposite manner, forcing a minimum exposure to the stimulus, instead of a maximum. With this goal, we also examine animals' performance in a task paradigm where they were expected to have a minimum RT.

Both of these temporal constraints have been studied before in different perceptual paradigms. For instance, in Kiani et al., 2008, it was shown that decreasing stimulus duration affected performance, decreasing accuracy; whilst increasing it resulted in increased accuracy, but only to a certain point, after which it reached a plateau. Decisions under short stimulus duration might also be related to urgency (for instance in the work of Salinas et al., 2014; Stanford et al., 2010) where it is suggested the perceptual evaluation period of a decision only corresponds to a small portion of the Reaction Time, highlighting the importance of non-decision time such as sensorimotor delays (Drugowitsch et al., 2012). While these works show how decision accuracy behaves for different duration stimuli, they do not fully characterise further performance parameters, such as RT, nor do they contextualise the findings in terms of a regularity such as Weber's Law. We seek to explain what takes place when the DV is still short of the decision bound at stimulus offset, and how this incomplete information may still result in a decision. By modelling our data for several short durations and experimenting different

outcomes for the evidence after stimulus offset, we managed to explain our data, accurately predicting performance both at the level of accuracy and RT. This hinges strongly on accurately determining the moment at which evidence is readout after stimulus offset, and in what way this is done. We determined that, even when stopping short of the bound, DVs for the shorter SDmax still manage to reach it. However, contrary to our initial hypothesis, when the DV does not reach the bound, the decision does not appear to be based on the sign of the DV. Instead, the choice seems to be determined randomly.

Although we accurately explain what seems to be taking place for short durations, further work is necessary to also explain what takes place for longer duration stimuli.

Ultimately, this work aims to contribute to a more nuanced understanding of perceptual dynamics within the framework established by the TIED and of how animals decide when they are not in control of the stimulus duration.

4.3 Methods

All procedures were reviewed and approved by the animal welfare committee of the Champalimaud Centre for the Unknown and approved by the Portuguese Direcção Geral de Veterinária (reference no. 0421/000/000/2019).

Experimental Animals

Experiments were performed on 11 adult female Long–Evans hooded rats. Animals were 12–13 weeks old and weighed between 250 and 300 g at the beginning of their experimental period. They were kept above 85% of the initial weight throughout. All animals were naive to any behavioural tests. Rats had free access to food, but water was restricted to the behavioural sessions, which were conducted during five consecutive days per week. Animals had access to water during the sixth day and were deprived of water for 24 h before each round of five

sessions. No statistical methods were used to predetermine the number of animals or the number of trials per animal, but our sample sizes were similar to those reported in previous publications (Uchida & Mainen, 2003). We used a 'within subject' design in which the animals were tested in all experimental conditions, so there was no need to apply blinding or randomization.

Auditory Stimuli

A percept of lateralization was created by presenting cosine-ramped (10 ms) broadband (5–20 kHz) noise bursts with different intensities to each ear (ILD) (Fig. 4.2 C). For initial training, stimuli were delivered by speakers placed on the side walls of the behavioural box; and for the latter stages of training, through speakers placed in headphones as described in Chapter 2. More details regarding the stimuli and its presentation available in Chapter 3.

Behavioural Apparatus and Headphone design

Rats were trained and tested on the sound lateralization task (Fig. 4.2 A, B) previously described in detail in Chapter 3.

4.3.1 Base Implant Surgery

Similar to what is described in detail in section 2.2.1 of Chapter 2 and in Chapter 3.

4.3.2 Behavioural Task

Rats started a trial by poking in the central port (Fig. 3.1 A) following the end of the Inter Trial Interval (ITI). After a short, variable Fixation Time (FT), the sound was played binaurally until the rat left the central port. Rats had to communicate whether the sound was louder to the left or right ear by poking in either the left or right side ports, respectively. Correct choices were rewarded with a drop of water and incorrect responses

were penalized with timeout, during which the rat was not able to start a new trial (Fig. 3.1 A, B).

Trials in which the rat failed to start a new trial within the start trial waiting time, broke fixation during the FT or failed to poke in either lateral port within the response waiting time were considered aborts. Aborts were repeated after a 1-s time penalty except for the fixation aborts, which had a 5 s time penalty.

Both ABL and ILD were changed pseudo-randomly for every trial. Typically, sessions lasted for 2 h and rats performed between 600 and 1,000 trials.

The task has been described in more detail in Chapter 3.

4.3.2.1 Task variants

This chapter focuses on the Final Training level for all cohorts analysed. This usually corresponds to the level of the final ILD set and different ABLs, but it was modified for the different cohorts in order to test various hypotheses. We modified the basic task in two ways for this chapter:

- Maximum sampling times: capped sound duration experiment with ABL = 20, 40 and 60 dB SPL. The range of maximum sound durations (SDmax) tested was 15, 30, 60, 120, 240, 480 ms and RT. Even though the rats were always free to leave the central port when they chose, the sound stimulus terminated after the selected duration even if the animal remained (Referred to as block type C2 in Chapter 3, as shown in Figure 4.2.

- Minimum Sampling times: minimum RT experiment with ABL = 20, 40 and 60 dB SPL. The animals were required to remain in the central port after the end of the fixation period for a certain duration, effectively providing them with a minimum sampling time of the stimulus. This minimum sampling time was determined on a trial by trial basis, sampled from an exponential distribution of 500 ms minimum and a mean of 200 ms and after this set duration,

stimulus would continue playing if the animal were to remain in the central port.

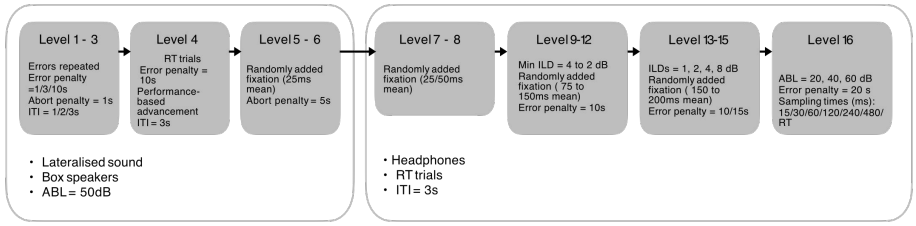

Figure 4.2: **Training structure for Sampling Duration Manipulation Task:** Scheme of the training progression for the animals in the studied task. The initial levels make use of the lateral sound speakers placed in the box, and the animals are exposed to fully lateralised sound only. After being fit with headphones, ILDs are introduced as well as the different ABLs. In the final level, after stable performance under the normal paradigm, as described in Chapter 3, different sampling durations to the sound were introduced.

4.3.3 Model fitting

In order to fit the model, we obtained the expressions for the likelihood of a given trial as a function of the model parameters and the experimental conditions (ILD, ABL, fixation duration, LED onset, if present), which simply put, corresponds to evaluating the theoretical RTDs at a given RT, and for a given choice. We consider it to be outside the scope of this Thesis to reproduce the mathematical expressions, but the way to obtain the combined likelihood from the race between the proactive and reactive process is explained in the supplementary material of Hernández-Navarro et al., 2021. The likelihood of reactive process following the TIED formulation is detailed in Pardo-Vazquez et al., 2019 (supplementary material). And the expressions for the distribution of the DV within the bounds are found in Ratcliff, 1980. We are currently finishing developing a bayesian pipeline to fit the parameters of the model in an automatized and fully principled way. For this Thesis, however, we opted for a simpler and more straightforward approach: we

manually varied the parameters of the model in order to obtain a good match between the empirical quantification of the data (RTDs, quantiles, psychometric and tachometrics) and the corresponding estimates from the model. This process, while not providing the same precision as an automated fitting, still was able to provide reasonable estimates of the parameters from which we could draw conclusions. In some cases, we only varied a small subset of parameters while keeping the rest fixed.

4.4 Results

4.4.1 Weber's Law breaks down for short duration stimuli

In order to study in more detail the behaviour in our task under conditions of limited Reaction Time, we first reproduced the results already shown in Figure 3 of Pardo-Vazquez et al., 2019. We tested the rats with more sound durations (SDmax) than contemplated in the published work, further probing the behaviour at both shorter and longer sampling times.

Discrimination accuracy degraded for short sampling times, for all ABLs, but it degraded faster for the lower ABL (Fig. 4.3). Our data show that the animals' performance is greatly decreased for the shorter durations and that it does not maintain its level invariance (Fig. 4.3 A.). It is noticeable that psychometric curves for the different ABLs no longer overlap as they did for the trails showing level invariance in Chapter 3 or in Pardo-Vazquez et al., 2019. This variance between the different ABLs is larger for the shorter SDmaxs, indicating animals are unable to reach the same level of performance for the lower ABLs when prevented from accumulating evidence for the required time.

The breakdown of WL for short, fixed-duration, stimuli but not RT choices is a good indicator the mechanism being used by our rats does not fit an explanation like SDT. In general, the lack of dynamics in SDT implies that the invariances of the behaviour need to be explicitly encoded in the form of the DV in models within this framework. Therefore, with an explanation that did not rely on bounded accumulation of evidence, one would not predict this breakdown, as it is the product of fewer DVs

reaching a bound during their trial.

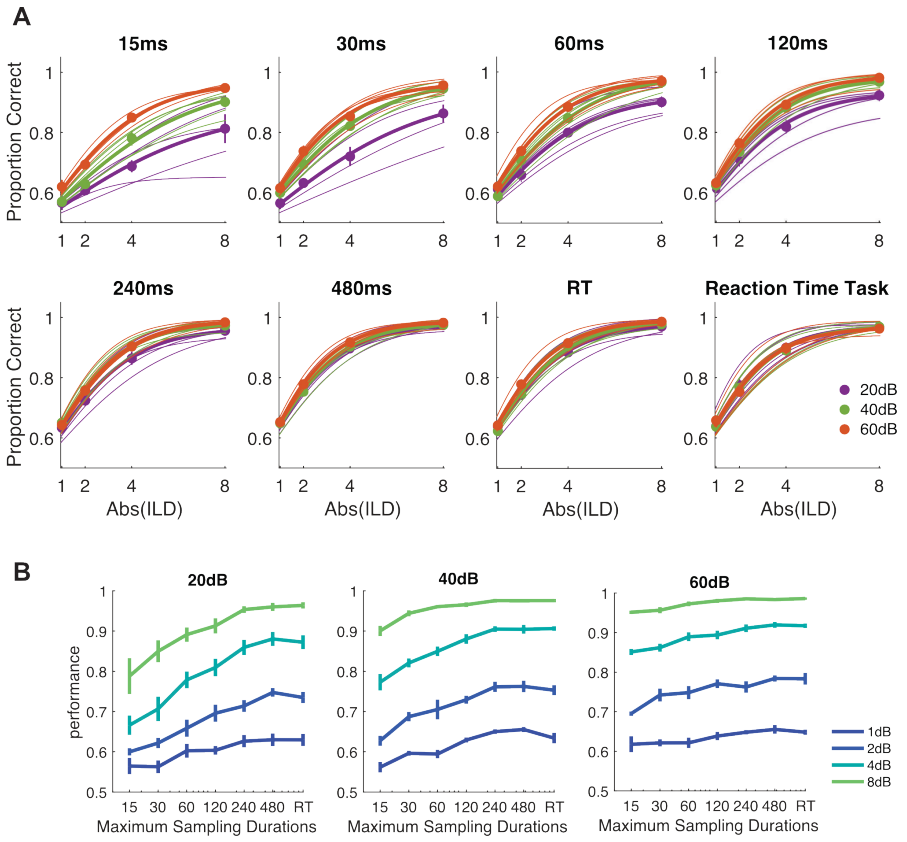

Figure 4.3: **Performance for different sound durations. A.** Proportion correct as a function of difficulty (absolute ILD) for the three tested ABLs (thin lines denote individual animals, thicker lines average across animals, N = 6 rats) for each of the SDmax. **B.** Variation of performance for the different ILDs across SDmax. Also noticeable is the bigger difference is discriminability between RT-trials and trials stopped by the experimenter. It is also clear how performance once again becomes similar for the three ABLs once the SDmax approaches normal RT and the difference between RT and SDmax trials tends to zero.

Level Invariance is Recovered as Sampling Duration Increases

Our data also show that animals are able to recover their level invariance gradually as sampling durations increase to values closer to their usual

Reaction Times, even before reaching trials with no maximum sampling duration. Performance rises as the duration of the sound is increased, reaching the same level of accuracy as in RT trials, invariance for 40 and 60 dB being recovered first (at around 120 ms – Fig. 4.3 A.) and with performance for 20 dB reaching the same level only later. In Figure 4.3 B. it is possible to notice how RT trial level performance is reached at different points for each ILD dependent on the ABL. For instance, for an ILD of 8 dB, 60 dB ABL performance reaches a plateau at a much earlier sampling time than for 20 dB.

4.4.2 Behavioural Characteristics Depend on Sound Duration

Previously, the behaviour of the animals performing the sound lateralisation task under conditions of limited sound duration was analysed focusing on the break-down of WL, but a more comprehensive characterisation of the behaviour in this paradigm had yet to be made.

Rats do not Leave the Central Port Immediately Following Sound Offset

Although the stimulus is interrupted, rats are not forced to respond at that point and may choose to remain in the port. In fact, they do not abandon the central port immediately to respond. Figure 4.4 A. shows the rats' Reaction Time Distribution (RTD) and how it relates to the duration of the sound for each SDmax. It is clear that for the shorter durations the animals react a vast majority of the time after the stimulus offset – as evidenced by the fact that the distribution peaks after the two vertical lines showing stimulus onset and offset. This means the animals remain in the port after the sound has ended. Although this could simply be due to rats not fully grasping the concept of this manipulation and remaining in the central port after stimulus offset in the hope that it might come back, we theorise this is not the case considering RTs were stable throughout this manipulation (data not shown) and if they were expecting to sample any further and thus waiting, we expect they would adapt quickly to this contingency. Remaining in the central port after

sound offset means the rats experience the end of the sound before they decide, something that they are not used to experiencing in the usual Reaction Time configuration. Furthermore, it seems like when they do, their RTs are significantly longer than the SDmax (Fig. 4.4 A).

As expected, the proportion of trials for which RT<SDmax increases as SDmax increases, making it so that the proportion of this type of trials for SDmax = 15 ms is close to zero and for SDmax = 480 ms it is close to one (Fig. 4.4 B).

Performance is Unexpectedly High for Shorter Sampling Durations

Something that can be noticed in Figure 4.3 is that the animals' performance was quite high – consistently above 50% for at the lowest ABL and shortest sampling time. This suggests animals are able to make correct choices with minimal amounts of evidence. Considering the usual peak of the RTD for RT trials is close to 150 ms, this would mean rats can still be well above chance with about 10 times less stimulus duration. For instance, 15 ms, the shortest sampling duration used, is close to the commonly accepted minimum latency for signals to reach the Inferior colliculus, or even the Auditory Cortex (ACx), from the inner ear (Heil & Irvine, 1997; Zhou et al., 2012). Which begs the question, how are the animals accumulating enough evidence when the stimulus is so short?

The initial portions of both RTDs (Fig. 4.4, A.) and tachometrics (Fig. 4.5) are similar for all sound durations, including RT trials, which suggests to us that the sound is responsible for setting in motion sensory response processes that extend over time and can account for these decisions.

Interestingly, it seems that for the RT trials that were randomly interspersed with the shorter SDmaxs, the rats showed even higher accuracy than for RT trials before being exposed to these shorter sampling trials (Fig. 4.3 A. panels for RT trials and RT sessions). This hints that perhaps they are still able to improve their performance when necessary, and that they recognise the possibility of the sound unexpectedly ending, adapting their execution of the task accordingly.

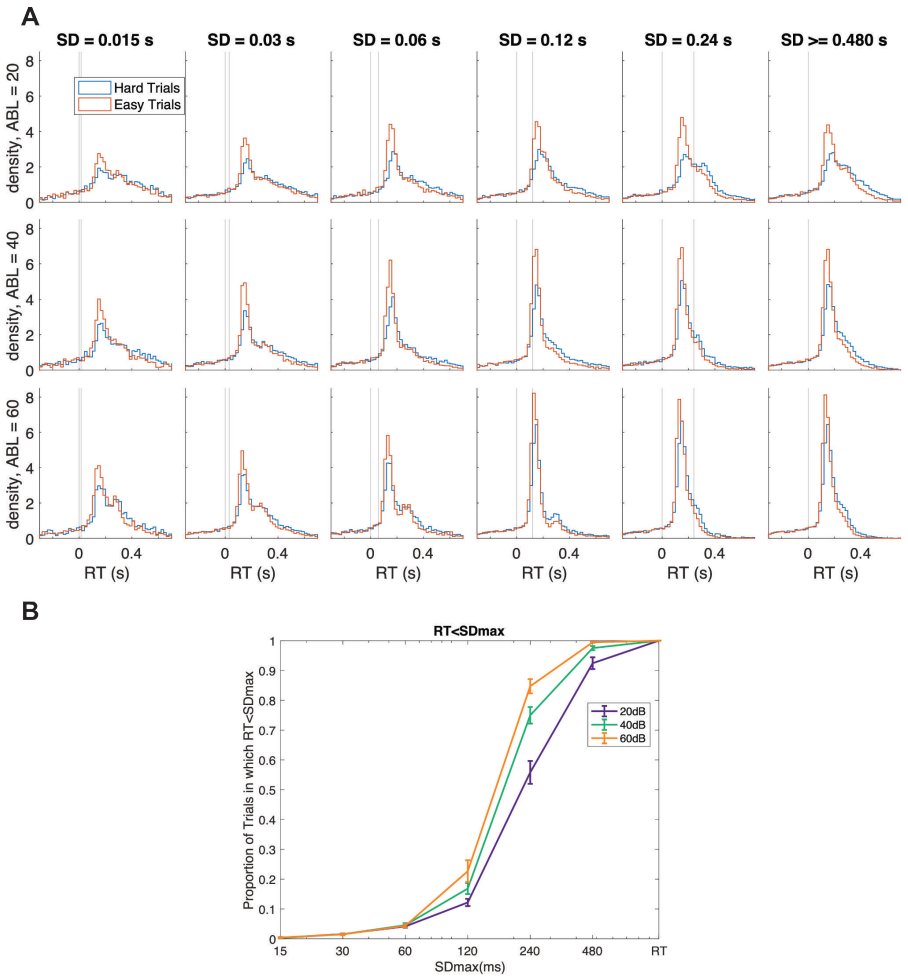

Figure 4.4: **Effect of sound duration on RT A.** Reaction Time Distributions (RTDs) for the different Sampling Durations (SDs). Each column corresponds to an SDmax and each row to an ABL. To make the plot easier to parse, we grouped trials into Hard and Easy, by grouping ILDs (1 and 2 dBs make up the Hard trials, and 4 and 8 dBs the Easy trials). Grey vertical lines on each panel denote the start and end of the stimulus, and bin width is of 20 ms. RT trials and SDmax = 480 ms were grouped due to their large similarity. **B.** Plot of the proportion of trials for each SDmax that were still reaction time-trials. As the SDmaxs are randomly assigned to each trial, it is possible for the RT that the animal would show for a particular trial to be shorter than the SDmax, leading to an RT trial.

Figure 4.4: (Continued caption) The proportion of these trials is small for the shorter SDmaxs, as expected, and, by default, 1 for the trials where the rats are in control of the sound duration.

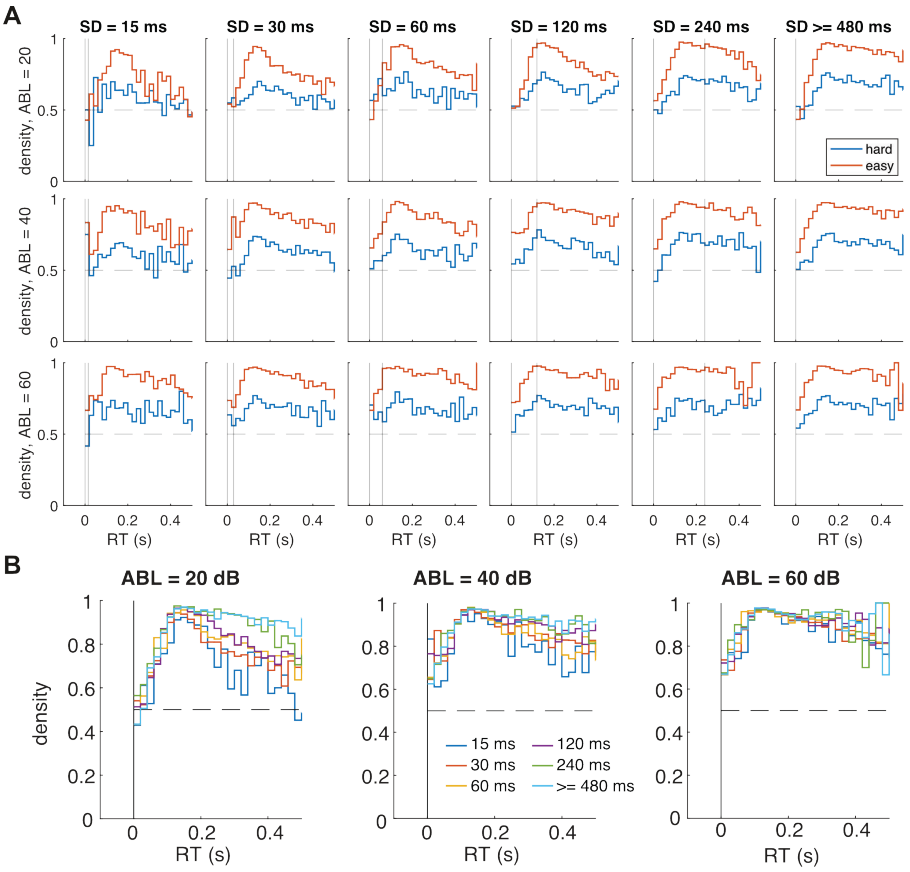

Figure 4.5: **Effect of maximum sound duration on performance according to RT A.** Tachometric function plots for the different SDs. Each column corresponds to an SDmax and each row to an ABL. As before, trials were grouped into Hard (1 and 2 dBs) and Easy (4 and 8 dBs). Grey vertical lines mark the start and finish of the sound stimulation for these trials, and a horizontal dashed line marks 50% performance. **B.** Tachometrics for easy ILDs (4 and 8 dBs) for each SDmax, divided by ABL, showing how the initial portion of the tachometric is conserved throughout the different sampling durations of the sound.

4.4.3 Proposed mechanism of how decisions are made for short duration stimuli

The data presented for this task paradigm shows considerable richness on the specification of choice and its timing when the sensory evidence has terminated (Figs. 4.3, 4.4, 4.5). However, if previously, in the RT configuration of this task, RT marked the moment we could associate to decision commitment, when and through which mechanisms this commitment takes place is now unclear. Previous work (Britten et al., 1993; Brunton et al., 2013; Kiani et al., 2008) has studied empirically how the accuracy of perceptual decisions depends on stimulus durations. In general, the typical assumption made for how choices are determined in these conditions (when the early termination of the stimulus prevents the decision variable from reaching a decision bound) is to assume that the subject commits to the choice associated to the decision bound that is closest to the instantaneous value of the DV when the stimulus terminates (Brunton et al., 2013; Kiani et al., 2008; Kopec et al., 2024; Stine et al., 2020). However, to the best of our knowledge, no previous work has systematically and quantitatively tested whether this decision rule is the most appropriate to describe choice commitment in conditions of early stimulus termination, nor how is the RT of the subject determined after the stimulus ends.

How could choice commitment be specified outside of reaction time context? Let us consider a situation in which the sensory stimulus is presented for a duration equal to t (according to the x-axis in Figure 4.6 A.), which is controlled by the experimenter. As we have been assuming throughout this Thesis, the task of the subject is to produce a binary categorical response. The simplest mechanism for this would assume no decision bounds. A DV reflects the accumulated evidence throughout the stimulus duration and a choice is made at the end of the stimulus based on the value of the DV at that time, as it would be done, for instance, in SDT (Green, Swets, et al., 1966). This has also been used in tasks where the duration of the stimulus was controlled by the experimenter (Britten et al., 1993; Brunton et al., 2013; Kiani et al., 2008). In this case (Fig. 4.6 A.

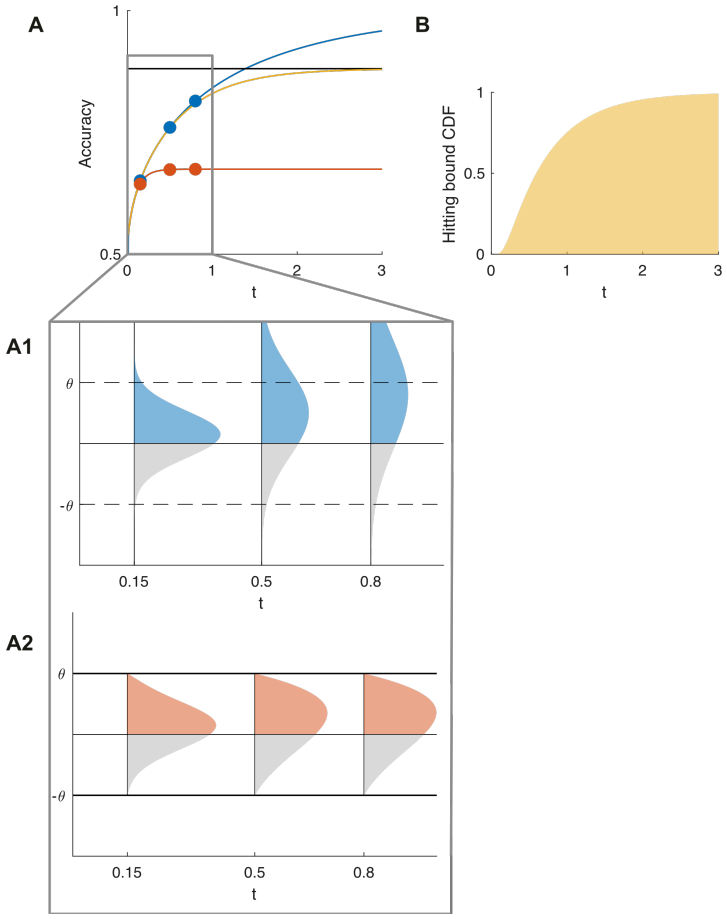

Figure 4.6: **Schematic Representation of Mechanisms of Choosing under fixed Stimulus Durations. A.** Accuracy of Readout at a given time for different models of choice under controlled stimulus duration. Blue trace corresponds to the model with no bounds. In this case, evidence keeps accumulating throughout its entire duration, and choice is determined by the sign of the DV at this point. Accuracy is determined by the area in blue in A1., the probability above 0. The orange process corresponds to the accuracy for the same process but under the presence of bounds that are not reached – this readout is still dependent on the sign of the distribution at the time of readout, now corresponding to A2. The horizontal black line corresponds to the accuracy of the trails that reach the bound, which is constant. For the yellow trace, one weighs the orange and black processes by their corresponding probabilities given by B.

Figure 4.6: (Continued caption) Markers correspond to times of the distributions in A1 (blue markers) and A2 (orange markers). **B.** Cumulative probability of DV reaching the bounds

blue trace), there are no bounds and evidence is accumulated throughout the duration of the stimulus. Across the trial, this causes the probability distributions of the responses for the trial to shift, more and more in the direction of the correct response (Fig. 4.6 A1.). Accuracy increases with RT and will, eventually, saturate at 1.

Now consider a case where decision bounds exist. Two situations might occur on a given trial. First, the bounds might be reached before the stimulus ends. In this case, choice is determined by which bound it reached first. Alternatively, the bound might not be reached for the duration of the stimulus. In this case, the choice must be based on the sub-threshold value of the DV. The standard assumption, as mentioned previously, is to chose based on the bound which is closest. Here, the probability of choosing UP would be the area under the probability distribution of the DV at stimulus offset above 0, as in A1. But critically, this distribution is different from the one in A1 because now the bounds exist, so one needs to condition on the bounds not being touched between 0 and t, causing the accuracy to saturate earlier than in the previous case and at a lower value. This distribution is shown in A2. For trials where the bounded process managed to reach the bound, accuracy is constant and higher, shown in black in A. The combined accuracy for a task including both trials that reach the bound and trials that do not, shown in A in yellow, will be then given by the weighted combination of the "within bounds" (orange) and "at bounds" (blue) accuracy curves, with the weight given by the cumulative probability of reaching the bounds, shown in B. One can notice that for a significant amount of time, the bounded total accuracy is almost as good as the readout without bounds.

In the previous chapter (Chapter 3) we mentioned the presence of stimulus independent choices in our data and how we have been investigating the use of a framework, the PSIAM, that models these as

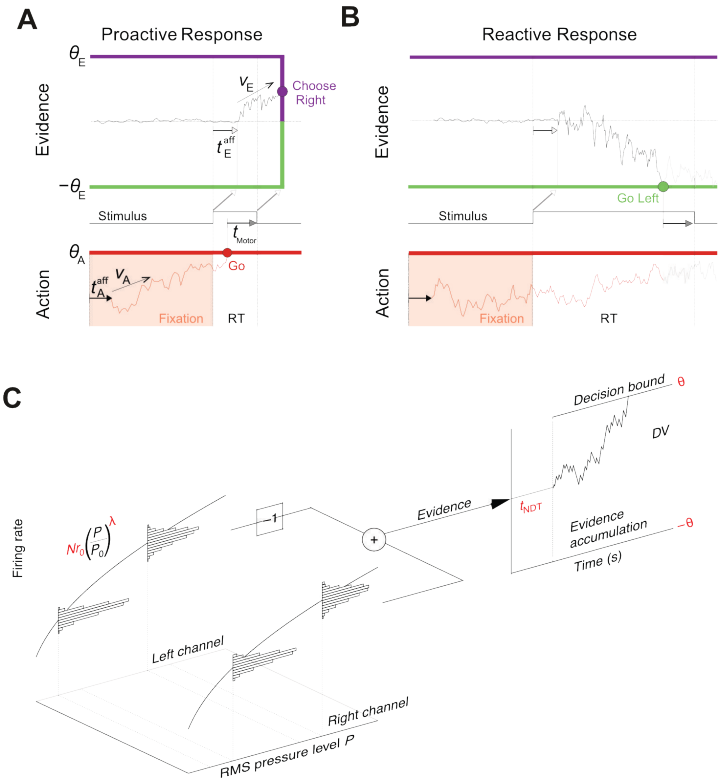

Figure 4.7: **Proactive and Reactive Processes A.** Example of a proactive response win, generated by the Parallel Sensory Integration and Action Model (PSIAM). The proactive process (in red), or action initiation, starts ramping up to a single bound even before the accumulation of sensory evidence begins (black trace). In this case, it also reaches its corresponding bound before the stimulus dependent DV, triggering a proactive response after a motor latency (tmotor). The stimulus-dependent process begins with a sensory latency (tEaff) and its response is decided by the final sign of the DV after the full stimulus has been integrated. **B.** Example of a reactive response also according to the PSIAM. If the reactive process reaches one of its two decision bounds, the proactive process plays no role. (Figure adapted from Hernández-Navarro et al., 2021) **C.** Model structure and parameters for the TIED model. Each stimulus is encoded through a compressive power-law non-linearity of gain r0 and exponent λ by a population of N neurons (a sensory channel) firing with Poisson statistics. The sensory evidence is the difference between the activity of each channel.

Figure 4.7: (Continued caption) The DV integrates the evidence until a constant bound at $\pm\theta$ is hit (decision time). RTs are the sum of the decision time and a stimulus-independent non-decision time tNDT. The four model parameters are shown in red (Pardo-Vazquez et al., 2019).

proactive responses (Hernández-Navarro et al., 2021). Because we believe these anticipatory choices to be an important part of the animals' behaviour, we will continue to include them in our analysis and have adapted our previous model (Pardo-Vazquez et al., 2019) to include both the stimulus-dependent (reactive) and stimulus-independent (proactive) processes.

Reactive process (TIED)

The reactive process is a DDM with two symmetric bounds, as illustrated in Figure 4.7 B. In this case, we employ the previously described TIED parametrization, with the parameters: The total baseline firing rate of the pool of sensory neurons in each channel (right or left), the power law exponent of the function transforming sound pressure to firing rate, the bound height, and the non-decision time (in this case the sum of an afferent sensory delay of the activity of the sensory neurons and a motor delay) (Fig 4.7 C.). These parameters in turn map onto the typical DDM parameters such as the drift rate, etc., and also determine the rescaling of the effective time unit as a function of ABL as described in Pardo-Vazquez et al., 2019.

Proactive process

The proactive process is a DDM with a single bound, as illustrated in Figure 4.7 A. The sufficient parameters to describe are the drift rate, the separation between the initial position and the boundary, and the non-decision time (the sum of an afferent delay which could be also thought as an initiation delay – the time taken by the animals to initiate the process, relative to the poke that initiates the trial –, and a motor delay).

Interaction between the two processes

The two processes race each other, such that the first one to hit a bound is the winner, determining the RT (which would be the bound hitting time plus the motor delay). Two situations can then happen, that will determine the choice, as illustrated in Figure 4.7 A. and B. The most straightforward one is the case in which the reactive process wins. In this case, the choice is simply given by which of the two reactive bounds was hit. The other case is when the proactive process wins. Here, the sensory evidence continues to accumulate for an additional period. In the original PSIAM formulation (Hernández-Navarro et al., 2021), this duration was always equal to the interval of time during which the stimulus was presented (regardless of the particular values of the sensory and motor delays), but we let it be a free parameter and become potentially longer or shorter. During this period, if the reactive DV reaches one of its corresponding bounds, that will determine the choice in the same way as when the reactive process wins. Otherwise, we apply a choice-readout rule.

Our strategy was to fix the values of the model parameters by fitting the RT trials – trials in which the SDmax was not capped, and the animal could decide freely – and then to consider how different choice commitment rules ("readout" mechanisms) in this model would fare in the limited duration trials.

We investigated different readout mechanisms. Starting by the case that most faithfully resembles the example shown in (Fig. 4.6) A2, we assumed that the choice readout happens at the moment of sound termination. This manages to replicate the choose-right probability Fig. 4.8 A.), but fails to account for the onset of the tachometrics (Fig. 4.8 B.). In our data, tachometrics become stimulus dependent right after stimulus onset, which is not replicated with this readout mechanism. We then considered a more complex readout rule – the one proposed in the PSIAM formulation (Hernández-Navarro et al., 2021).

This rule assumes that the evidence is allowed to continue evolving after RT, for as long as the remaining sensory information is left to be

processed, due to sensory and motor delays. This prolonging of the integration process allows more instances to reach the bound, increasing accuracy. Applying it provides a better explanation of the data, both at the level of the psychometric fittings and at the level of the tachometrics (Fig. 4.8 C. and D., respectively). Besides these two, we considered a third readout alternative, in which, instead of determining the choice by the sign of the DV, even though it had not reached the bound, the readout was random for this sub-threshold trials. This results in fits very similar to the previous rule (Fig. 4.8 E. and F.) if the latency between the proactive choice to leave the central port and the moment of evidence readout is allowed to increase (from 75ms to 130ms).

Because these rules and their good fits to the tachometrics and psychometric do not influence RT – this has been decided at the moment the race between proactive and reactive was won by one of the processes –, after focusing on accuracy we can then verify how well the RTs are captured (Fig. 4.9). We can confirm that the rules that allow us to closely replicate the accuracy data (Fig. 4.8 C. D. and E. F.) also replicate the RT data faithfully, indicating that they are both a good description of the sensory-motor transformation in the trials in which the animals control the stimulus duration.

Once the parameters that explain the RT trials were set, we attempted to explain the performance for the shorter SDmax trials by considering different scenarios describing how the sensory evidence unfolds once the stimulus is terminated (Fig. 4.10). We started by testing different ways for the evidence to behave following sound termination (Fig. 4.10) at an example SDmax of 30 ms. Firstly, we modelled the accuracy for these trials in the situation where, following sound termination, the evidence immediately decays to 0 (Fig. 4.10 A.). Due to this instantaneous decay, we could not use the random readout rule (described in figure 4.8 E. and F.), as accuracy would, in that case, be 50% by design. Thus, we used the sign readout rule instead – choice is decided by the sign of the DV at the time of decision readout. For this instance, psychometric fittings are not able to replicate the slope our data shows (Fig. 4.10 B.), nor the pattern of the tachometrics (Fig. 4.10 C.), with the model showing a fast stimulus

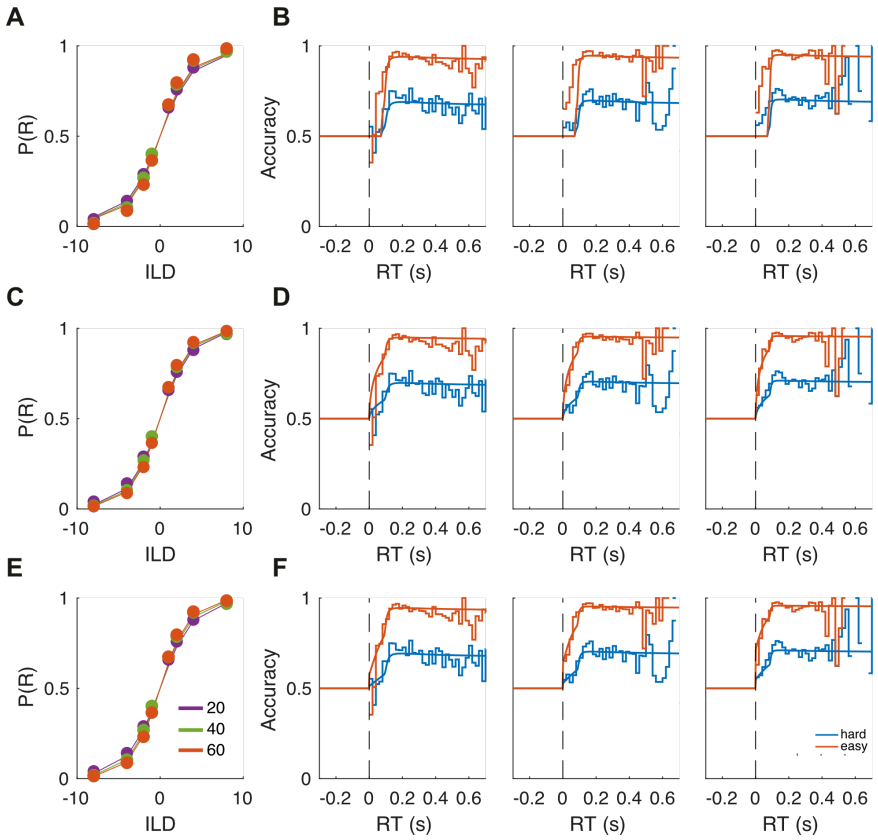

Figure 4.8: **Model Fits of psychometric and Tachometric According to Choice Readout. A.** Psychometric fittings based on a readout as described in Figure 4.6 A. Markers correspond to actual data points for RT trials. **B.** Tachometrics for easy (orange) and hard trials (blue) and corresponding model fittings (overlaying lines) taking into account the readout process in A. For these modelled trials, the bound exists, yet it has not been reached, limiting accuracy. It is also apparent that the tachometrics are not replicated, because stimulus dependence is only clear much later for the fittings than for the real tachometrics. **C. D.** Like A. and B. but taking into account the impulsive choices and a readout similar to what is employed in the PSIAM – choice is dependent on the sign of the DV at the moment of readout –. This more accurately explains the tachometrics we have observed for the animals.

Figure 4.8: (Continued caption) **E. F.** Like C. and D. but considering an alternative choice readout in which case, if the bound is not reached, the choice is random. This method works as well as the previous one if the latency between proactive choice and evidence readout is allowed to be longer.

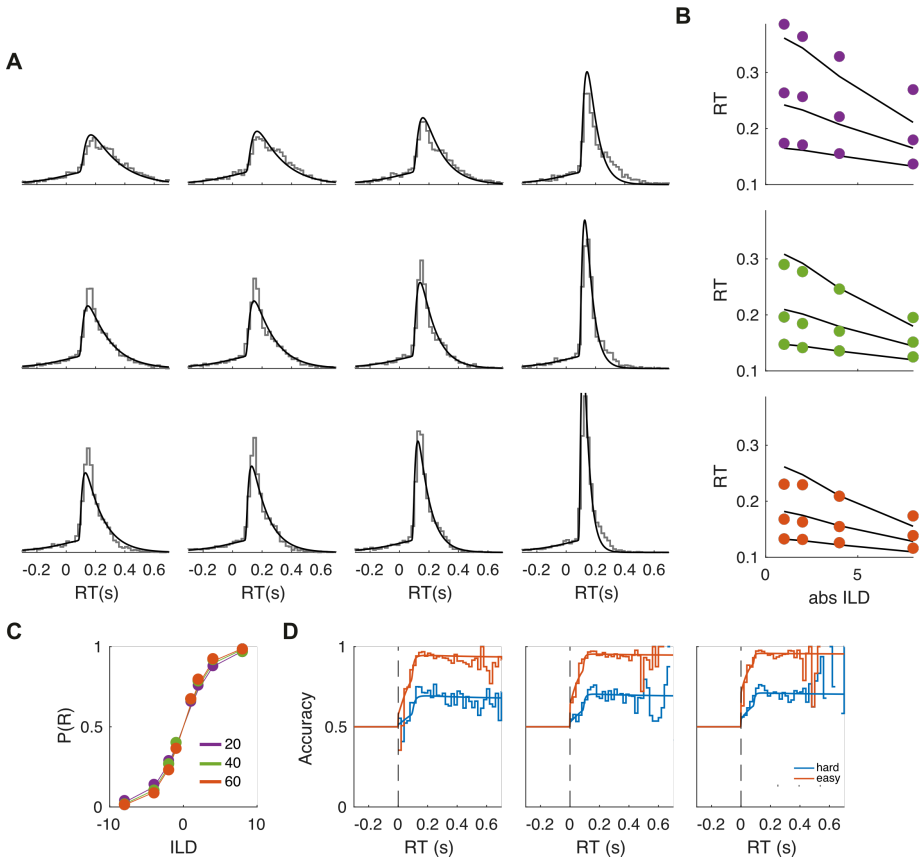

Figure 4.9: **Model Fittings for Reaction Time Trials for the model in Fig. 4.8 E. F. A.** RTDs for the different ABLs (rows) and ILDs (columns) in grey and fits in black. **B.** Reaction Time data divided by quantile for the different ILDs. Each plot corresponding to an ABL. The black lines correspond to the model fittings. **C.** Choice-right probability for the different ILDs (markers) and model fittings for the psychometric functions for the three ABLs. **D.** Tachometrics for easy (4 and 8 dB, orange) and hard (1 and 2 dB, blue) trials with model fits the overlaying lines. Similar results are obtained with model 4.8 C. D.

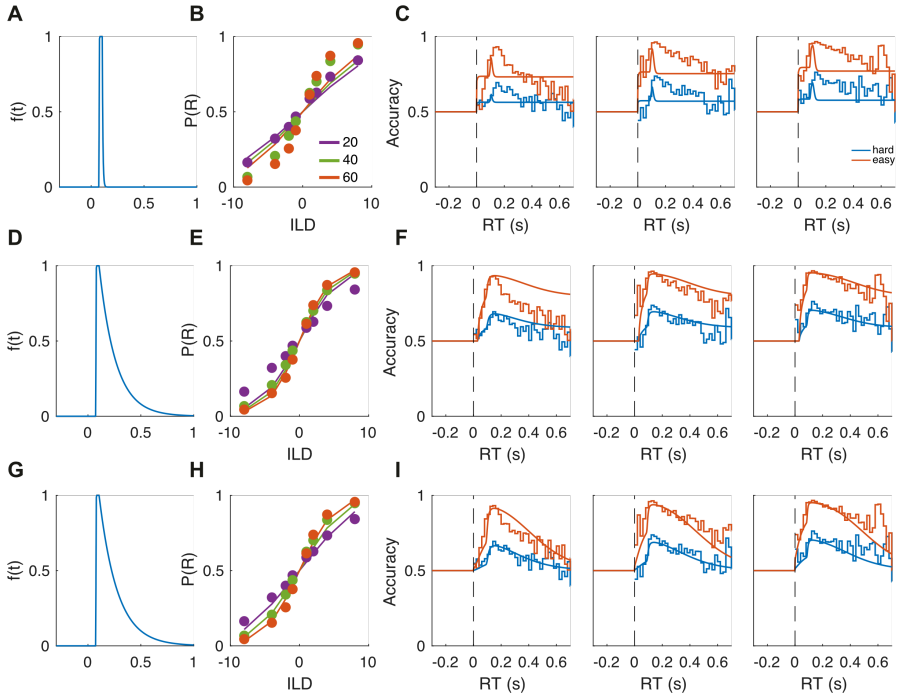

Figure 4.10: **Model Fittings for SDmax = 30 ms Assuming Different Evidence Decays A.** Evolution of evidence during the RT period. For this instance, we explored instant decay of evidence after sound termination. **B.** Psychometric fittings for the different data points for choose-right probability at the different ABLs for SDmax = 30 ms, considering the evidence decay profile in A. **C.** Tachometrics for the different ABLs for SDmax = 30 ms, considering the evidence decay profile in A. **D.** Evolution of evidence during the RT period. For this instance, we explored exponential decay of evidence after sound termination. **E.** Like B. but considering the evidence decay profile in D. **F.** Like C. but considering the evidence decay profile in D. Panel **G.** is the same profile as in D., but for this row, the readout of choice when the bound is not reached is random. **H.** and **I.** like E. and F. but for the profile described in G.

dependent differentiation and a peak some time after sound onset that will then decay to a plateau value maintained through the remaining RTs. We then tested an exponential decay to the evidence following stimulus offset (Fig. 4.10 D.), maintaining the same sign readout rule. This slower decay better approximates the accuracy of our animals – the slope of the psychometric fits are closer to our data points (Fig. 4.10 E.) and the rise and peak of the tachometrics (Fig. 4.10 F.) are also better approximated. However, the decay of the tachometrics is not well reproduced, as the data decays faster than the model fits. This is especially apparent for the leftmost tachometric, at 20 dB ABL, matching the psychometric being also the least accurate. This overestimation of accuracy at 20 dB ABL put into question the readout rule of following the sign of the DV, and invited us to consider the previously mentioned random readout rule (described in figure 4.8 E. and F.).

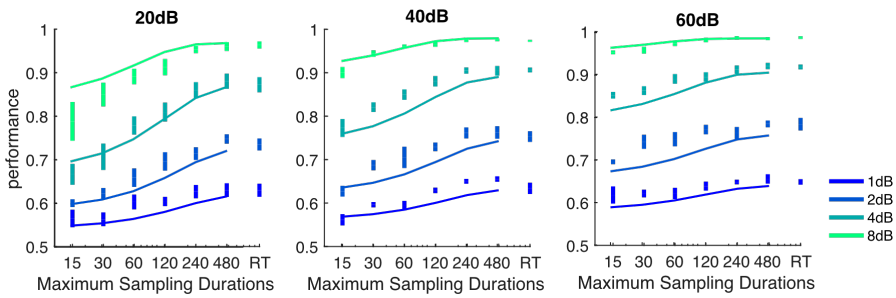

Figure 4.11: **Accuracy of model fits for all SDmax conditions** Accuracy of the model fits as a function of SDmax for the different stimuli (one panel per ABL, different coloured lines per ILD). The vertical lines correspond to the data and thinner, horizontal lines to the model fits.

The reasoning is that, given that for the lowest ABL the process of evidence accumulation happens slower, it is expected it will reach the bound less often for shorter sounds, indicating our current rule to deal with trials that fail to reach the bound is overestimating accuracy. To account for this faster decay noticed in the data, we maintained the same decay pattern for the evidence (Fig. 4.10 G.) but replaced the readout rule with the random choice at readout time. This change allowed for a

much more accurate fitting of the psychometric function (Fig. 4.10 H.), even for 20 dB ABL, and for the tachometrics, now better replicating the decay of the different ABLs for longer RTs (Fig. 4.10 I.).

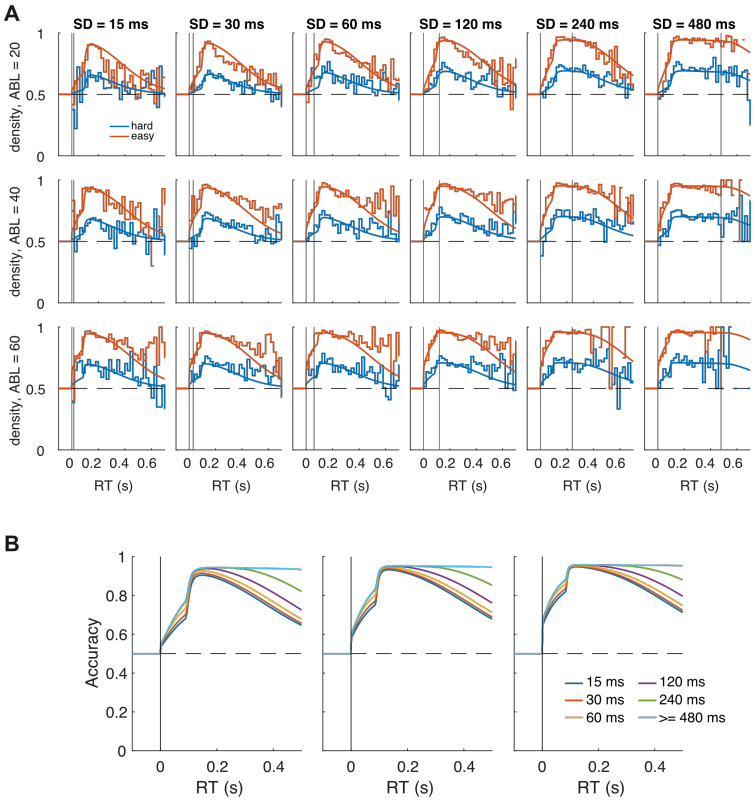

Figure 4.12: **Tachometrics of model fits for all SDmax conditions** Tachometrics of the model fits as a function of SDmax for the different stimuli (smooth lines) compared to data. Each column is a different SDmax and each row is different ABL. The blue and red colours represent hard and easy ILDs, as in (mention previous figure). **B** For each ABL and easy ILDs, tachometrics of all the SDmax together.

Once we settled on the model specifications, we fitted the data for the remaining SDmax. Importantly, the parameters were constrained to be the same for all the conditions, with only the duration of the stimulus itself changing. In Figures 4.11 and 4.12 we summarize the model fitting results. Figure 4.11 shows that the accuracy is reasonably

well captured across conditions, reproducing the increase as a function of duration, and the breakdown of WL for shorter durations. Figure 4.12 shows a remarkable agreement for the tachometric functions across the board. While the model doesn't reproduce all the details of the decay patterns for each individual conditions, nor the exact dependency of accuracy on duration (Figs. 4.3 B. and 4.11), overall it quite captures well how accuracy, RT and the tachometric functions change as the stimulus duration decreases.

4.4.4 Complementary Manipulation with Minimum RT does not Improve Accuracy

As a complementary manipulation, we trained a different animal cohort in the same task but in a paradigm where they instead had a minimum Reaction Time, and they had to remain in the central port while the sound played. This minimum RT was selected from an exponential distribution (minimum 500 ms and a mean of 200 ms), similar to the one employed for the determination of the Fixation Time (Methods). If the animals left the port before the end of this period (signalled by an LED in the port going off), the trial was classified as an abort, but if the animals remained in the port the sound would remain on. A possible outcome to this manipulation, ensuring the stimulus to remain on for longer than the average RT, could be that accuracy increases, since longer fixed-duration stimuli are expected to lead to higher discrimination accuracies under evidence accumulation (Brunton et al., 2013; Burr & Santoro, 2001; Kiani et al., 2008). However, it is possible to notice from Figure 4.13 that that is not the case. While rats are still showing an ILD-dependent performance (Fig. 4.13 A. and C.) i.e. a difference both at the level of the tachometric and psychometric for the different ILDs, it is also clear they are not as accurate as previous cohorts, with lower accuracy for 20 dB ABL and clear lack of level invariance.

Their RTDs are also clearly distinguishable from those of previous batches (Fig. 4.13 B.). These animals show some aborts at early fixation times, as previous batches, but minimal aborts during sound. However,

they do show a nearly Gaussian RTD that starts rising close to the minimum RT.

It seems to us like animals trained in this task paradigm are very clearly timing the end of the RT mandatory period in order to respond, anticipating the moment they are able to answer. However, one might expect their performance to be much higher, taking into account their minimum RT is now longer than average RTs from previous cohorts (that show both higher performances and level invariance). This suggests that animals are not able to accumulate evidence for a period of time much longer than the typical time needed to discriminate the stimulus, and that forcing them to longer sound exposures might not have a positive impact on their performance (Kiani et al., 2008). While our results agree with previous work with regard to not having a continuous improvement of performance (Kiani et al., 2008), a possible explanation for the decrease in accuracy we actually observe is that rats seem to have focused on remaining in the port until they are allowed to leave, instead of focusing on the stimulus being presented. Their decision seems to be regarding leaving the port, and only at this time do they briefly shift their attention to the sound. This limited sampling would explain their lower performance, and also why it is not level invariant in these conditions. It remains a subject of future work to attempt to apply the same modelling strategies that resulted successful in the case of stimuli with short, controlled durations.

4.5 Discussion

The findings presented in this chapter provide significant insights into the dynamics of decision-making in perceptual tasks, particularly concerning the breakdown of WL and the implications of constraining the evidence accumulation period. By investigating how auditory stimuli of different ABLs influence decision-making when presented for durations shorter than typical RTs, we have expanded upon the framework established in the work from Pardo-Vazquez et al. (2019). Our results indicate that the invariance of performance across different ABLs is disrupted

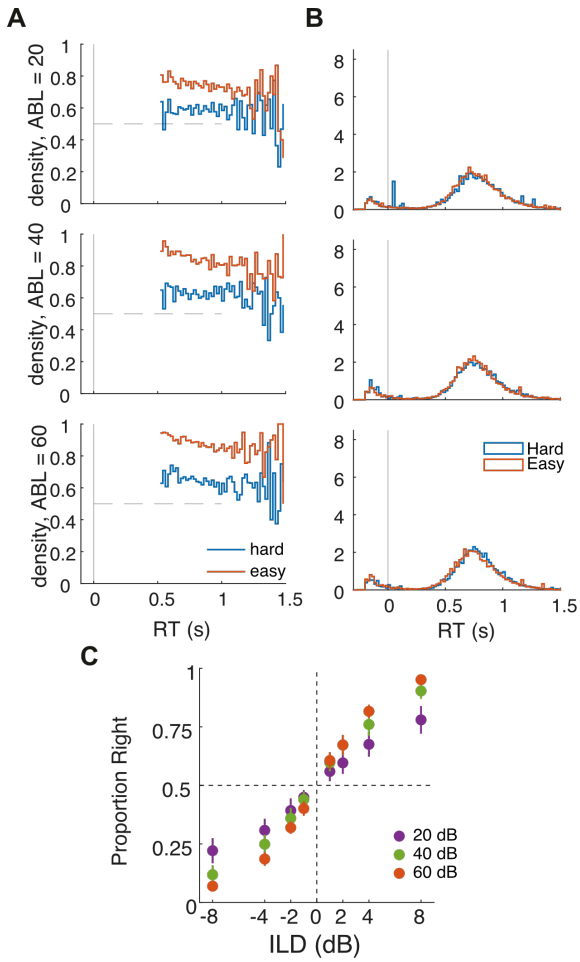

Figure 4.13: **Effect of minimum sound duration on performance A.** Tachometric functions for the animal cohort experiencing minimum Reaction Time trials (N = 5 rats), each plot for an ABL and divided into easy (1 and 2 dBs) and hard (4 and 8 dBs) as previous tachometrics. For these animals the tachometrics only start at RT = 500 ms as the previous RT duration was a mandatory portion of the trial. **B.** Reaction Time Distributions (RTDs) for the same animal cohort. Separate distributions for hard and easy trials are overlapping for these animals. **C.** Choose-right probability (mean ± SEM across rats) for animals experiencing this minimum RT paradigm. For this cohort, there is little overlap between the different ABLs.

under these conditions, suggesting that bounded accumulation of evidence plays a critical role in this process. We have also observed that the decisions that occur based on this incomplete evidence might still be able to reach the decision bound and also be employing a somewhat unexpected decision rule when that does not occur. Furthermore, when we imposed a minimum duration to the stimulus as opposed to a maximum one, accuracy did not improve and in fact animals also showed the breakdown of WL in these conditions. One of the most striking outcomes of our experiments is the degradation of discrimination accuracy for both shorter SDmaxs and long RTs (Fig. 4.3 and 4.13). While the TIED predicts a breakdown of WL for short sound duration, we had not addressed what might happen for longer RTs, although other works might hint that animals would, if possible, improve (Brunton et al., 2013; Kiani et al., 2008).

Larger sound intensities result in more activity in the sensory channels, leading to faster accumulation of sensory evidence toward the bound and faster RTs. However, as long as the four conditions described in Pardo-Vazquez et al., 2019 – power-law relationship between physical stimulus intensity and its internal neural representation; trial-to-trial variance of the sensory evidence proportional to the mean; exact accumulation of evidence by the DV; and a constant decision bound – are fulfilled, the relative probabilities across time of hitting the bound at any particular moment become independent of overall intensity. As mentioned, this is formally equivalent to a change in the units of time of the discrimination process (Pardo-Vazquez et al., 2019). The significant effect on performance of interrupting the RT demonstrates that, when sounds are abruptly terminated, the ability to accurately lateralise sound sources diminishes, particularly at lower ABLs, causing the breakdown of WL. This finding provides model-independent evidence that this task depends on bounded accumulation of evidence, as this result would not be predicted in cases of "static" explanations for WL (Green, Swets, et al., 1966). As predicted, our data show that performance improves as stimulus duration increases, ultimately reaching levels comparable to RT-trials when sufficient time is allowed for evidence accumulation.

Interestingly, while performance was significantly affected by SDmax as we had predicted, our observations regarding Reaction Times also reveal an effect, and a somewhat unexpected one. The fact that animals often did not immediately exit the central port following stimulus termination (Fig. 4.4) suggests a puzzling interplay between evidence accumulation and decision timing. We were initially surprised with the high performance demonstrated by our animals for the shorter SDmax, as well has the longer RTs observed when stimuli were interrupted early on. We hypothesised animals might engage in additional processing even after the sound has ceased, and realised the moment of sound offset might not in fact correspond to the moment of termination of evidence accumulation or choice readout in these conditions. Most studies that employ stimuli with fix duration (for example Britten et al., 1993; Brunton et al., 2013; Kiani et al., 2008; Stine et al., 2020) assume that the choice is based on the state of the readout immediately preceding stimulus termination: the response is rightward or leftward according to the sign of the decision variable at this moment. Here, we have provided evidence that this is not sufficient to explain the performance of the animals, and that further post-stimulus processing is required in order for the accuracy to increase to the levels that we observed. A way to capture this is through the mechanisms proposed in models such as the PSIAM, where the authors explicitly delineate the dynamics of the readout following a decision whose timing is not determined by the sensory evidence having reached a bound. There, the sensory and motor delays play an important role in specifying how much additional time the evidence has to keep evolving.

We employed an adaptation of the PSIAM in order to reconcile subthreshold decisions with those of full evidence accumulation for the same task (Hernández-Navarro et al., 2021; Molano-Mazón et al., 2024). The notion that two competing DDMs operate concurrently — one accumulating evidence until a bound is reached and another signalling the choice to act — provides a framework for understanding how decisions can be made rapidly, and independently of the stimulus, or in the absence of complete information. The PSIAM employs a specific rule for

sub-threshold responses, according to which the choice is defined by the sign of the DV at the time of the readout. While applying this model and this readout rule to our data effectively captures the dynamics observed in RT trials (Fig. 4.8 C. and D.), we realised it overestimated accuracy for short duration trials.

According to our work, responses at short durations are better described through an evidence accumulation process that does not terminate immediately at stimulus offset, but that, through exponential decay of the evidence available and due to sensory delays, continues in time. Occasionally, the bound will still be reached, even after stimulus offset, explaining high accuracy. However, when the bound is not reached, the data is better described by a model where the animals do not read out the sign of the DV, and instead choose randomly (Fig. 4.9 G. – I.).

This is, however, only true if sufficient time is allowed for integration to continue. This could involve leveraging neural signals not usually necessary or accessible at the moment of choice – such as offset signals in the auditory pathway, specifically in the ACx. Neuronal responses to sound are not confined to its onset nor to its duration and in fact cells continue to fire in diverse profiles while sound is being played and after, even for temporally confined sounds such as clicks (Bagur et al., 2018; Bondanelli et al., 2021; Hartley et al., 2011; Lütkenhöner & Patterson, 2015; Takahashi et al., 2004). Offset signals are generated when the sound ends (Bondanelli et al., 2021; Hartley et al., 2011; Joachimsthaler et al., 2014) and although previously studied, their perceptual meaning and behavioural impact is still poorly understood. However, they have been suggested as forming the basis of duration selectivity, both in the visual and auditory pathways, and contributing to sound source localisation, and auditory memory – more recent events are recalled with greater accuracy – (Glanzer & Cunitz, 1966; Hartley et al., 2011; Stecker & Hafter, 2009). During RT-trials the animals only experience these signals after they have made their decision and as they abandon the central port in the direction of the chosen response port and the sound is terminated. For short SDmax-trials, however, these signals happen before the DV reaches a bound, and they may be integrated, as a source of extra evidence

to help explain the unpredicted accuracy shown, or used as a biasing mechanism for the motor response (Hartley et al., 2011). It is important to mention that we have yet to focus on accurately reproducing the RTs for the short duration trials. It might be the case that while the standard readout rule appears to overestimate accuracy and choosing randomly allows us to reproduce it, intermediate values might be necessary for the reproduction of the full behavioural phenotype. Although the data are fairly well captured by a rule where, in the absence of hitting the bound, choices are random, this does not exclude a situation in which, if sufficiently close to the bound, factors such as biases might cause a choice to not be random. This does, however, seem to suggest that at least in our task, not only the threshold crossing determines the time of commitment, but it also is a necessity for evidence to guide choices at all. We also hypothesise the random choice at sub-threshold readout situations might not mean an equal percentage of choices for both sides, and biases might impact the response at this level as well.

Our findings replicate an ecologically meaningful paradigm of auditory perception and decision-making: in natural environments, animals frequently encounter transient sounds and must make rapid decisions based on incomplete information. The results from our study highlight how these real-world scenarios might differ from the laboratory conditions and allow for the investigation of how animals adapt their decision-making strategies in response to varying temporal constraints.

Although we had not made specific predictions regarding the animals' performance for the task paradigm where we imposed a minimum RT, we aimed at testing whether imposing a long RT would do away with impulsive decisions. If proactive choices corresponded exclusively to the shortest RTs, imposing a minimum RT that is longer than the average would abolish these. In this ideal situation, the expected RTD would be similar to the one for the WL animals in Figure 3.2 C., just shifted accordingly in time. And accuracy would also be similar or even improve considering impulsive behaviour might occur even at longer RTs, but would be avoided in this case as one is forced to remain accumulating evidence. However, while, by design, short RTs are abolished, it does

not seem like the animals are behaving as they did in the original task without proactive responses. The RTDs are shifted, but they are the same for all ILDs and show little difference between ABLs as well. It seems as though the animals still accumulate evidence during the same period of time as before, but then have to remain in the central port, potentially storing their choice in memory for the remaining duration of the RT and focusing on not leaving the port until cued. An alternative to maintaining the choice in memory until response time would be to increase the bound for evidence accumulation, but this would potentially also lead to better accuracy levels than the ones registered. This breakdown of WL and decrease in performance, especially for the smallest ABLs, reinforces one of our conclusions from Chapter 3, according to which animals seem to be behaving at psychophysical threshold. Their slight improvement for RT conditions (Fig. 4.3 A. might be a reflection of increased experience in the task or suggest that when exposed to a harder version of the task (unexpected and random stimulus durations), they might transfer whatever mechanisms allow them to excel in those trials to the regular ones, as if transferring between two tasks (Kurt & Ehret, 2010).

In conclusion, this chapter contributes to a deeper understanding of auditory decision-making processes by highlighting how Weber's Law breaks down under conditions of constrained stimulus duration. Our findings further highlight the significance of temporal dynamics in shaping perceptual outcomes, and suggests questions regarding the connection between memory and intensity, concerning whether the memory of fainter sounds degrades faster than that of higher intensity. Future research should continue to explore these dynamics and our advanced modelling approach and potentially neuronal manipulations of key brain regions. By doing so, we can further elucidate the neural mechanisms underlying auditory perception and decision-making, ultimately enhancing our understanding of cognition in both humans and animals.

4.6 Acknowledgements

We thank the vivarium staff, and histopathology and microscopy platforms for their help.

4.7 Author Contributions

Alfonso Renart (A.R.), José Pardo-Vazquez (J.L.P.-V.), Juan Castiñeiras (J.R.C.-d.S.) and Mafalda Valente (M.V.) designed research, M.V. performed research, J.R.C.-d.S., M.V. and Ulysses Tsai (U.T.) analysed the data.

5

Role of Auditory Cortex in Sound Source Lateralisation

5.1 Summary

After establishing our ILD-based auditory discrimination task as a robust framework for studying perceptual decision-making in previous chapters, and gaining insight into the mechanisms underlying its performance, this chapter addresses the central question of this thesis: the role of the Auditory Cortex (ACx) in auditory decision-making.

While subcortical structures such as the Superior Colliculus (SC) and Inferior Colliculus (IC), as well as associative cortical areas such as the Lateral intraparietal cortex (LIP) are known to contribute to auditory behaviours and decision-making, the necessity of ACx for simple auditory tasks in rodents remains uncertain. Although ACx is involved in processing interaural level differences (ILDs) cues, previous studies suggest it may not be essential for task execution. However, cortical impairment often leads to performance deficits, particularly when high accuracy is required.

To investigate this, we assessed the effects of ACx lesions and optogenetic inhibition on task performance. Our results show that rats with ibotenic acid lesions in ACx maintained similar performance levels to

control animals, suggesting that ACx is not essential for task completion. However, optogenetic silencing of ACx led to a marked decline in accuracy and changes in Reaction Time (RT), indicating a role for ACx in optimizing performance rather than being essential for sound lateralization

Here, we examine how ACx disruption influences both accuracy and RT, exploring the temporal dynamics of decision-making under cortical manipulations. Although rats can still perform the task following ACx lesions or inactivations, their performance deteriorates, suggesting that ACx plays a role in higher-order processing. By combining our well-characterised task with computational modelling, we revealed that cortical silencing not only disrupted evidence accumulation and, thus, the reactive process, but also influenced the proactive decision-making process.

These findings suggest that while ACx is not strictly required for auditory decision-making, it plays a modulatory role in refining and optimizing the process. Our study contributes to the broader understanding of sensory cortical involvement in perceptual tasks and highlights the importance of using both behavioural techniques and computational approaches to uncover the neural contributions of target brain regions.

5.2 Introduction

The main goal of this thesis has been to investigate the role of ACx in a sound lateralisation task. For this, we have analysed our Interaural level differences (ILD)-based task in depth, in order to establish it as a robust and reliable perceptual task for the study of decision-making. We have shown the behaviour is stable and reproducible and that it consistently obeys Weber's Law (WL) and the Time-Intensity Equivalence in Discrimination (TIED), and thus can be modelled through similar processes to the ones previously shown (Pardo-Vazquez et al., 2019). Indeed, we are now able to incorporate both reactive and proactive trials within the same model in an attempt to explain the full behavioural repertoire of these animals during our task. With these accomplished,

we are now able to focus on our study of the neural underpinnings of this behaviour.

While multiple structures contribute to both sound lateralisation and decision-making, both from the sensory and motor pathways, the ACx presents a unique opportunity to explore cortical contributions to this behavioural task. From the motor end, the cortex projects to structures shown to be involved in decision-making and lateralised responses (Malmierca, 2015; May, 2006), such as the SC (Basso & May, 2017; Felsen & Mainen, 2008; Koyama & Pujala, 2018; May, 2006; Mysore & Knudsen, 2012; Redgrave et al., 1986, 1993), and, through it, the Reticular Formation in the brainstem, that have been shown to be critical for orienting movements such as the ones in our task (Cregg et al., 2020; Usseglio et al., 2020). From the sensory end, the Auditory Cortex is the final stage of the ascending auditory pathway and receives input, both direct and indirect from the IC (Bajo et al., 2007; Malmierca, 2003, 2015), one of the earliest stages encoding ILDs (Grothe & Pecka, 2014; Grothe et al., 2010; Severo et al., 2024). The ACx has commissural connections and has been shown to respond to ILDs in this exact paradigm (Kobak et al., 2019), yet its precise role in guiding perceptual decisions remains unclear. Given its position as a sensory-motor interface and its accessibility for neural manipulations, ACx is a compelling target for investigating how cortical computations shape auditory decision-making.

The ACx is sensitive to ILDs and sound azimuth cues, especially through a strong contralateral tuning in A1 (Campbell et al., 2006; Heffner & Heffner, 2007; Stecker et al., 2005; Yao et al., 2013). Although it has been shown that neurons in A1 and surrounding areas also respond to ipsilateral sounds (Kobak et al., 2019; Myoga et al., 2023), indicating the often observed preference for contralateral sounds in the auditory cortex is not a hard-wired property. Other auditory areas contribute to sound localization through more complex processing mechanisms. For instance, the posterior auditory field (PAF) is implicated in integrating spectral and temporal information about sounds, enhancing the ability to localize sounds based on frequency characteristics (Doron et al., 2002). However, despite research indicating the ACx is sensitive to ILDs, it is

unclear how this area contributes to sound localisation in rats.

Previous studies, in other model animals, suggest that ACx is important for sound localisation (Heffner & Heffner, 1990; Jenkins & Merzenich, 1984; Kavanagh & Kelly, 1987; Nodal, Kacelnik, et al., 2010). In these, decorticated or otherwise cortically lesioned animals showed large deficits in their ability to localise the source of a sound in the azimuthal plane. However, studies in the rat actually suggest that the region might not be necessary for simple auditory behaviours such as sound source localisation, or lateralisation, in the horizontal plane, like the task we are studying. It has been shown that ACx is not strictly necessary for the performance of tasks pertaining to sound localisation (Kelly, 1980; Kelly & Masterton, 1977), or other simple auditory tasks, such as frequency discrimination (Pai et al., 2011). Chemical or optogenetic inactivation of the ACx also show little effect on discrimination of sound duration or frequency (Ceballo et al., 2019; Gimenez et al., 2015; O'Sullivan et al., 2019). In these previous studies, manipulations of the ACx did not destroy the full behaviour – the animals were still able to perform in the different tasks –. However, these cortical manipulations did still have a noticeable effect on the rats' performance. Subtle deficits in accuracy and response dynamics indicate that cortical processing might play a role in refining auditory-guided decisions, particularly under conditions requiring high precision. Previous studies have shown that ACx neurons encode not only sensory features but also task-related information (Jaramillo & Zador, 2010), suggesting a broader role in shaping decision strategies beyond basic stimulus encoding. Moreover, projections from ACx to the striatum and frontal cortex have been implicated in sensory-to-motor transformations, reinforcing the idea that ACx contributes to higher-order decision-making processes (Guo et al., 2017; Znamenskiy & Zador, 2013).

Our approach builds upon these foundational studies by leveraging our robust behavioural task, combining lesions, optogenetic silencing, and computational modelling to dissect the role of ACx in auditory decision-making. Specifically, we examine whether ACx influences accuracy, response timing, and anticipatory behaviour. By making use of our

knowledge regarding how Reaction Times are affected by our stimulus and how they, in turn, affect accuracy, we aim at understanding the role of the ACx in further detail, providing a principled and thorough examination of the effect of cortical perturbations in our sound lateralisation task. Thus providing us with a solid basis to launch our study of the neural underpinnings of this perceptual decision-making task in the near future. We employ two different strategies in this chapter, namely we started out performing lesions in rat auditory cortex, in order to ascertain the necessity of this structure for the performance of our task. However, lesions only allow us to assess necessity and the role of cortex as it cannot be taken over by compensatory mechanisms, and thus, we have also employed optogenetic silencing of the cortex in order to specifically study its role during our task. Utilising our thorough readout of the animals' behaviour, we modified the previously utilised model that explains the non manipulated trials in order to match the behaviour of the rats when ACx is silenced. Through the model changes needed to match the data we infer that silencing ACx has non-specific effects over anticipation, as well as sound processing specific effects, relating to the firing rate of sensory neurons and the noise in the evidence accumulation process.

5.3 Methods

All procedures were reviewed and approved by the animal welfare committee of the Champalimaud Centre for the Unknown and approved by the Portuguese Direcção Geral de Veterinária (reference no. 0421/000/000/2019).

5.3.0.1 Experimental Animals

Experiments were performed on 14 adult female Long–Evans hooded rats. Animals were 12–13 weeks old and weighed between 250 and 300 g at the beginning of the experiments. They were kept above 85% of the initial weight throughout. All animals were naive to any behavioural tests. Rats had free access to food, but water was restricted to the behavioural

sessions, which were conducted during five consecutive days per week. Animals had access to water during the sixth day and were deprived of water for 24 h before each round of five sessions. No statistical methods were used to predetermine the number of animals or the number of trials per animal, but our sample sizes were similar to those reported in previous publications (Uchida & Mainen, 2003). We used a 'within subject' design in which the animals were tested in all experimental conditions, so there was no need to apply blinding or randomization.

5.3.1 Auditory Stimuli

A percept of lateralization was created by presenting cosine-ramped (10 ms) broadband (5–20 kHz) noise bursts with different intensities to each ear (ILD) (Fig. 3.1 C). For initial training, stimuli were delivered by speakers placed on the side walls of the behavioural box; and for the latter stages of training, through speakers placed in headphones as described in Chapter 2. More details regarding the stimuli and its presentation available in Chapter 3.

5.3.2 Behavioural Apparatus and Headphone design

Rats were trained and tested on the sound lateralization task (Fig. 3.1 A, B) previously described in Chapter 3. The headphones utilised followed the same design detailed in Chapter 2, with added implanted LEDs as shown in Figure 5.1 A.

5.3.3 Behavioural Task

Rats started a trial by poking in the central port (Fig. 3.1 A) following the end of the Inter Trial Interval (ITI). After a short, variable Fixation Time (FT), the sound was played binaurally until the rat left the central port. Rats had to communicate whether the sound was louder to the left or right ear by poking in either the left or right side ports, respectively. Correct choices were rewarded with a drop of water and incorrect responses

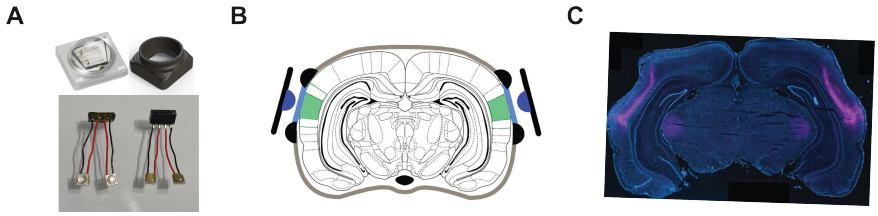

Figure 5.1: **Task and Implant for Optogenetic stimulation A.** top – LED and 3D printed holder used for the optogenetic-capable implants. The LED was placed into the 3D base and then attached to the glass cover slips that made up the window to the brain. Bottom – for binaural stimulation, two LED were implanted for all animals. These were attached to a 4-pin female connector that was implanted in the skull, posterior to the regular base for the headphones. **B.** Scheme of the cranial windows on top of the ACx and the LED pointing at these. **C.** histology image for one example animal, showing virus expression for stGtACR2, in magenta.

were penalized with timeout, during which the rat was not able to start a new trial (Fig. 3.1 A, B).

Trials in which the rat failed to start a new trial within the start trial waiting time, broke fixation during the FT or failed to poke in either lateral port within the response waiting time were considered aborts. Aborts were repeated after a 1-s time penalty except for the fixation aborts, which had a 5 s time penalty.

Each session was divided into blocks of 96 trials. For some sessions, the animals were experiencing the trials in blocks, within each block, the Average Binaural Level (ABL) was kept constant, while the ILD changed pseudo-randomly from trial to trial; for no-block sessions, both ABL and ILD were changed pseudo-randomly. Typically, sessions lasted for 2 h and rats performed between 600 and 1,000 trials.

The task has been described in more detail in Chapter 3.

Training variations: lesioned animals. Due to the base implant covering bregma and other landmarks of the skull, accurately targeting ACx for lesioning after the animals had been implanted would have been difficult, therefore the group of animals that was lesioned after learning the task experienced several ILDs before surgery and, as shown

in Figure 5.2, for these animals level 12 still had being emitted from the side speakers in the box. This level was also then experienced with headphones after implant surgery. For pre- and post-lesion comparisons, we will focus on level 12. For the remaining groups of animals, we will still focus on level 16.

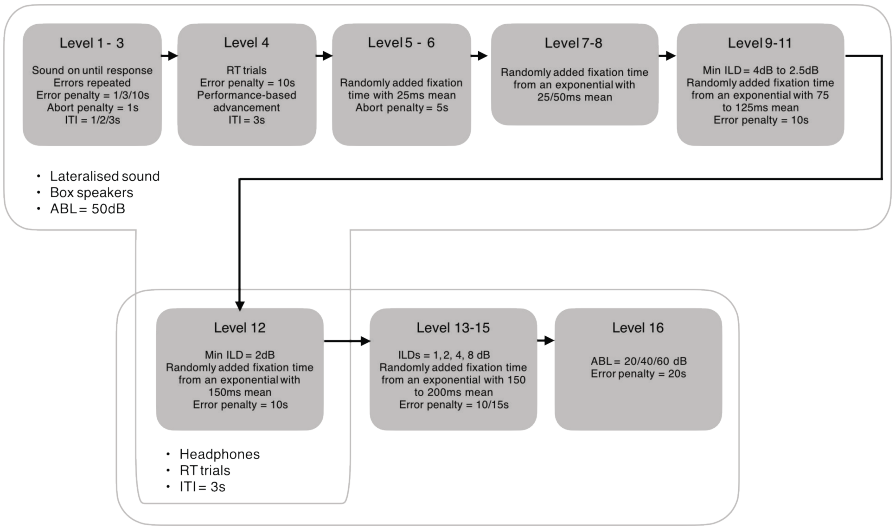

Figure 5.2: **Training progression for lesioned animals** Scheme of the progression of training levels the animals that underwent cortical injections of ibotenic acid for the lesioning of ACx. For these animals, the box side speakers were used to transmit the sound up until level 12. Such change was made in order to approximate, as much as possible, the levels in which performance and other parameters of the task, were to be compared in the setting of pre lesion with post lesion. As such, we have data for level 12 with and without headphones.

Training variations: Optogenetic manipulations. The animals were trained and previously described and after performing at level 16 in a stable manner, once their headphones were attached, a second cable for the LED control was also connected and attached to the 4 pin connector accessible posterior to their headphones base (Fig. 5.1 A bottom). LEDs were turned on either bilaterally or unilaterally, in 30% of trials. Several manipulations were employed, but for this chapter we will focus on

a single one. To allow for a broader range of fixation times for the optogenetic inhibition to start, we raised the mean of the exponential added fixation time up to 400 ms for training level 16, as shown in (Fig. 5.3). For these sessions, the LED was turned on for 30% of trials at a time during fixation and left on during the RT or during a period of 1 second.

Training variations: Maximum sampling times. This manipulation was experienced by the same animals that experienced the optogenetic manipulation above. Capped sound duration experiment with ABL = 20, 40 and 60 dB SPL. The range of maximum sound durations (Maximum Sampling Duration (SDmax)) tested was 15, 60, 120 ms and RT. Even though the rats were always free to leave the central port when they chose, the sound stimulus terminated after the selected duration even if the animal remained.

5.3.4 Surgical Procedures

5.3.4.1 MRI anatomical scans and coordinate determination

A maximum of a week before surgery, animals were scanned and a whole brain, high-definition anatomical T2-weighted set of images was acquired. Rats were anaesthetised briefly with 5% isoflurane (VIRBAC, Carros cedex, France) maintained by a vaporizer (VETEQUIP, Livermore, CA United States) in a custom-built box. The isoflurane concentration was reduced to 4% after roughly 2 min, and the animals were quickly moved to the cryocoil animal bed and stabilized with a nose cone and a bite bar. An anatomical T2-weighted Rapid Acquisition with Refocused Echoes (RARE) sequence was used (TR/TE = 1600/36 ms, RARE factor = 8, echo spacing = 9 ms; averages = 3; FOV = $18 \times 16mm2$, in-plane resolution = $168 \times 150\mu m2$, slice thickness = $800\mu m$, tacq = 1 min 3 s). These images were then aligned and superimposed with an atlas reference for stereotaxic coordinates (Paxinos & Watson, 2006) for surgery planning as shown in Figure 5.4.

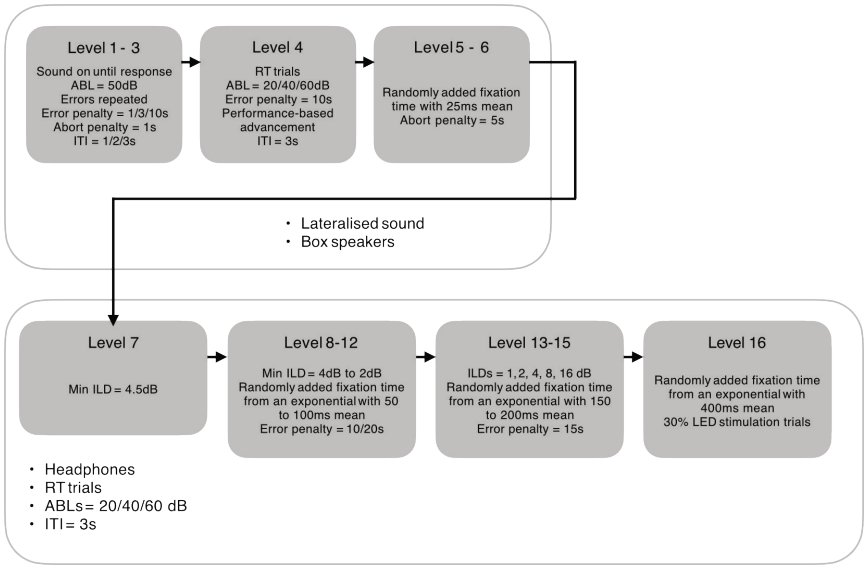

Figure 5.3: **Training progression for Optogenetic stimulation animals** Scheme of the progression of training levels the animals that underwent optogenetic stimulation went through. As usual these animals started hearing fully lateralised sound from speakers placed in the behavioural box, even though, for these animals, the implant surgery for the headphone base had already taken place. These animals also experienced 5 absolute ILDs as a final set of stimuli (1, 2, 4, 8 and 16 dB). At their final level they experienced 30% of trials with LED ON in order to inhibit ACx.

5.3.4.2 Surgery for ACx substance injection

Anaesthesia was induced by inhalation of isoflurane at a concentration of 5% (oxygen at 2 L per min) and maintained at 3/2.5% during surgery. The percentage of isoflurane could be increased if the animal exhibited signals of pain or discomfort. The animal's head was shaved, and the rat fixed to the stereotaxic frame through a mouthpiece and ear bars. Eye ointment (Bepanthen® Augen und Nasensalbe, Bayer, Germany) was applied to the eyes. Lidocaine (0.2 ml) was injected subcutaneously at the incision site before the incision was made, for local anaesthesia. The skin was cleaned using iodine, an incision was made along the midline of the skull from just behind the eyes until between the ears, and the skin

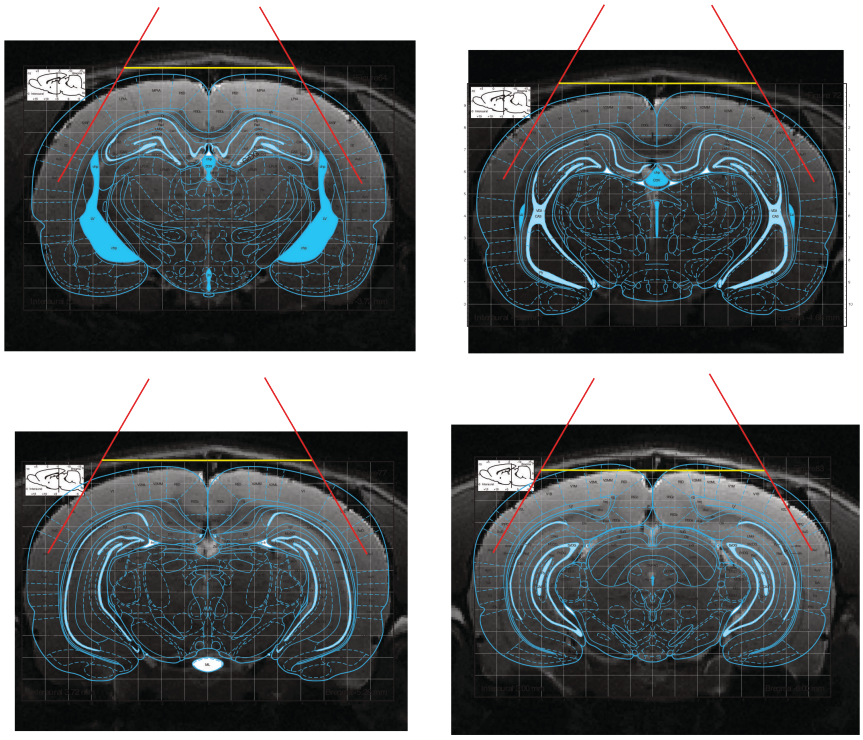

Figure 5.4: **Projection of Lesion Coordinates and Outcome of Injection** Overlay of atlas (Paxinos & Watson, 2006) to MRI scans of the corresponding coordinate. These overlays were made using an image editing program and relied on linear transformations for the match between atlas and scan. After this match, lines at an angle of 30 degrees with the vertical direction were placed, targeting the centre of the ACx and the corresponding coordinates in the skull for the craniotomies determined. This allowed us to generate very precise coordinates for each individual animal, taking into account individual differences.

was displaced laterally, exposing the surface of the skull. After cleaning the top region of the skull by blunt dissection, the skull was aligned using a rat alignment tool (Model 944 Rat Alignment Tool, Knopf®) and the bregma and lambda landmarks. Subsequently, five craniotomies were drilled (drill bit: Meisinger HM1 014), per hemisphere, according to the coordinates found through the process described in 4.3.2.1. These injections were performed with a Nanoject2 (Drummond scientific) using a thin glass capillary (Drummond scientific, 100 μm outer diameter pulled to $\sim$ 15 μm) at a 30-degree angle with the vertical direction.

For the injection of ibotenic acid (1 mg/100 μL) in solution with 0.1M NaOH, we injected 4 pulses of 32 nL per injection site, at a 23 nL rate. The different pulses were separated by 5 seconds and after completing the pulses for each site there was a 10-minute waiting period. After completing the full injection protocol, the craniotomies were subsequently covered with silicone elastomere (World Precision Instruments) and the animal was implanted with the base for the headphones according to the protocol detailed in Chapter 3, section 3.3.1.

For virus injection into the auditory cortex, we used three different approaches.

1. Parietal bone injection: The craniotomies (5 per hemisphere, with a 1 mm spacing) were performed on the parietal plate of the skull and the glass pipette of the injector was inserted into the brain at an angle of 30° to the perpendicular axis. For this approach, the pipette was inserted down to the most ventral dorsal-ventral coordinate previously calculated according to section 4.3.2.1 (Fig. 5.4) and there we would inject 2 pulses of 9 nL at a 23 nL/s rate, wait for 5 seconds and move dorsally 100 μm, to inject again, until reaching the more dorsal coordinate. At the most dorsal coordinate, the glass pipette would remain after injection for a 10-minute waiting period. The amount injected was different for each animal depending on their size, but not exceeding 1 μL per hemisphere.

2. Temporal bone injection : Craniotomies were opened on the temporal skull plate after the dissection of the temporal muscle. For these, we made only two craniotomies per hemisphere, at -4.5 AP and

-5.5 AP; and injected 180 μL in each location. The pulses were of 18 nL at a 23 nL/s rate, with a 5 second inter pulse interval. After the last pulse, the pipette remained in the craniotomy for 10 minutes before removal.

3. Cranial window injection: While the animal was still fixed with ear bars, the placement for the craniotomies was marked at -4.5 AP and -5.5 AP, and -4.5 DV on the surface of the temporal bone after temporal muscle dissection. The craniotomies were then drilled when the animal's head was turned on the side and the injections were performed perpendicularly to the surface of the ACx. The pulses were of 18 nL at a 23 nL/s rate, with a 5 second inter pulse interval. After the last pulse, the pipette remained in the craniotomy for 10 minutes before removal.

We injected the following virus for our study:

stGtACR2: AAV1-CKIIa-stGtACR2-FusionRed was injected into the ACx. This soma targeted *Guillardia theta* anion-conducting channelrhodopsin is an inhibitory opsin capable of targeting the membrane of neuronal somas of pyramidal cells due to the CKIIa promotor (Mahn et al., 2018). It was injected in a PBS 1x solution in order to reach a titter of 10^{12} vg/mL.

5.3.4.3 Base Implant surgery for headphones only

Similar to what is described in section 2.2.1. Anaesthesia was induced by inhalation of isoflurane at a concentration of 5% (oxygen at 2 L per min) and maintained at 3/2.5% during surgery. The percentage of isoflurane could be increased if the animal exhibited signals of pain or discomfort. The animal's head was shaved and the rat fixed to the stereotaxic frame through a mouthpiece and ear bars. Eye ointment (Bepanthen® Augen und Nasensalbe, Bayer, Germany) was applied to the eyes. Lidocaine (0.2 ml) was injected subcutaneously at the incision site before the incision was made, for local anaesthesia. The skin was cleaned using iodine, an incision was made along the midline of the skull from just behind the eyes until between the ears, and the skin was displaced laterally, exposing the surface of the skull. After cleaning the top region of the skull by blunt dissection, four drilling holes were made and stainless

steel screws (length, 3 mm; thread diameter, 1.6 mm) were attached to the skull, allowing for most of their length to remain outside. Cement was poured on top of these screws, ensuring it reached the space between the screws and the skull for a secure attachment. A 3D printed oval well was placed on top of the cement layer and filled with more cement, the resin base, already fitted with the cubic magnet (Fig. 2.2 A), which was then placed inside the well, and more cement was added around the lower part of the base until it was covered. The displaced skin was then stitched around the base, only allowing the necessary structure for the attachment of the headphones to remain visible (Fig. 2.2 B, C). Antibiotics (8 mg/kg, s.c.; cefovecin, Convenia) and analgesics (5 mg/kg, s.c.; carprofen, Rimadyl) were administered after the surgery and the animal remained single housed after this procedure.

5.3.4.4 Base Implant surgery with cranial window and LED implants

For the animals that went through optogenetic manipulations of the auditory cortex, their implant surgery also included the chronic implant of bilateral LEDs. For this effect, the same procedure as describe in Chapter 2 section 2.2.1 and the previous section took place but with some modifications. After cleaning of the conjunctive tissue on the skull, the temporal muscle of the animal was dissected and removed. After placement of the attaching screws, the limits of cranial windows and craniotomies for injection were marked for reference. For craniotomies in the temporal bone and outside the cranial window, as described in technique 2. of section 5.3.4.2, these were fully drilled and the animal was injected. After this, the rat was removed from the ear bars and their head turned in order for the temporal bone to be exposed and fully visible. Using a handheld contralateral angle drill, craniotomies were drilled if using the technique 3 described in section 5.3.4.2 and the animal was injected. And a 4 mm diameter cranial window was drilled into the temporal bone. To compensate for the different thicknesses of the skull on this location, the bone was polished so that a stack of 3 glass plates – two 3 mm ones and a 4 mm one – could lay flat. The glass window

was fixed with agarose and superglue. On top of the glass plates, a 3D printed piece was glued, and the LED was then placed on top (Fig. 5.1 B.). The structure was then covered in opaque black dental cement (Contemporary Ortho-Jet™, Lang Dental Manufacturing Company, Inc., Illinois, USA). Once the LEDs were implanted on both hemispheres, the animal was returned to a straight position and the headphones base was implanted as described in Chapter 3 without the oval well, due to space constraints and including the LED connector, Figure 5.1 A., placed posterior to the base. The remainder of the surgery and post-operative care follows what was mentioned in the previous section.

5.3.5 Histology

Histological processing was performed for all injected animals, unless impossible due to health issues. Animals were perfused transcardially, 24 h hours after lesion. First with a PBS 1X solution, followed by 4% PFA. The brain was extracted and kept in 4% PFA for approximately 12 h. After this, the brain was placed in a 30% sucrose solution for a minimum of 4 days, after which the tissue was embedded in a frozen section compound (FSC 22, Leica Biosystems, Nussloch, Germany) and sliced on a cryostat (Leica CM3050S, Leica Biosystems, Nussloch, Germany). After sectioning, the brain slices were stained with cresyl violet (for lesion studies) or DAPI (for optogenetics studies) and mounted with mowiol mounting medium (Fig. 5.1 C.).

5.3.6 Model fitting

Similar to what was employed for Chapter 4. We obtained the expressions for the likelihood of a given trial as a function of the model parameters and the experimental conditions (ILD, ABL, fixation duration, LED onset, if present), which simply put, corresponds to evaluating the theoretical RTDs at a given RT, and for a given choice. We manually varied the parameters of the model in order to obtain a good match between the empirical quantification of the data (RTDs, quantiles, psychometric and tachometrics) and the corresponding estimates from the model. This

process, while not providing the same precision as an automated fitting, still was able to provide reasonable estimates of the parameters from which we could draw conclusions. In some cases, we only varied a small subset of parameters while keeping the rest fixed.

5.4 Results

To study the role of the ACx in our sound lateralisation task we performed both lesion and optogenetic studies. We will focus on each of these separately.

5.4.1 Ibotenic acid effectively lesions the ACx but has little effect on behaviour

In order to lesion the ACx we injected ibotenic acid, an excitoxic agent that damages cells by causing a large influx of calcium into the cell, thus creating excessive activation while mainly targeting excitatory neurons (Neves et al., 2023; Raghavendra et al., 2013). These lesions usually encompass an area of sparser cell density or even with no intact neurons with a diffuse border (Pai et al., 2011; Wallace et al., 1990).

Animals injected with ibotenic acid were found to have severe lesions of the ACx, as shown in Figure 5.5 A. The extent to which the ACx was lesioned varied from animal to animal and also between hemispheres within the same animal. As mentioned, Ibotenic acid targets mainly excitatory cells, which is not meant to cause the absence of tissue we can notice in our histological images. However, the animals were perfused for the acquisition of these images months after the lesion, at which point the area had lost structural integrity and was obliterated while slicing the perfused brain.

We lesioned animals both after learning our sound lateralisation task and while naive, and analysed how they behaved once ACx was impaired. We compared the animals within the first cohort to themselves, pre- and post-lesion, and also to the naive cohort. We focused on the

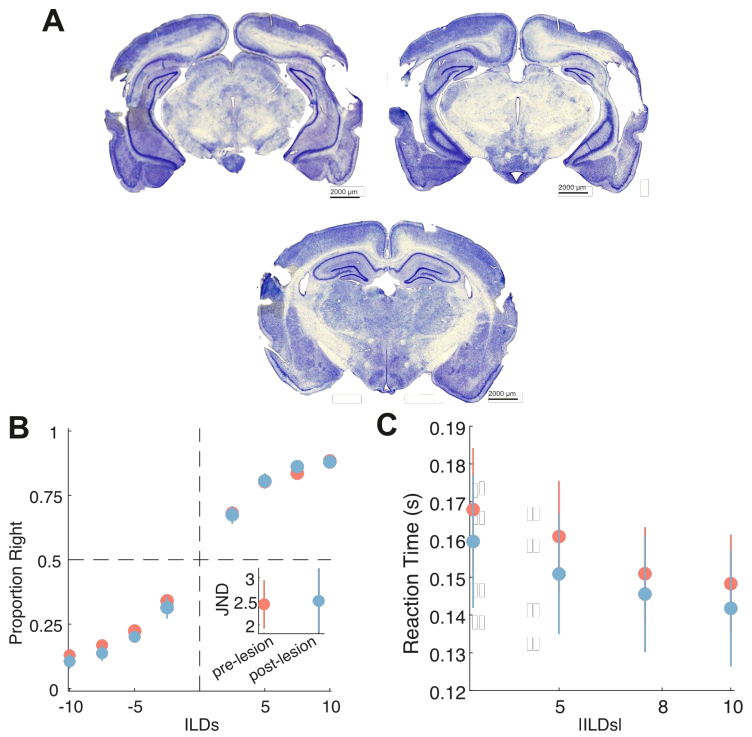

Figure 5.5: **Post-Learning Lesions A.** Histology images of animals in which we performed excitotoxic lesion in ACx. Brain slices are stained with a Nissl stain, and shown are three different coordinates that include ACx. **B.** Choose-Right probability for animals lesioned after learning the task, pre- and post-lesion (in orange, and violet, respectively). These animals were trained without headphones and then lesioned and implanted on the same surgery. When they were returned to training, the same level, still without headphones, was compared to their last sessions pre lesion. **Inset**: comparison of the Just Noticeable Difference (JND)s pre- and post-lesion for these animals. **C.** Chronometric for the animals pre- and post-lesion (in orange and violet, respectively) for the comparison level shown for the absolute ILDs. Data shown for N = 5 animals, error bars denote the standard error of the mean.

measurements of performance used in previous chapters for accuracy and RT.

Post-learning Lesions of the ACx have little to no effect on performance

We trained animals in the regular sound lateralisation task as detailed in previous chapters (Chapter 3, but allowed them to reach a later level working with side speakers instead of headphones, for comparison of performance pre- and post-lesion (see Methods, Fig. 5.4).

Animals that underwent these lesions in ACx after learning the task show no large differences in performance when comparing their choose-right probabilities (Fig. 5.5 B.) for training level 12 (the training level animals experienced both pre- and post-lesion) (Fig. 5.4). These animals also show little effect at the level of their RT pre and post lesioning, and although it is possible to notice a decrease in RT (Fig. 5.5 C.), it seems to be related to the animals' increase in experience performing the task. This was confirmed by analysing the animals' behaviour across sessions before and after lesioning (Fig. 5.6). Both RT and accuracy are similar across sessions before and after the lesion (marked with a vertical dotted line); indeed, RT seems to be even slightly longer immediately after the lesions, which might be explained by the time taken for surgery recovery. For Figure 5.5 B., we took into account all sessions before and after the lesion, and this included sessions where the rats were still improving their performance. As shown in Figure 5.6 B., manipulating the amount of sessions taken into account can change the relation between pre- and post-lesion performance, but taking into account a larger window of 10 sessions, allows considering sessions where the performance is already stable post lesion, while also no longer including session during which the animals were still perfecting their behaviour before the lesions.

Post learning Lesions of the ACx are comparable to naive lesions

As previously mentioned, another animal cohort was lesioned while naive to the behavioural task. These animals were also trained in the

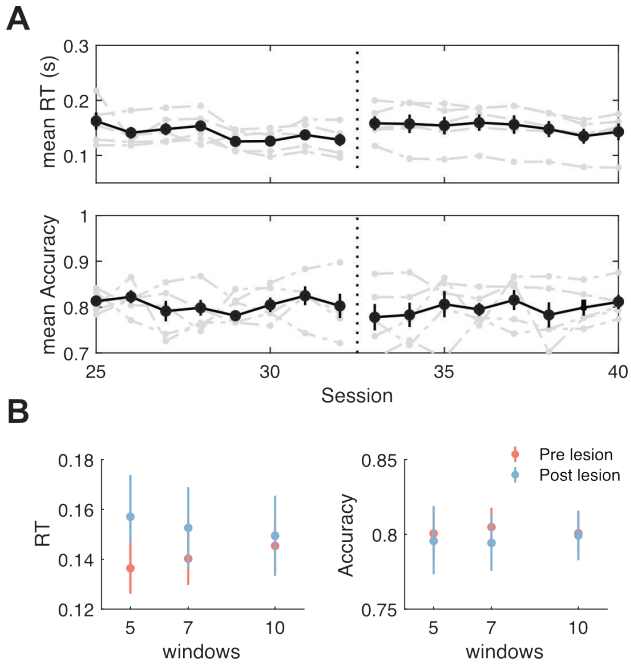

Figure 5.6: **Comparison of RT and Accuracy pre and post ACx lesion A.** RT (top plot) and Accuracy (bottom plot) across session immediately before and after ACx lesion. Grey lines denote the different animals and black line the mean of the different animals. Error bars correspond to the standard error of the mean and the dotted vertical line to the training interruption due to the lesion. **B.** Average RT and Accuracy over animals for different number of sessions pre- and post-lesion. Each dot is the mean RT or Accuracy over session and animals, with the error bars corresponding to standard error of the mean. Any larger differences seem due to a small window of sessions being used.

same way (ILDs were presented without headphones) even though the lesion had already been completed, in order to more easily compare them to the post-learning lesioned cohort.

These animals showed similar performance, both at the level of accuracy and RT to animals lesioned after training (Figure 5.7 A.). Both cohorts were then fitted with headphones and started performing the task at the same level, level 12, with them (Figure 5.7 B.). As expected, performance suffered some changes from the use of headphones, as these, as suggested in Chapter 2, allow for a more accurate and precise stimulus delivery. Both cohorts show a decrease in lapses, and although they are slightly biased in these conditions (as shown in Figure 5.7 B.), their JND remains comparable (Figure 5.7 B. inset). This bias is later abolished, as shown in Figure 5.7 C. Panel C. shows choose-right probability and RT for the same cohorts but at the final training level, at which point the animals are considered to be behaving at psychophysical threshold and can be compared with other cohorts, such as the ones shown in Chapter 3. In this last training level, both cohorts perform to a very similar level and have comparable JND to each other (Figure 5.7 C. inset). They also show accuracy and RT similar to previous groups (Fig. 5.8).

Although RT is lower for animals that were lesioned after learning (Figure 5.7 B. Right plot) this difference is then not present for the final training level in Figure 5.7 C. or Figure 5.8, so it might be due to some temporary difference that is then abolished.

Both lesioned cohorts indicate that the ACx is not necessary for the performance of our sound lateralisation task. Animals are still able to perform this task even though ACx is impaired. There is also little difference between the two batches in the final level of training, indicating they can reach similar levels of performance regardless of the time at which their ACx was lesioned. A small effect on accuracy was expected from existing literature (Kelly, 1980; Kelly & Glazier, 1978; Kelly & Masterton, 1977). But our animals show even less of a behavioural effect than expected. This might be due to the fact that previous tests were done on sound localisation instead of lateralisation, with multiple speakers throughout an arena and not a binary response. However, it might be

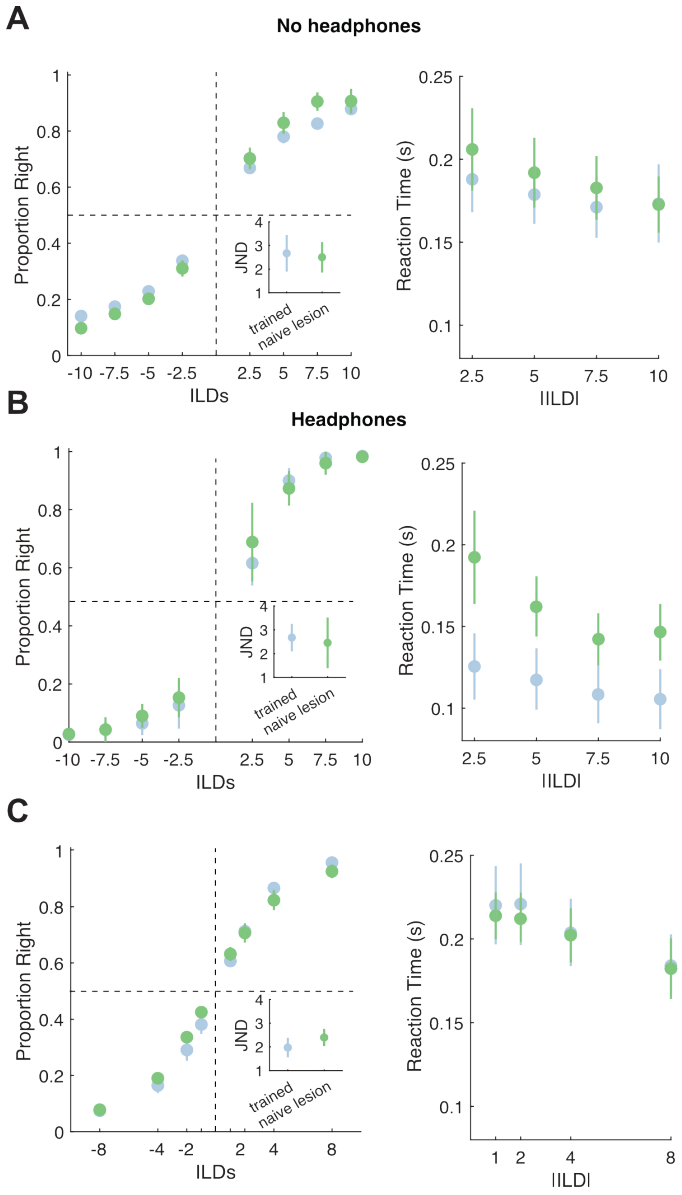

Figure 5.7: **RT and Accuracy for trained and naive lesioned animals** Comparison between animals that were lesioned after learning the task (N=5) and a cohort that was lesioned while naive (N=3) and followed the same training protocol. **A.** shows the comparison of accuracy and RT for the same training level post lesion and without headphones. **B.** Continues the comparison in level 12, but now with headphones.

Figure 5.7: (Continued caption) **C.** Shows the accuracy and RT for the two cohorts of lesioned animals in the final level of training, with headphones. **Insets:** show the comparisons of the JNDs between the two cohorts.

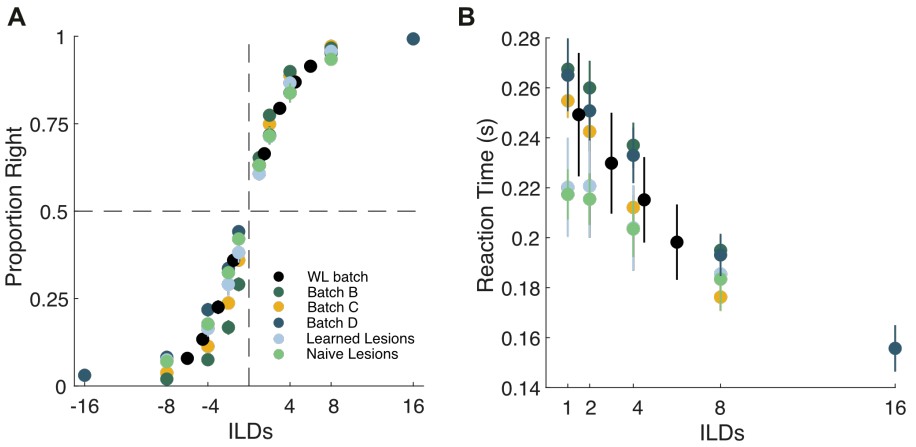

Figure 5.8: **Comparison of Lesioned Cohorts to Previous cohorts A.** Choose-right probability for several batches. Lesioned animals (lesioned after learning the task, light blue, N = 5 rats; and lesioned naive, light green, N=3 rats) show comparable performance to other batches. **B.** Reaction times per ILD for the same cohorts as in A. Lesioned cohorts are also comparable in terms of RT, although they perhaps show shorter RTs for the smaller ILDs.

worth it to further investigate the resilience shown in the case of lesions, especially in light of the following optogenetic silencing results.

Although lesions offer an important way to test the necessity of a structure in a certain behaviour, they allow for compensatory strategies to take place, such as plasticity. Furthermore, they do not allow us the study of a brain region with fine temporal resolution, for instance, to test the role of ACx during a specific point of the decision-making process for our task, such as during sound presentation. For this, different strategies are necessary.

5.4.2 Optogenetic inhibition of Auditory Cortex

In order to investigate the role of the ACx in our task under normal conditions, we employed optogenetics. We targeted the A1 area of Auditory Cortex (Fig. 5.1 B.), and expressed the opsin stGtACR2 in the cortex of rats (example animal with broader expression in Fig. 5.1 C.) implanted with cranial windows so that light from externally implanted LEDs reached the cortex and manipulated the opsin-expressing cells (Fig. 5.9). The remaining sections of the Results for this Chapter will be focused on these LED-implanted animals.

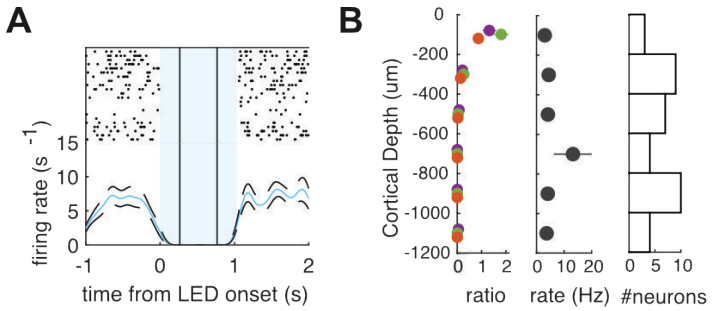

Figure 5.9: **Silencing of ACx with stGtACR2 A.** Testing the efficiency of stGtACR2 (Mahn et al., 2018), to silence the rat ACx. We injected an AAV driving expression of stGtACR2 under the CamKII promoter in the ACx, and after 4 weeks recorded from the same rat under urethane anaesthesia using a linear probe. We presented the same broadband noise bursts using during behaviour at an ABL of 60 dB SPL to the contra-lateral ear. In half the trials, we silenced the ACx shining blue light with an LED. Plot shows an example raster and PSTH of a single neuron. **B.** Summary statistics of silencing the ACx. Left, ratio of firing rate in light-on relative to light-off trials, as a function of cortical depth. Middle, mean firing rate across neurons at each depth. Right, number of neurons recorded at each depth. stGtACR2 is effective at silencing the ACx.

5.4.2.1 These animals reach similar levels of performance as previous Batches Without Optogenetic Stimulation

In order to attempt a mechanistic explanation of how silencing the ACx affects the performance on this task, it was important to first confirm

the animals perform the behaviour to the level of the previously studied batches when they are free of optogenetic manipulations.

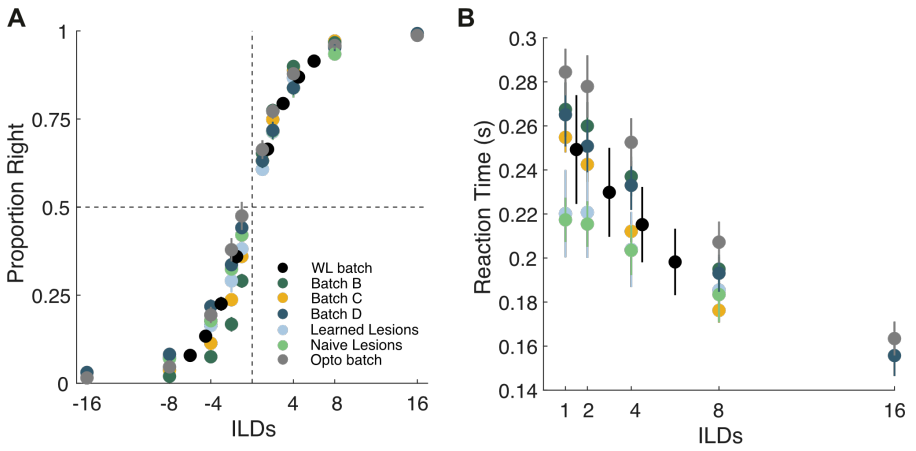

Figure 5.10: **Comparison of LED Cohort to Previous cohorts A.** Choose-right probability for several batches, including the batch we will be focusing on for this chapter (Last LED, grey markers, N = 8 rats, error bars correspond to SEM across rats). **B.** Reaction times per ILD for the same cohorts as in A. LED cohort is also comparable in terms of RT, although they perhaps show higher RTs due to the increased Fixation Time this animals experience when compared to the previous cohorts.

These animals are comparable to previous cohorts (Fig. 5.10) both in terms of accuracy and RT. Although RTs are longer for these rats, we believe this is due to their longer Fixation Times (Fig. 5.3).

We analysed these animals' performance as we did for the lesioned animals in the previous section, and we show their overlapping psychometric curves for the different ABLs (Fig. 5.11 A.). Furthermore, their Reaction Times (Fig. 5.11 B.) show an evident speed accuracy tradeoff and ABL dependency, much like the animals for which the TIED model was fitted on.

Looking at the Reaction Time Distribution (RTD) for these animals, one can still observe the bimodality mentioned in Chapter 3, as well as the responses before sound onset – negative portion of the RTD (Fig. 5.11 C.). Tachometric curves (Fig. 5.11 D.) also present the usual pattern as in previous chapters and show a clear distinction between hard and easy

trials but similarities between the different ABLs.

We have established the consistency between the results of this cohort and previous ones, which gives us confidence on having a handle on the mechanisms underlying their behaviour under no optogenetic manipulation. We will now focus on the trials where the LED is on.

5.4.2.2 Optogenetic silencing of ACx has a clear effect on performance

Animals from the cohort being analysed for this chapter had bilaterally implanted LEDs, as described in Methods. The LEDs were turned on in 30% of trials and each it would be randomly decided if both LEDs would turn on or if stimulation would be unilateral. We will be focusing on LED ON trials in which bilaterally stimulation occurred (1/3).

In previous experiments, we noticed the animals would react quickly in response to the LED turning on, originating very short RTs that were both ILD and ABL independent (data not shown). This created a confound where we could not disentangle the effect of the LED from the effect of these extremely short RTs, on performance. In order to understand if the animals were able to remain in the central port after LED onset, we anticipated this moment so that it took place during the Fixation period, at a variable moment. In this way, extremely short RTs would culminate in a Fixation Time abort. Animals managed to remain in the port and listen to the sound after LEDs were turned on, and so, we adopted this configuration for LED ON trials. The LEDs were then turned on before sound onset, and remained on, either until the animal left the central port – turning off at the same time as the sound –, or for one second, regardless of what the animals were doing at the time. These two paradigms were studied sequentially and did not alter the animals' performance.

For trials in which the LEDs were turned on and the ACx inhibited through this optogenetic manipulation, the animals showed clear differences in their behaviour. Namely, their accuracy decreased, as shown in

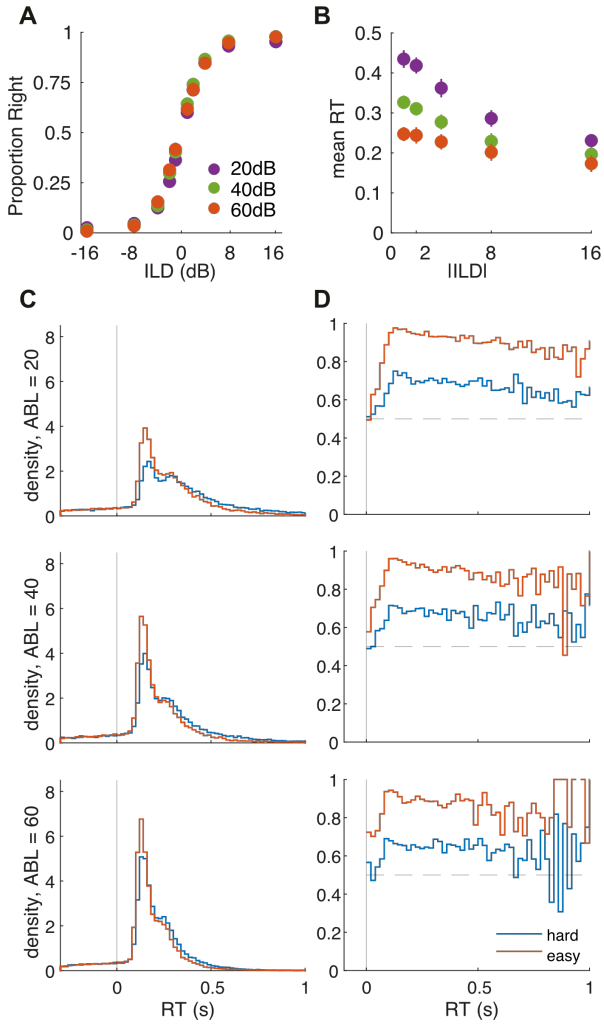

Figure 5.11: **Accuracy and Reaction time in trials without LED stimulation A.** Psychometric curves for the animals performing the task with optogenetic stimulation, for the LED OFF trials (N = 8 rats). Each colour corresponds to a different ABL and error bars correspond to SEM. **B.** Like A. but chronometric curves. The Speed Accuracy Tradeoff (SAT) is clear as smaller ILDs show higher RTs when compared to larger ILDs. The presence of an ABL effect on RT is also clear, as the faintest overall sound level – 20 dB – shows a larger RT. **C.** Reaction Time Distributions for hard (1 and 2 dBs) and easy (4 and 8 dBs) trials of the different ABLs. These distributions include negative RTs corresponding to Fixation Time aborts. **D.** Like **C.** but tachometrics for the same conditions.

Figure 5.12 A., where it is clear LED ON trials – blue markers – correspond to a lower accuracy than that for LED OFF trials – grey markers –. This difference in accuracy seems to also bee ABL-dependent, being more pronounced for 20 dB and less evident for 60 dB ABL. There is also a clear effect on RT, which once again decreases across all conditions, but retains its speed accuracy tradeoff. This decrease is particularly apparent for the smaller ILDs, and in an ABL-dependent manner. Crucially, these differences are not present when comparing LED ON and LED OFF trials for rats that underwent the same surgery and LED implantation, as well as the same percentage of LED ON trials, but had no opsin expressing on their cortex (Fig. 5.12 C. and D.). Even though some variation might be expected for LED ON trials – as a light turns on while the animals are usually working in darkness –, this variation is not consistent with the one verified for opsin-expressing rats, indicating the LED alone turning on during a trial is not sufficient to generate the effect observed in the rats' performance.

While the decrease in accuracy is to be expected under the silencing of ACx, it is less clear how to interpret the decrease in RT. We expected that, if ACx provides key sensory information for making a decision, RTs would increase if the activity of this area and its sensory neurons were reduced.

5.4.2.3 Fixation Time Aborts Increase for LED ON trials

Why would animals respond faster when ACx is perturbed? Looking at the shape of the RTDs (Fig. 5.13 A. and B. top rows, blue line) provides clues as to what might be going on. Rats are leaving the central port earlier in LED ON trials, even before the sound is presented (i.e., trial aborts) in some cases. The animals seem to become more impulsive for LED ON trials, as it is clear from Figure 5.13 C, where the cumulative probability of a Fixation Abort is consistently higher for LED ON trials (in blue).

It is clear aborts before LED onset are the same for LED ON and LED OFF trials, as expected considering there is no cue regarding the trial

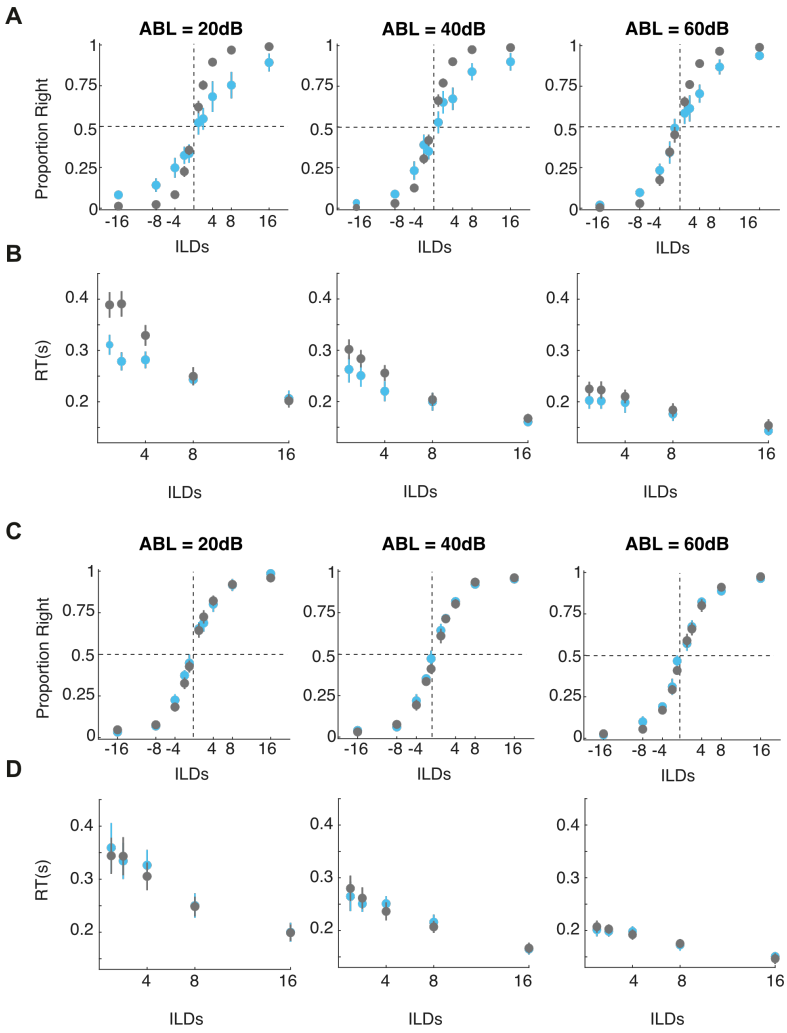

Figure 5.12: **Comparison of Accuracy and RT for LED ON and LED OFF trials A.** Choose-right probability for the different ABLs. Each plot includes the choose-right probability for LED OFF (grey) and LED ON (blue) trials. Error bars correspond to SEM across animals (N = 6 rats) expressing the opsin in ACx. Accuracy for LED ON trials is lower than for LED OFF, and this effect appears to be ABL-dependent. **B.** Same as A. but for RTs. RTs are lower for LED ON trials, specifically for the lower ILDs, and the difference also seems to be ABL-dependent. **C.** Like A., for control animals (N = 6 rats) not expressing the opsin). **D.** Like B., for control animals. Accuracy and RT do not seem to suffer simply due to the implanted LEDs being ON.

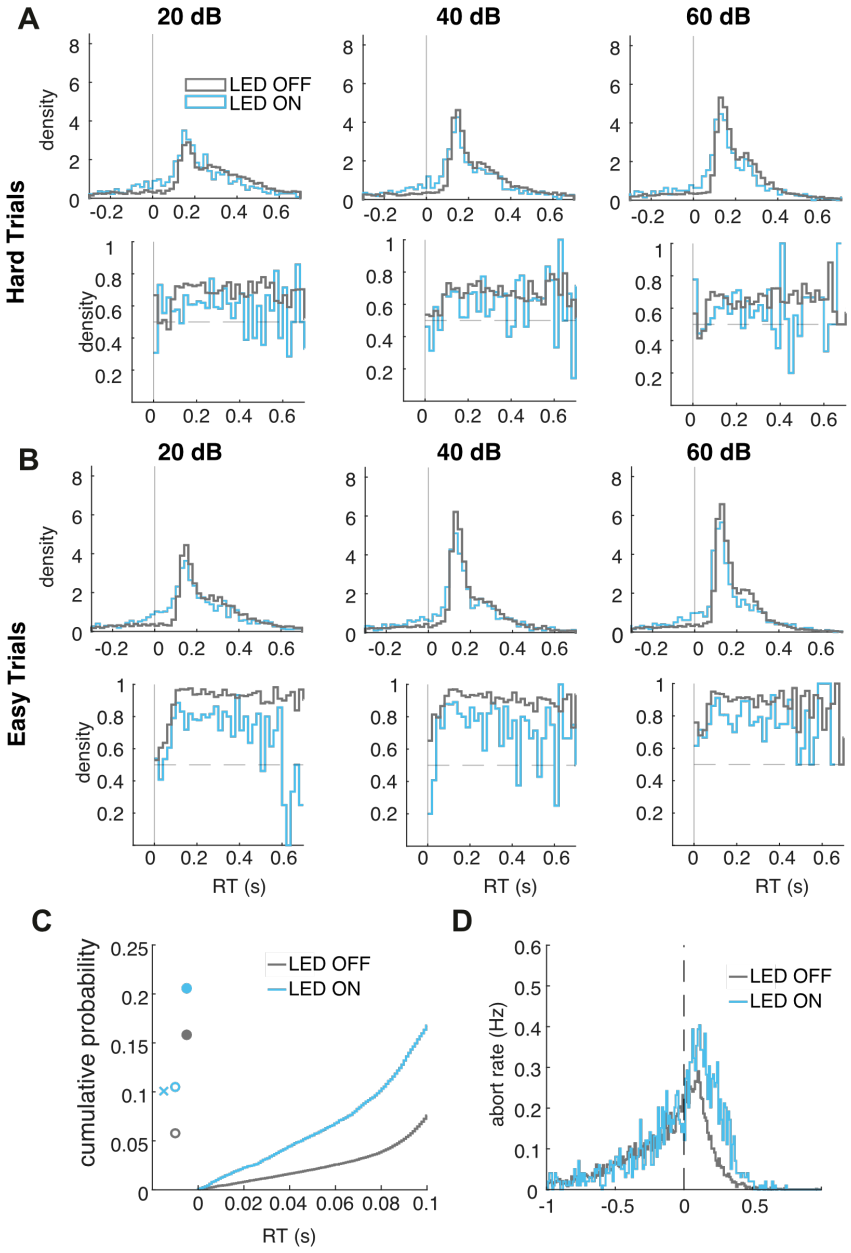

Figure 5.13: **Effect of LED on RTDs and Tachometrics. A.** Reaction Time Distributions (top) and Tachometric functions (bottom) showing accuracy according to RT for LED ON (blue trace) and OFF (grey trace) for hard trials (1 and 2 dB).

Figure 5.13: (Continued caption) **B.** Similar to A., for easy trials (4 and 8 dB). Each column corresponds to one ABL and tachometrics are shifted in order to align with RTDs for easier interpretation. **C.** Cumulative distribution function (CDF) for RTs of trials with LED ON and trials with LED OFF. The markers indicate cumulative distribution of aborts, RT<0, also for the different trial types. The crosses (overlapping) denote abort rates before the LED onset; open circles denote abort rate restricted to after LED onset; and filled circles denote total abort rate. **D.** Abort rate, aligned to LED onset, for trials with LED ON (blue) and LED OFF (grey).

type before the LED onset. The aborts only differ if they occur after LED onset (Fig. 5.13 D.). The remaining RTDs are similar for the two types of trials, and present bimodality for both trial types, much like previous cohorts showed. This is an indication that perturbing the ACx causes an increase in anticipatory trials.

From these data, we wondered if the observed loss of accuracy was only due to an increase in the number of trials where RT is set by the proactive process. In this case, for short RTs, we would expect little difference, as those choices already corresponded to a large percentage of proactive trials. For longer RTs, however, one would expect a mild decrease in accuracy, considering that the Decision Variable (DV) would now reach the bounds for fewer trials, and when the bound is not reached the choice readout is random (see Chapter 4). This could be qualitatively consistent with the tachometrics (Fig. 5.13 A. and B. bottom rows), but we do not know if changes at the level of the proactive process would be enough to explain the recorded differences in a quantitative manner. In order to understand if the increase of the proactive process represents the main effect of ACx silencing, and is responsible these effects on RT and accuracy, we used the model previously mentioned in Chapter 4.

5.4.3 Modelling the Effect of Silencing ACx in our Task

5.4.3.1 Fitting aborts

As mentioned in previous sections, we noticed an increase in both early RTs and Fixation Time aborts for trials with LED on. These short RTs and

aborts have previously been linked to a proactive process (see Chapter 3) (Hernández-Navarro et al., 2021). In order to isolate this proactive component, we opted to first fit our model to Fixation Time aborts only.

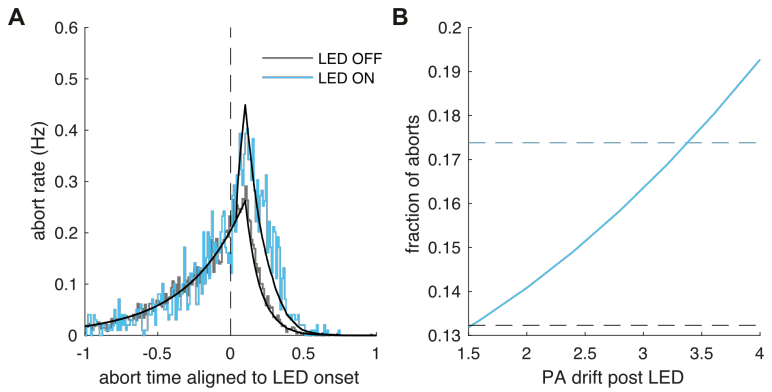

Figure 5.14: **Abort Rate for LED ON and LED OFF trials. A.** Abort rate, aligned to LED onset, for trials with LED ON (blue) and LED OFF. Model fittings correspond to the smooth overlaid grey lines. **B.** Evolution of the fraction of aborts given by the change of the drift rate of the proactive process. Dashed lines correspond to fraction of aborts for LED ON (blue dashed line) and LED OFF (grey dashed line at the bottom)

This can be accomplished with just three parameters: the proactive drift, bound and delay. We were able to obtain an accurate fit for LED OFF data (Fig. 5.14 A. in grey and overlaid black). The fact that this fit is so accurate, not only supports our theory that Fixation Time aborts are caused by a proactive process parallel to evidence accumulation, but also suggests that this parallel process does not require more complex parameters, such as a time-varying drift-rate or bounds, and it can be modelled similarly to what Hernández-Navarro et al., 2021 showed.

We then turned onto the LED ON data. When aligned to LED onset, the abort rate shows a clear change (Fig. 5.14 A. in blue), being noticeably higher for trials with LED ON. We wondered whether an increase in the proactive drift would be sufficient to capture this effect. In order to obtain this, we increased the drift of the proactive process until the abort rate equalled the one we recorded for LED ON trials (Fig. 5.14 B.). Matching the overall abort rate was sufficient to provide an accurate description of

the data (Fig. 5.14 A. in black), capturing the increase and shape of the abort rate for LED ON trials. This provides evidence that silencing the ACx has an effect on anticipation, that is well described by a step change in the amount of anticipatory evidence.

5.4.3.2 Fitting valid trials

We have demonstrated that, consistent with previous work (Carrillo-Reid et al., 2019; Chong et al., 2020; Luis-Islas et al., 2021; Marshel et al., 2019; Ortiz-Rios et al., 2023; Sun et al., 2021; Weible et al., 2020), the perturbation of a cortical area has an effect on behaviour, in our case a tendency to break fixations.

Presumably, this could, at least partially, explain the decrease in RT in LED ON trials. It might even explain the decrease in accuracy for the same trials. In order to understand if it is so, we attempted to, first, provide an accurate fit of our data in control trials – LED OFF –. Our strategy was then to explore the effect of the perturbation on the anticipatory process only, and evaluate if this is sufficient to explain the observed effects of the perturbation on accuracy and RT. If, on one hand, this is so, we would conclude that the impairment in sound lateralisation caused by the perturbation does not really reflect an impairment in the processing of sound, but instead an unspecific behavioural reaction to the experience of the perturbation. If, on the other hand, the effects of the perturbation on sound lateralisation cannot be fully reproduced by a model including the observed change in anticipation, then this suggests the perturbation might impair actual processing of sound, and that neurons in the ACx might provide sensory evidence relevant for choice in our task.

As we have shown in previous chapters, model fits of the LED OFF trials produced a good match to the experimental data (Fig. 5.15) at the level of both Reaction Times and accuracy. As mentioned, we first attempted to model LED ON data for valid trials by applying the same changes to the proactive process drift that allowed us to reproduce Fixation Time aborts (Fig. 5.14). As Figure 5.16 shows, changes to the

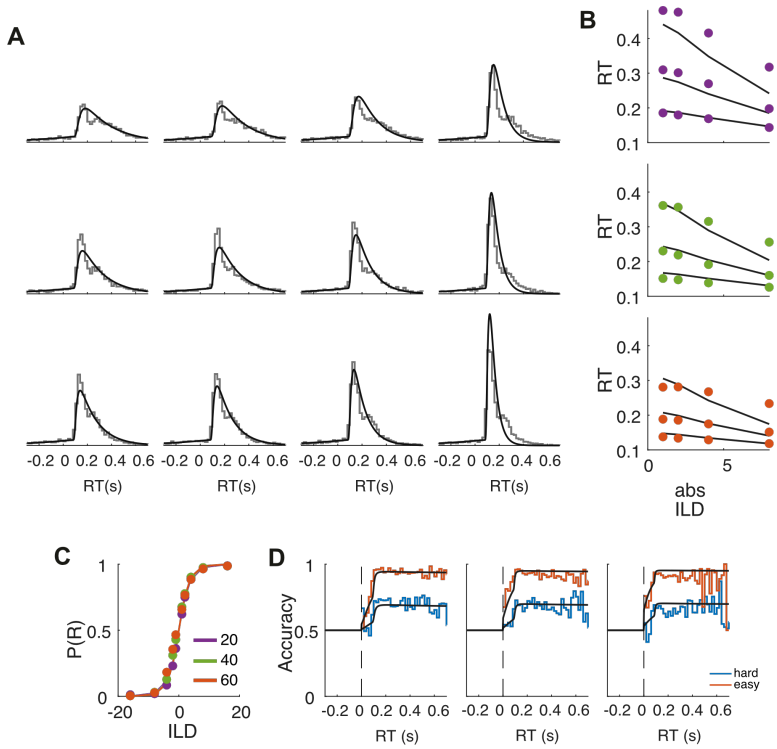

Figure 5.15: **Model Fitting for LED OFF trials. A.** RTDs for the different ABLs (rows) and ILDs (columns) in grey and fits in black. **B.** Reaction Time data divided by quantile for the different ILDs. Each plot corresponding to an ABL. The black lines correspond to the model fittings. **C.** Choose-right probability for the different ILDs (markers) and model fittings for the psychometric functions for the three ABLs. **D.** Tachometrics for easy (4 and 8 dB) and hard (1 and 2 dB) trials and model fits in black.

proactive process are able to reproduce some of the changes caused by the LED. Namely, the general shape of the RTDs, especially the initial part of the distribution, and the RT for the lower quantiles (Fig. 5.16 A. and B.). However, we could only produce a very modest decrease in performance (Fig. 5.17 shows the comparison between the fits for LED ON and OFF). The predicted psychometrics (Fig. 5.16 C.) show higher predicted performance than our data, and even though the initial portion of the tachometrics is captured by the model, the predicted accuracy for the longer RTs is also higher than what the animals were able to show.

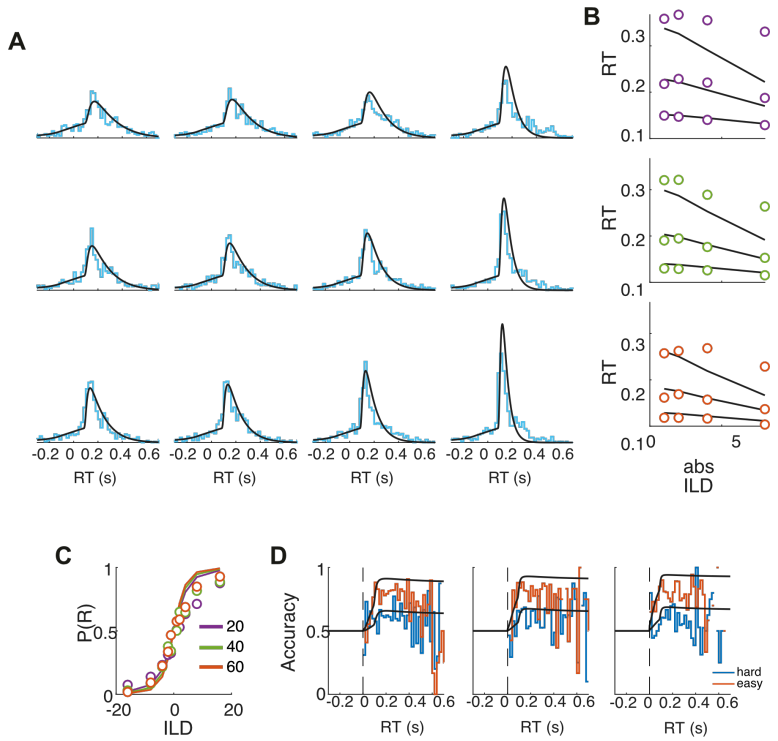

Figure 5.16: **Model Fitting for LED ON trials, changing the proactive process alone. A.** RTDs for the different ABLs (rows) and ILDs (columns) in blue and fits in black. **B.** Reaction Time data divided by quantile for the different ILDs. Each plot corresponding to an ABL. The black lines correspond to the model fittings. **C.** Choose-right probability for the different ILDs (markers) and model fittings for the psychometric functions for the three ABLs. **D.** Tachometrics for easy (4 and 8 dB) and hard (1 and 2 dB) trials and model fits in black. RT data and distributions are similar to the data, but accuracy is overestimated.

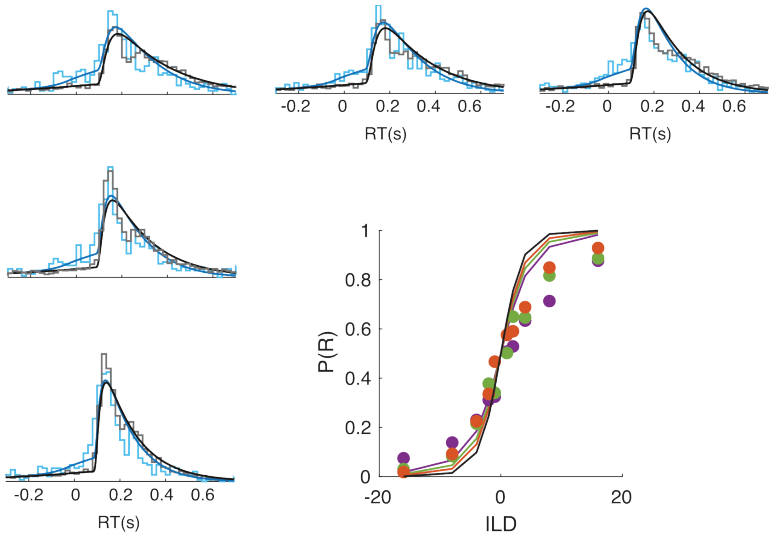

Figure 5.17: **Comparison of Model Fittings for LED ON and LED OFF trials, changing the proactive process only.** Example RTDs with the data corresponding to LED ON trials in blue and LED OFF trials in grey histograms. Lines on top correspond to the model fittings following the same colour code. Choose-right probability for the different ILDs (markers) and model fittings for the psychometric functions for the three ABLs. It is noticeable the fittings closely match the early portion of the RTDs, but not the accuracy data.

These results show that salient effects of the perturbation are not accounted for by a change in anticipation, and suggests that silencing the ACx does impair the processing of sound in our task. We do not, however, know what specific form or forms these impairments in the processing of the sound take. This meant we needed to ascertain what other parameters needed to be altered, in addition to the proactive drift, in order to reproduce the effects of silencing the ACx the behaviour of the animals. In order to understand this, we modified different aspects of the way the sensory evidence shapes choice and RT in the model, attempting to match the effects of the perturbation as accurately as possible. We reasoned that, since we were inhibiting neurons in a sensory cortex, it would be sensible to expect a decrease in the overall firing rate of the sensory neurons that provide the evidence in our model. Thus, we

attempted to lower the values of the parameter that represents the firing rate of the sensory neurons in our model (Fig. 5.18).

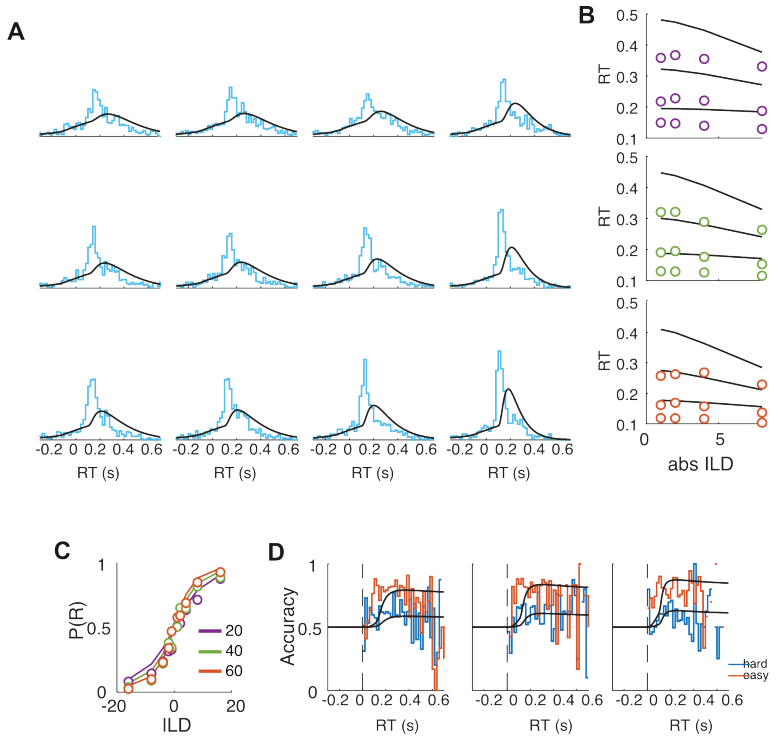

Figure 5.18: **Model Fitting for LED ON trials, changing the proactive process and firing rate. A.** RTDs for the different ABLs (rows) and ILDs (columns) in blue and fits in black. **B.** Reaction Time data divided by quantile for the different ILDs. Each plot corresponding to an ABL. The black lines correspond to the model fittings. **C.** Choose-right probability for the different ILDs (markers) and model fittings for the psychometric functions for the three ABLs. **D.** Tachometrics for easy (4 and 8 dB) and hard (1 and 2 dB) trials and model fits in black. It is clear that although accuracy can match the data, RTDs and RTs for the same conditions are overestimated.

Altering both the proactive drift and the firing rate of sensory neurons, we achieved comparable levels of accuracy to the data, as shown in Figure 5.18, panels C. and D. The psychometrics are no longer overlapping for the different ABLs, as seen in the data and tachometrics reach lower levels of accuracy. However, at the level of the RTs, changing the drift of

the proactive process and decreasing the firing rate did not manage to accurately reproduce the data. By decreasing the firing rate, the model predicts RTs would increase, to an extent that is clearly larger than what we observe (Fig. 5.18 A. and B.).

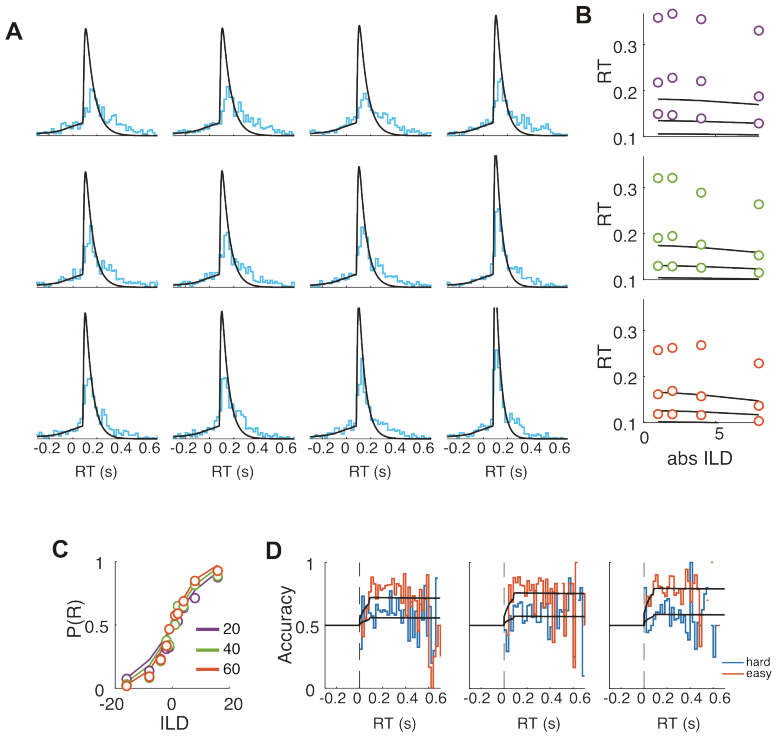

Figure 5.19: **Model Fitting for LED ON trials, changing the proactive process, and Noise level. A.** RTDs for the different ABLs (rows) and ILDs (columns) in blue and fits in black. **B.** Reaction Time data divided by quantile for the different ILDs. Each plot corresponding to an ABL. The black lines correspond to the model fittings. **C.** Choose-right probability for the different ILDs (markers) and model fittings for the psychometric functions for the three ABLs. **D.** Tachometrics for easy (4 and 8 dB) and hard (1 and 2 dB) trials and model fits in black. These modifications to the model allow us to better match for accuracy, but there is a large underestimation of the RTs.

Another parameter of relevance is extra noise added to the process of evidence accumulation. This can mean, for instance, that, under normal circumstances, ACx creates representations of the different aspects of

the sound, that we modify or contaminate through our cortical silencing. We attempted to alter noise alone in our model (Fig. 5.19) and once again managed to match the data in terms of accuracy (Fig. 5.19 C. and D.), However, at the level of the RTs, we have the opposite effect observed when changing the firing rate (Fig. 5.18) and now RTs are underestimated (Fig. 5.19 A. and B.).

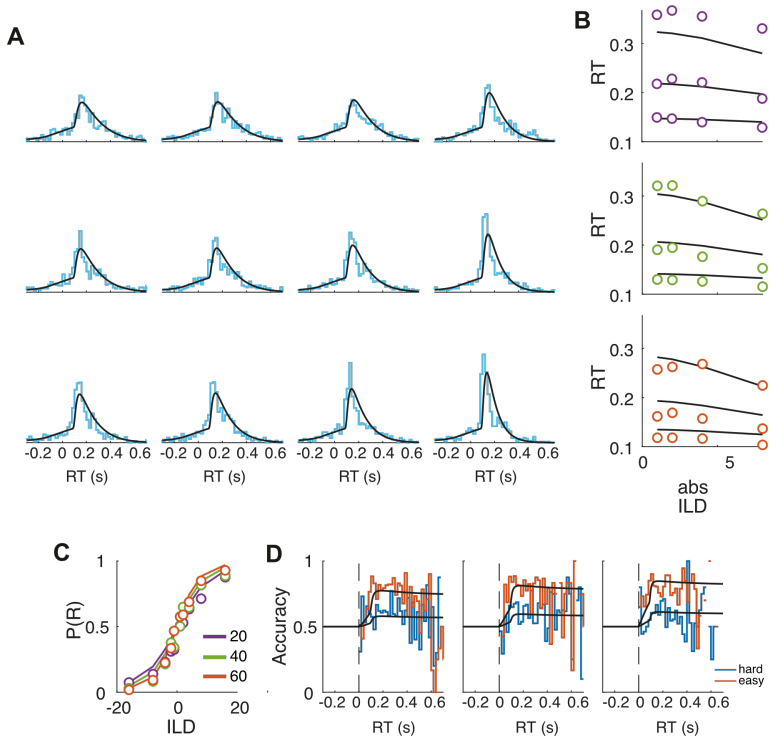

Figure 5.20: **Model Fitting for LED ON trials, changing the proactive process, firing rate, and Noise level. A.** RTDs for the different ABLs (rows) and ILDs (columns) in blue and fits in black. **B.** Reaction Time data divided by quantile for the different ILDs. Each plot corresponding to an ABL. The black lines correspond to the model fittings. **C.** Choose-right probability for the different ILDs (markers) and model fittings for the psychometric functions for the three ABLs. **D.** Tachometrics for easy (4 and 8 dB) and hard (1 and 2 dB) trials and model fits in black. These modifications to the model allow us to better match both accuracy and RTs.

Since both decreases in firing rate, and increases in noise, decrease accuracy, but have opposite effects on RT, we reasoned that a combination of both manipulations might produce the desired effect: a clear decrease in accuracy (which was not accomplished by changing anticipation alone) with only a moderate effect on RT (as the increase in anticipation already largely explained the observed decrease in RT due to the perturbation). Indeed, using a combination of increased proactive drift, decreased firing rate of sensory neurons, and increased noise in the process of evidence accumulation, was finally sufficient to produce a good fit of the accuracy and RTs observed in the LED ON data (Fig. 5.20). This adaptation of the model nicely captures the overall shape of the RTDs (Fig. 5.20 A.), offering a good approximation of the RT quantiles (Fig. 5.20 B.), while also matching the data in terms of accuracy, both at the level of the psychometrics – showing a lack of ABL invariance and overall lower accuracy (Fig. 5.20 C.) –, and at the level of the tachometric, reproducing the rise in accuracy for the early results and the lower accuracy for the longer RTs (Fig. 5.20 D.).

Reproducing the effect of silencing the ACx through our model allows us to interpret the behavioural results and infer what might be happening in the brain as a result of our manipulation of the Auditory Cortex. Our data shows that silencing the ACx has an impact on anticipation, potentially a non-specific effect; but also modifies the way the sensory evidence guides behaviour, suggesting that ACx conveys sensory evidence that is relevant for sound lateralisation under normal conditions.

5.4.4 The Breakdown of Weber's Law for Short Durations is Worsened Under LED Inhibition

Similarly to Chapter 3, we also tested these animals in conditions where the sound was terminated for durations shorter than the average RT, different SDmax. Our goal was to test whether the breakdown of WL worsened with the silencing of the ACx through optogenetics, in order to understand the predominance of the effect of the LED on the proactive process. Considering our findings detailed in Chapter 4, most trials for

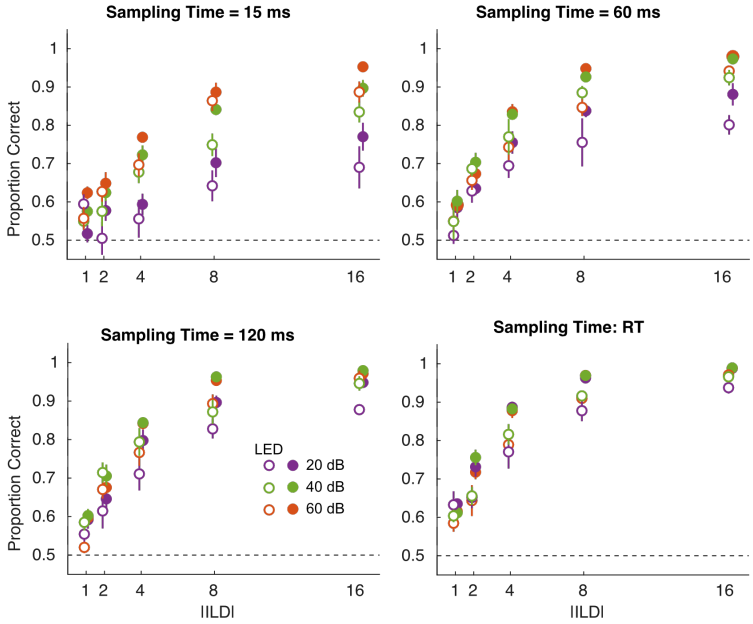

Figure 5.21: **Comparison of accuracy of LED ON and OFF Trials for the different SDmaxs.** Each panel corresponds to a different SDmax and shows the accuracy for LED ON (open circles) and LED OFF (filled circles) trials. ABLs are denoted by the different colours N = 5 rats, error bars correspond to the SEM.

short SDmaxs will predominately correspond to proactive trials – the evidence is short and therefore there is no time for the DV to reach the bound. For this reason, if the main effect of silencing the ACx were related to affecting the proactive process, there would be little difference in accuracy for LED ON trials.

For this, we reproduced the protocol from Chapter 4 with SDmaxs of 15, 60, 120 ms and RT trials interspersed through the session (see Methods section). Results show that indeed, performance for short SDmax is affected by optogenetically silencing the ACx (Fig. 5.21). Accuracy is consistently lower for LED ON trials (Fig. 5.21, open circles) when compared to LED OFF.

Similarly to what happened for this type of trials in Chapter 4, RTDs show an increase of longer RTs for the shorter duration trials, and that

remains true for the LED ON trials as well (Fig. 5.22). Interestingly, for the LED ON RTDs (in blue) the second peak of the RTD becomes less pronounced, hinting that perhaps silencing the ACx might help us understand this bimodality we have seen in the RTDs for the longer and more variable Fixation Times.

5.5 Discussion

The findings presented in this chapter provide critical insights into the neural mechanisms underlying the auditory decision-making task in study, particularly regarding the role of the ACx in perceptual decision-making tasks. Our experiments, which involved both ibotenic acid lesions and optogenetic inhibition of the ACx, showed that while the cortex is not essential, its manipulation affects performance of our sound lateralisation task. While the cortex is traditionally considered non-essential for simple auditory behaviours (Ceballo et al., 2019; Gimenez et al., 2015; Kelly, 1980; Kelly & Glazier, 1978; O'Sullivan et al., 2019; Pai et al., 2011), its manipulation still affects performance. In spite of still being able to perform the task above chance level, our animals show clear decreases in accuracy and changes at the level of Reaction Times. This is a somewhat surprising result, considering the prevailing notion that subcortical structures may play a more prominent role in processing auditory information for localisation and lateralisation of sound (Kelly, 1980; Kelly & Masterton, 1977), and guiding decisions based on that information. However, we show that there appears to be a role for ACx, seemingly related to both specific and non-specific effects regarding sound processing that show an influence in our task.

Cortically Lesioned Rats Achieve the Same Level of Performance as Non-Lesioned Rats

The results from our lesion experiments indicate that animals with significant damage to the ACx (Fig. 5.5 A) exhibited no substantial differences

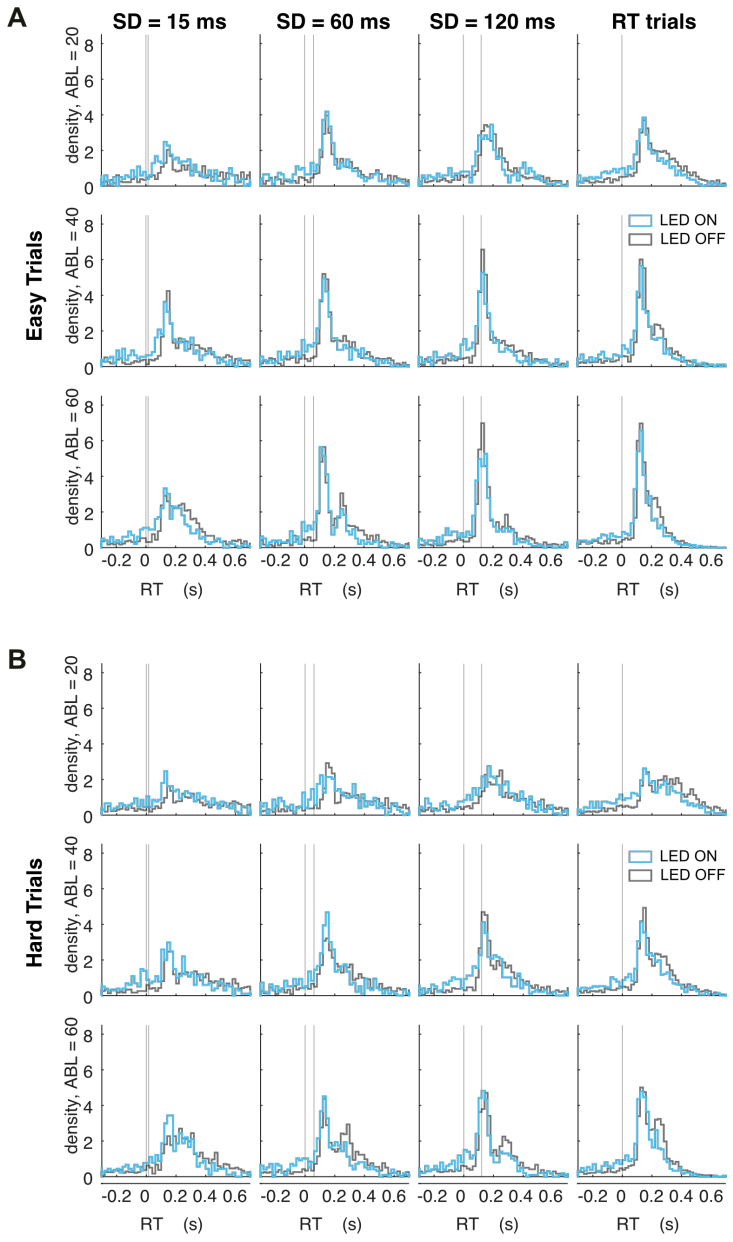

Figure 5.22: **Reaction Time distributions for LED OFF and LED ON trials. A.** Reaction Time Distributions (LED ON in blue and LED OFF in grey) for easy trials (4 and 8 dBs) as a function of SD (columns) and ABL (rows). **B.** As A. but for hard trials (1 and 2 dBs).

in performance compared to pre-lesion baselines (Fig. 5.5 B). This finding is in agreement with previous research showing that the ACx is not essential for sound lateralisation (Kelly, 1980; Kelly & Glazier, 1978). This suggests rats may rely on alternative neural pathways or compensatory mechanisms to localise sound sources in space, highlighting the plasticity of auditory processing systems. Previous studies have demonstrated that subcortical structures, such as the IC and Medial Geniculate Nucleus (MGN), are capable of processing auditory information and contributing to sound localization (Grothe et al., 2010). Indeed, the IC is one of the first structures to encode ILDs (Grothe et al., 2010; Malmierca, 2015; Severo et al., 2024). This is also an area potentially capable of compensating for sensory deficits caused by the loss of auditory cortex, considering its preponderant role in the auditory pathway (Malmierca, 2015; Malmierca & Ryugo, 2010).

The ability of rats to maintain performance despite ACx lesions implies that these structures could be sufficient for basic auditory decision-making. However, it has been shown that rats and other rodents have difficulties reaching their maximum accuracy with a lesioned ACx (Kelly & Glazier, 1978; Pai et al., 2011; A. L. Smith et al., 2004). This does not seem to be the case here, but perhaps only because our comparison was performed for a set of ILDs still far from the animals' psychophysical threshold, at which point they may still not require cortical processing for high performance. This was done due to the technical challenge of presenting difficult sounds in a precise manner without fitting the rats with the headphones. At the same time, it is difficult to lesion the ACx after the animals have been implanted with the headphone base, as the cement covers the top of the skull. However, we proceeded with the normal training for these animals after training level 12. As shown in Figure 5.7, lesioned animals were trained until they were performing with the smallest ILDs that we use for this task (1, 2, 4, and 8 dBs) and their performance was comparable to animals that were lesioned naive and also to non lesioned animals (Fig. 5.11) It is also possible the lesions were not as extensive, and it would be needed to have an impairment for this task, for some animals lesions were very confined which might

have contributed to the negligible difference found. To account for these factors, ideally, the experiment would be repeated in a way for the animals to experience smaller ILDs both before and after the lesion, and with a better targeting of the area. Although not performed for this Thesis, we plan on turning our modelling approach also to the lesion data. While more data is necessary in order to fit the model, we would like to understand the effect of this perturbation in further detail as well.

Optogenetic Silencing of the Auditory Cortex Affects Performance

Cortical lesions allow us to address the necessity of ACx for our task, however, they do not allow us to understand its role under normal circumstances, and only the extent to which other structures are unable to take over. In order to minimise these compensatory mechanisms, we employed optogenetic manipulations as well. In contrast with the results from the lesions studies, our results from optogenetic silencing of the ACx provide evidence of the nuanced role of the ACx in auditory tasks. While animals exhibited similar baseline performance levels when not subjected to optogenetic inhibition – suggesting the more invasive surgery and chronic implantation of LEDs, as well as opsin injection and expression do not affect their behaviour –, activation of the expressing opsin stGtACR2 led to marked declines in accuracy and alterations in RTs (Figs. 5.12 and 5.13). This indicates that while the ACx may not be essential for sound lateralisation, its perturbation has clear effects on task performance, even though it does not fully preclude it, consistent with previous studies (Kelly & Glazier, 1978; Pai et al., 2011; A. L. Smith et al., 2004; Tai & Zador, 2008; Talwar et al., 2001).

Because it is hard to make clear inferences regarding physiological manipulations in a behavioural task, our goal for this work was to leverage our thorough measurements in our behavioural task and the model we have developed to explain normal behaviour of the animals. This model encompasses some biological notions regarding how the brain makes the transformation of sensory stimulus to evidence that is then accumulated,

offering us a glimpse into the sensorimotor transformation necessary for this task. Therefore, modifying the parameters of this model can help us interpret the effects of the optogenetic manipulation. We modelled how these optogenetic manipulations may affect the dynamics of the decision-making process during our task exploring several hypotheses through an adaptation of the Parallel Sensory Integration and Action Model (PSIAM) (Hernández-Navarro et al., 2021) (similar to what we employed in Chapter 4).

Increase in Anticipatory Behaviours Explains the Increase in Aborts

We first focused on an observed increase in the rate of Fixation aborts during optogenetic inhibition trials. We considered this increase in Fixation Time aborts – animals leaving the central port before stimulus onset – when LED is turned on might have at least two possible causes: silencing the auditory cortex creates a percept that causes the animals to react; alternatively, the ACx might have a default suppressive role on anticipation, and silencing of the ACx would unveil anticipatory processes. It has been shown that optogenetic manipulations may be perceived by the animals as external stimuli (Carrillo-Reid et al., 2019; Chong et al., 2020; Luis-Islas et al., 2021; Marshel et al., 2019; Ortiz-Rios et al., 2023; Sun et al., 2021; Weible et al., 2020), and it is plausible this perception could motivate the animals to abandon the central port, as if responding, but aborting the trial. It is, however, complicated to assess if this is happening or not.

We focused on the possibility that silencing the ACx has an impact on anticipatory behaviours, and this seems to explain some of the changes we recorded. Anticipatory responses are characterised by self-initiated actions that can occur independently of external stimuli. We have previously modelled these trials through a proactive process that is parallel to the usual evidence accumulation process (see Chapter 4). We modified our model, specifically as it pertains to the proactive process, in order to reproduce the abort rate of LED ON trials and managed to reproduce abort rate and shape (Fig. 5.14).

Increase in Anticipatory Responses Alone Cannot Account for the Behavioural Deficits

Besides the increase in aborts mentioned above, that naturally become associated to an increase in shorter RTs, we also observed a decrease in accuracy during LED ON trials. Because lower accuracy could simply be a by-product of increased anticipatory responses, we attempted to match our model to the data in valid trials as well. While increasing the drift rate in proactive processes successfully reproduced the increase in fixation aborts, it did not account for all the changes in accuracy or RTs during completed LED ON trials. It was able to reproduce the earlier RT quantiles but it did not accurately account for the longer ones (Fig. 5.16 B.), nor was it able to reproduce the effects of the LED at the level of the psychometric (Fig. 5.16 C.) or the latter portion of the tachometrics (Fig. 5.16 D.). Thus, while it is clear that, when the ACx is inhibited, animals may rely more heavily on these proactive strategies, leading to impulsive responses and premature decisions, it is also clear this is not enough to describe the behaviour of the animals under these conditions. This implies an interplay between proactive and reactive decision-making strategies, complicating our understanding of how decisions are formed, and highlighting the need for further investigation into how these processes coexist within the brain.

We have shown that the behavioural outcomes of silencing the ACx are not fully explained by an increase in anticipatory decisions, actually point to effects at the level of sound processing – a decrease in the firing rate of sensory cells and an increase in sensory noise (Fig. 5.20). This suggests that silencing the ACx does disrupt ongoing processes involved in evidence accumulation and decision-making – the auditory cortex seems to be part of the pathway that links sound and action in our task. These findings also align with previous research indicating that activity in the ACx can modulate perceptual decision-making by influencing how sensory information is integrated (Xiong et al., 2013; Znamenskiy & Zador, 2013).

Optogenetic Silencing of the Auditory Cortex Appears to do More than Decrease Activity

The fact that we could not reproduce the behavioural outcome of this manipulation by solely lowering the activity of sensory neurons in our model also lead us to an interesting conclusion – this perturbation appears to not only lower activity, but also give rise to *different* activity patterns.

It seems logical that silencing a brain region would lead to lower firing rates of its cells. In fact, we show this to be the case in ACx (Fig. 5.9). But because this does not fully reproduce the effect of this cortical silencing, we hypothesised the concurrent increase of noise. This suggests that cortical circuits may act to enhance the signal-to-noise ratio in auditory spatial computations, enabling more reliable discrimination of subtle ILD differences. Or the presence of network effects from the cortical silencing, that may bring noise in the decision-making process.

It has been shown that transient inactivations of certain regions of the brain, while indeed silencing said region, may either not do so fully (Talwar et al., 2001), creating aberrant forms of the original activity, or create off-target effects – network effects (Otchy et al., 2015).These can modify the animals' percepts, potentially giving rise to the noise we had to increase in the evidence accumulation process to reproduce our data.

Interestingly, these network effects raise some other questions. They might arise in the auditory cortex itself – considering we are silencing this area through direct silencing of pyramidal cells, this is probably not a pattern of activity that occurs naturally, potentially giving rise to further abnormalities in surrounding cells. However, we cannot exclude these networks effects to take place elsewhere along the auditory pathway, including subcortical structures. The descending auditory pathway connects the ACx back to the subcortical structures that relayed the auditory signals to the cortex in the first place (Malmierca, 2003, 2015; Malmierca & Ryugo, 2010; Winer, 2005; Winer & Lee, 2007). And it is possible these areas are the ones with an abnormal activity pattern during LED ON trials, adding noise to the process. Several areas could be

affected by these off-target effects, such as the SNr, MGB, or, for instance, the IC. The IC is as a main hub in the auditory pathway (Malmierca, 2003, 2015) and receives corticofugal input that influences its activity (Bajo et al., 2010; Blackwell et al., 2020). However, recent work actually ascribes a much smaller role to descending cortical input into the IC (T.-Y. Lee et al., 2024), and although this does not discount abnormal patterns to become more relevant due to their unusual profile, it suggests that perhaps another structure might be the target of these network effects. It is also meaningful that the IC is one of the first structures to show encoding of ILDs (Grothe et al., 2010; Malmierca, 2015; Severo et al., 2024), which makes it possible that affecting it would create a much larger deficit in terms of performance than the one we have shown in this work. Importantly, it is unclear the extent to which these effects are specific to auditory tasks. Network effects that create noise in the sensory pathway might impact performance in a variety of scenarios and sensory modalities through processes of attention/distraction. A similar paradigm of silencing ACx while animals behave in a task of a different modality might help answer this question.

Further evidence in favour of the ACx having an effect not only at the level of the proactive process but also for reactive choices as well is the fact the effect remains for LED ON trials in the context of a short duration manipulation such as the one employed in Chapter 4. In this case, if the proactive process were the one mainly affected, we would expect a more subtle effect for LED ON trials, which did not occur. Interestingly, the RTDs for these trials show a decrease in the bimodality we have been observing, potentially linking it to the proactive process, although more data is required for a more concrete finding to take form.

5.5.1 The role of Auditory Cortex in Auditory Decision-Making

Recent studies have significantly advanced our understanding of the neural mechanisms underlying auditory decision-making in rodents, highlighting the intricate interplay between cortical and subcortical structures.

Gimenez et al., 2015 demonstrated that extensive lesions to the ACx in rats did not impair their ability to adapt to new sound-categorization contingencies, with only minimal effects on discrimination performance. In contrast, lesions to the auditory thalamus resulted in significant impairments, suggesting that subcortical pathways can independently mediate rapid adaptation in sound interpretation. This aligns with the long-standing idea that subcortical structures such as the IC and SC play essential roles in auditory processing and behavioural responses (Kelly & Masterton, 1977; Malmierca, 2015).

The role of ACx in decision-making has been further explored by studies recording single-unit activity in rats engaged in a self-initiated go/no-go auditory task (Jaramillo & Zador, 2010). These results showed that ACx neurons encode not just sound features but also task-related information, linking auditory processing to behavioural context. In a related study, Guo et al., 2017 found that choice-selective neurons in ACx encode both sound identity and expected reward magnitude, reinforcing the idea that ACx contributes to decision-related computations beyond basic auditory processing.

Other research has focused on the transformation of auditory representations into motor commands. Corticostriatal projections play a key role in linking sensory input with motor responses during auditory decision-making tasks (Znamenskiy & Zador, 2013). Inactivation of corticostriatal pathways biases decisions, suggesting that these projections serve as a critical interface between perception and action.

Similarly, studies on evidence accumulation have demonstrated that both rodents and humans can optimally integrate discrete pulses of sensory evidence (Brunton et al., 2013), most likely passing through the ACx before reaching downstream decision areas. Additionally, research on multisensory decision-making has shown that both rats and humans integrate auditory and visual cues to enhance perceptual accuracy (Bizley et al., 2013). This highlights the flexibility of cortical processing, as ACx is involved not only in sound perception but also in cross-modal integration.

Our research offers a nuanced perspective, demonstrating that ACx

disruption affects both accuracy and RTs in an ILD-based auditory lateralisation task. In this study, optogenetic silencing of ACx did not fully abolish task performance but led to increased impulsivity – more frequent fixation breaks and shorter RTs –, and decreased accuracy, suggesting that ACx plays a role in shaping auditory decisions. This builds on previous work by reinforcing the idea that ACx is not strictly necessary for sound lateralisation but contributes to optimising evidence accumulation and response timing, particularly when high accuracy is required. This is also reminiscent of findings in visual decision-making tasks, where perturbations of sensory cortical areas affect the dynamics of decision formation (L. N. Katz et al., 2016; Koay et al., 2020). It also relates to previous research suggesting that ACx might be more relevant for more difficult discrimination tasks (Talwar et al., 2001), while its impairment might leave simpler tasks unaffected (Ceballo et al., 2019; Slonina et al., 2022).

Additionally, our research expands on the work from Jaramillo and Zador, 2010 on temporal expectation, showing that disrupting ACx activity does not simply affect sensory processing but alters decision-making strategies. Specifically, ACx silencing increased anticipatory responses, shifting the balance between proactive and reactive choices, leading to more responses before sound onset and shorter RTs, which could be seen as evidence of involvement of ACx in temporal expectation. Taking it further, one could argue that one of the goals of the proactive process is to time the decision to ensure that a response is given so the least amount of time is wasted – occasionally making an impulsive choice is actually the optimal behaviour. In this case, ACx might be part of this process, judging whether to remain accumulating evidence or when to abandon the stimulus-based decision in order to terminate the trial. A compelling complementary experiment would be to introduce temporal expectation into our task and assess whether rats require ACx to take advantage of this information.

To gain deeper insight into how ACx influences response selection beyond its effects on RTs, future analyses should focus on unilateral silencing of ACx to determine whether and how asymmetry in cortical

activity impacts decision-making and response patterns. Our findings suggest that ACx may play a role in stabilising evidence accumulation by filtering out irrelevant noise, a function that may have been underappreciated in previous models. Alternatively, ACx manipulations could induce perceptual distortions that directly influence decision-making. Unilateral inactivations may introduce systematic biases in the animals' choices, particularly in trials where the accumulated evidence had not reached the decision bound and responses are otherwise random. In such cases, silencing one auditory cortex could shift the balance, leading to a consistent bias in the decision outcomes. Though our task is solely based on auditory decisions, the observed effects of ACx impairment on decision noise and anticipation suggest a potential key role that might affect other modalities, which should also be assessed.

It is useful to draw a parallel between auditory decision-making in rodents and decision-making processes in primates. In primates, evidence accumulation is believed to occur in the lateral intraparietal cortex (LIP) (Kiani et al., 2008; Shadlen & Newsome, 2001). However, LIP does not receive direct visual input; instead, it receives preprocessed, task-relevant sensory information from upstream areas of the visual cortex, such as MT/V5 for motion perception. These sensory areas extract and refine relevant stimulus features before passing them to LIP, where evidence is integrated over time to form a decision variable.

Similarly, in rodents, ACx does not appear to be the site of evidence accumulation but may instead filter and refine auditory information before passing it to downstream decision-making circuits. Several candidate regions could serve as the auditory equivalent of LIP, with ACx functioning more like early sensory cortices in visual decision-making—providing graded, decision-related sensory input but not integrating it over time.

5.5.2 Conclusion

This chapter provides a detailed behavioural and computational analysis of how ACxmodulates perceptual decision-making. While previous research established that subcortical structures can compensate for ACx

lesions, our findings show that ACx plays a more intricate role in shaping decision dynamics, modulating both accuracy and RT through its influence on evidence accumulation and proactive responses. By integrating behavioural data with computational modelling, we contribute to a deeper understanding of auditory decision-making, providing a principled framework for how cortical computations influence performance in a sound lateralization task, offering new insights into the function of ACx in sensory-guided behaviour.

5.6 Acknowledgements

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5.7 Author Contributions

Alfonso Renart (A.R.), Juan Ramón Castiñeiras de Saa (J.R.C.-d.S.), and Mafalda Valente (M.V.) designed research; M.V. performed the experiments, M.V., J.R.C.-d.S., Raghavendra Kaushik (R.K.) analysed the data.

6

Final Discussion

In this thesis, we have explored the decision-making process for an auditory perceptual task, and this study has highlighted the intricate relationship between sensory processing and behavioural outcome and, especially, how important temporal dynamics are to define this outcome.

Throughout this work, we have investigated how auditory stimuli influence decision-making dynamics, focusing on the role of the Auditory Cortex (ACx) and the implications of temporal constraints on evidence accumulation. By integrating findings from both behavioural experiments and computational modelling, we have gained valuable insights into how decisions are formed in response to auditory stimuli and how these processes may differ under varying conditions.

6.1 Ratphones Allow for Precise Stimuli Presentation

A vital point in the study of perception and perceptual decision-making is the certainty one is presenting the correct stimuli to the subject. For this, the development of the Ratphones, as described in Chapter 2, represents a significant advancement in auditory research methodology for stimulus presentation to freely moving rats.

The Ratphones address a critical challenge in auditory research with

freely moving, small animals, enabling precise control over sound presentation. We can adjust the headphones individually to each animal and make sure that for every sound presentation they are in the appropriate position. By ensuring the Interaural level differences (ILD) and Average Binaural Level (ABL) are presented to the animal in a precise and accurate manner, we can then study the decisions made based on these stimuli.

Headphones allow for more naturalistic experimental paradigms, bridging the gap between highly controlled but constrained head-fixed preparations, and less controlled but free-movement experiments. This had been accomplished for larger animals (Fishman & Steinschneider, 2009; Schroeder et al., 2001), but for smaller rodents there was still a lack of adjustable means to present sound that were also easy to place on the animal and remove. Previously, sound presentation has been done though several designs. Speakers placed inside the ear using, for instance, otoscope speculae (Hartley et al., 2011; Walker et al., 2011), afford a high degree of precision with respect to the sound that reaches the inner ear but it requires anaesthesia, not allowing for a behavioural study.

For both anaesthetised and awake animals, perhaps the most commonly used technique in auditory research is to have speakers fixed in the arena/setup used for the study. In this configuration, the animal might be freely moving or head-fixed. For head-fixed animal, speakers may be placed in any position in relation to the ears and sound presentation will be reliable and precise. However, head-fixation requires habituation, surgery for the implantation of a head-bar or similar apparatus for the fixation and does not allow for the animals to move, requiring unnatural responses that are hard to learn, when employed during behavioural task, and requiring sometimes lengthy habituation for the animals to be comfortable being fixed (Deneux et al., 2019; Joachimsthaler et al., 2014; Oviedo et al., 2010). Recent work has attempted to bridge this gap between head-fixed animals – allowing for increased accuracy and precision in stimuli presentation as well as limiting movement in order

to facilitate measurements and manipulation –, and the naturalistic responses of freely moving animals by creating arenas suspended on air flow that allow for the illusion of movement and naturalistic responses for head-fixed animals (Jordan et al., 2021; Krumin et al., 2018; J. J. Lee et al., 2022; T.-Y. Lee et al., 2024; Nashaat et al., 2024) but these are hard to employ for rats, as they are not easy to head-fix. For freely moving animals, the speakers are placed along the arena through which they move, and so there is little need for extensive habituation (Keating et al., 2013; Nodal, Keating, & King, 2010; Wesolek et al., 2010). However, it is hard to control the exact sound that is presented to the animal at a given time, especially in terms of magnitude considering the distance to the speakers changes as the animals move. It is especially difficult to precisely present stimuli that differ between the ears in a controlled manner. For larger ILDs side speakers placed in the box can be precise enough for accurate delivery to the animal, even at larger distances, however, for smaller differences between the ears it becomes difficult to accurately calibrate the speakers and small differences in the angle of the head of the animal with relation to the speaks will have a large effect. This makes it very complicated to use this configuration for the type of stimuli used in our task and guarantee that animals are experiencing what we intend to present. The need for headphones appropriate for smaller rodents and other animals has been a reality for auditory research for a long time, and there is a previous example of adjustable removable speakers that were placed on the animal (Otazu et al., 2009). These differ from our Ratphones in that the implantable portion consisted of plastic rings placed close to the pinnae and to which the speakers were screwed on at the beginning of each behavioural session. Even though the animals did not need to be anaesthetised for the speakers to be fixed, and they could behave, this was still a harder and more stressful process than simply snapping the headphones into place with magnets as we established with the Ratphones.

Our design, featuring a skull-implantable base and magnetically attached adjustable structure, offers several advantages that complement our approach:

- Minimally invasive: The small implantable base is less disruptive to the animals, potentially reducing confounding factors in behavioural studies.

- Flexible: The magnetic attachment allows for easy application and removal, facilitating repeated testing sessions without additional surgical procedures.

- Precise: The adjustable but fixed structure ensures consistent speaker positioning relative to the ears, crucial for accurate ILD presentation.

The ability to deliver binaural stimuli with high precision is particularly relevant to our investigations of sound lateralization tasks and ILD. And the ability to do so while the animal is freely moving is crucial to the study of the temporal dynamics of decision-making in a more naturalistic manner. The Ratphones' flexibility and the fact they are minimally invasive allows us to make use of additional tools and manipulations in the task we are studying, such as optogenetics and lesion studies. Even though the skull might be inaccessible after the implantation, the implantable base for the Ratphones is compatible with other implants such as fibres or LEDs.

However, the headphones we have been using do bring some constraints to the behaviour we are studying. The fact they require cables means the animals are always somewhat tethered and if the cables and headphones are not light and properly fixed they might impact the animals' movements. Besides this, the animals will occasionally protest having a cable protruding from their head and attempt to destroy it, which means constant supervision is required, at least for the first weeks of training. An improvement to the existent design could be to make them wireless, using, for instance, Bluetooth technology. However, we strive for an accurate sound presentation in the temporal dimension, having a small and consistent lag between the time the animals fixate and the start of the sound and this was not easy to achieve through Bluetooth.

It is also worth mentioning that although so far animals have been amenable to headphones being placed on their heads with no special handling, habituation, or training, it might be the case that some animals will become stressed by this process, for instance, animals with autistic phenotypes might react negatively to this manipulation, raising the need for a different means of speaker placement. Ideally, speakers would be placed in the setup, but not on a far wall, merging the freedom of non head-fixed animals with the precision of headphones. As the animals are required to poke in a central port for sound presentation, if one managed to equip this poke with speakers and make sure the animal would poke in a stereotypical manner every time, sounds could be presented precisely but without the need for implants. This is something we are currently focusing on developing and that would allow for expedited training without the need for implant surgeries and recovery time and also for the parallel training of a larger number of animals, without the need for such strict supervision.

In conclusion, the development and application of the Ratphones have provided a crucial methodological foundation for the subsequent chapters of this thesis. The Ratphones represent a significant step forward in auditory research methodology, aligning well with our goal of understanding auditory perception and decision-making in more naturalistic contexts. They facilitated our investigations into the complexities of auditory decision-making, the role of the auditory cortex, and the interplay between proactive and reactive decision processes.

6.2 Short Duration Stimuli as a Window for Decision Readout

6.2.1 Short Duration Paradigm Lends Further Evidence into Weber's Law depending on Accumulation of Evidence

Chapter 4 of this Thesis focused on how subjects make decisions based on short duration sounds. Besides allowing us to study perceptual decision-making in a scenario that better approximates natural decisions – as

animals in the wild often times have no control over the duration of a stimulus need to inform their behaviour –, this was a key manipulation for the understanding of how decisions are made. Our first goal regarding this behavioural paradigm was to understand how Weber's Law (WL) would behave. Model predictions for short durations, consider that if WL is based on bounded accumulation of evidence, then it should break down, as less and less trials have their DV reach the decision bound. Our findings do indicate that the WL breaks down when auditory stimuli are presented for durations shorter than typical Reaction Time (RT), suggesting that the invariance of performance across different ABL is disrupted under conditions of limited stimulus duration. The Time-Intensity Equivalence in Discrimination (TIED) framework is based on the fact that the Decision Variable (DV) reflects the temporal accumulation of evidence, evolving more rapidly for louder sounds. Our results further support this hypothesis, by demonstrating that performance improves with longer stimulus durations, ultimately aligning with RT trials when sufficient time is allowed for evidence accumulation. According to this, WL and the TIED, are dependent on the subject being able to adapt their RT to the ABL being experienced – if WL did not suffer for these conditions, it would indicate the level invariance that characterises this behaviour is not dependent on the accumulation of evidence, perhaps corresponding to some intrinsic property of sensory neurons, such as an ability to normalise sound level –. However, due to the observed breakdown of this psychophysical law, we can conclude that this is most likely not the case – an invariance in behaviour does not imply an invariance in the neural activity –, and that the difference between ABLs corresponds to the speed at which evidence is able to be accumulated, thus the invariance hinges on reaching the bound, which will take longer for lower ABLs.

6.2.2 Short Duration Stimuli as a Window for Decision Readout

Our work further highlights the difference between RT decisions and the ones with shorter durations, as we were able to focus on how these trials still result in a response even though the evidence does not seem to be enough for the decision bound to be reached. Initially, we were surprised by the relatively high accuracy displayed by our animals for the shorter stimuli durations. It seemed hard to understand how they could accomplish it with so little evidence. How could a 15 ms sound actually result in above chance level accuracy when sensory delays are of this order? We speculated rats might be employing several different processes, such as remembering the stimulus and revisiting it during the longer RTs displayed for these trials or somehow adapting their accumulation of evidence to this new task paradigm once they realised the sound would be terminated without them being in control. In the end, we were able to infer that rats might still be able to have the DV reach the decisions bound even after the stimulus has stopped and those trials are governed by the same rules as normal RT trials, ensuring a relatively high performance, dependent on the percentage of trials that are in this situation. However, this is not true for all trials. Some will truly not reach the bound, and those are the ones that cause the decrease in performance. These trials are still somewhat of a mystery, but they appear to follow a random decision rule.

Several interesting points are raised by this model.

Decay of activity following sound offset

The fact that in some trials the DV still manages to reach the decision bound even after sound has ceased relies on the principle that even after sound stops, neural activity does not.

Neurons in the auditory cortex exhibit distinct temporal activity patterns in response to auditory stimuli, which are influenced by the duration and temporal structure of these stimuli. The temporal characteristics of auditory stimuli significantly influence the activity patterns

within the ACx. Low-rate noise burst trains elicit sustained responses over extended periods, whereas high-rate trains induce phasic responses characterised by pronounced peaks immediately after stimulus onset and offset. These findings suggest that the auditory cortex differentiates between continuous and rapidly changing stimuli, adapting its neural activity accordingly (Harms et al., 2005; Scholl et al., 2010; Takahashi et al., 2004). Silencing the auditory cortex can alter these temporal response patterns, potentially disrupting the processing of temporal information, affecting auditory perception and processing.

We attempted to model the accuracy our animals exhibited under the assumption that all sensory activity used as evidence would stop instantly when sound stopped, and realised this is not the case. This is an interesting point, hinting at the fact that even after sound is terminated there is still evidence integrated. This might originate from an interplay between sensory delays and neural activity – persistent neural activity is still integrated and used for the decision due to the delays between sound onset and evidence accumulation onset, as well as sound offset and stopping of the DV drift –.

The occurrence of persistent neuronal activity even after stimulus offset makes sense considering the response profile of auditory cortex cells. While a larger number of cells show a response to sound onset, some cells exhibit responses to the offset of sound (Hartley et al., 2011; Takahashi et al., 2004). These are characterised by poor tonotopic organization and have little spatial overlap with onset responses, occurring at the edges and forming inhibitory sidebands (Takahashi et al., 2004). The amplitude of these responses is influenced by sound intensity, the duration, and fall time of the stimulus. Shorter fall times originate more synchronous and larger OFF responses, something we may expect in our task (Hartley et al., 2011; Takahashi et al., 2004). And, although longer stimuli tend to originate larger OFF responses, there seems to be some preferential durations, with some research showing connections to the durations of conspecific calls, for instance (Wang et al., 2016). These responses might then be responsible for the longer duration of the neural activity in relation to the stimulus, or for the decay profile observed.

The duration sensitivity of OFF responses highlights the fact that some cells in the auditory pathway, including the Auditory Cortex of rats, show duration selectivity. Some neurons show a marked preference for short duration sounds and it is speculated that this might be due to a precise balance of excitation and inhibition in the auditory cortex (Wang et al., 2016). And we speculate that perhaps these duration sensitive neurons also influence cortical responses for the shorter Maximum Sampling Duration (SDmax).

Although it is unclear at which point in the sensory pathway do the relevant computations take place – what are these neurons that show something that can be approximated as exponential decay? –, our optogenetic manipulation in conjunction with the short duration manipulation might help this understanding. By optogenetically silencing auditory cortex, we worsened the effect of limiting sound duration. While we have yet to model this manipulation, we can speculate that this might be due to the perturbation of the excitation-inhibition balance in the cortex and that that might affect both offset responses and duration selectivity of cells, potentially affecting this persistent activity after stimulus offset and the ability to have the DV still reach the bound.

A limited window of integration appears to be essential

Our data allow us to infer a particular time-course of evidence decay after stimulus offset, which can only be integrated if the process of integration does not stop at sound offset either. Drift Diffusion Model (DDM) predict the existence of non-decision time, which reflects stimulus encoding and motor preparation and execution, and serves as proxy for cognitive processing factors during decision-making (Palmer et al., 2005; Pardo-Vazquez et al., 2019). A stimulus may only influence a decision after this time has elapsed (Hernández-Navarro et al., 2021, and our own work in Chapter 4). According to our model, the integration of any of the previously mentioned signals that occur after stimulus offset seems to hinge on the fact that these sensory delays take place. But we also concluded that, besides these expected delays, the duration of the window of integration

seems to be important as well. This period of stimulus integration where, according to our model, the DV is now integrating the neural decay post stimulus offset, is required to have a specific duration, not accounting for the entire decay. This might be the case if, due to time constrains and in an attempt to optimise time spent in each trial, animals opt to not spend more than a certain period integrating residual information. Animals might also only engage in this extended integration of residual evidence until they have to commit to a side for their response – in this case, the maximum duration of this window is constrained by the logistics of our task and until when the animal might not need to commit to one side for their final response. Conversely, it might be that our exponential decay approximation is not entirely accurate and there is instead more of an offset peak with a faster decay, meaning the full information might instead be integrated. It is remarkable, nonetheless, that we were able to account for the tachometrics across all duration with the same common, simple decay.

Due to this extended integration post stimulus offset, the DV reaches the decision bound more often than what we first expected, accounting for the high accuracy even for shorter stimulus durations. However, we realised that in trials where the bound was still not reached, the decision is better matched assuming the readout is random. This is still not a perfect match to the data, however, it seems to capture the behaviour fairly well. If so, it suggests that crossing the threshold limits not only the timing of the decision, but also whether evidence can guide decisions at all. We have all made decisions under uncertainty where we would have described our answer as "random"; and perhaps that is more accurate than we would have thought. This goes against Signal Detection Theory (SDT)-like rules – the animal does not seem to have direct access to the distribution of accumulated evidence (Fig. 4.6 A1). Being able to use the evidence in order to make a decision seems to be predicated upon reaching the bound, which is not predicted in SDT (Green, Swets, et al., 1966) or in other models that do not take into account these limitations in the readouts (Brunton et al., 2013).

6.3 Animals Decide on What and When

Starting the study of the proactive decisions in our task made us aware of the importance of understanding how decisions are still made under evidence insufficient for the DV to reach the bound. How are these decisions made? To understand this, we focused on exploring the decision rules for these, and at what moment that rule was applied – the readout moment and what it comprehends. At the moment sound ceases, several things will take place in the context of our task, but importantly, sensory representations of the ILD in the brain will no longer have an external stimulus to encode.

It also highlights the importance of the temporal dynamics of decision-making, as decisions made with incomplete evidence – short sound duration in spite of RT – are not the same as decisions made on short RT. Our work evidenced the fact that proactive and reactive decision-making processes in auditory decision-making have different dynamics and give rise to different decisions. While prior research often treats these processes as distinct strategies employed in separate contexts (Carpenter, 2001; Carpenter & Williams, 1995), our findings suggest that they should instead be applied and interact within a single framework. The ability to initiate responses based on internal goals or predictions about upcoming stimuli — proactive responses — can lead to quicker decisions but may also increase the likelihood of errors when sensory evidence is compromised. This duality complicates the understanding of how decisions are formed and emphasizes the necessity of considering both proactive and reactive strategies when studying auditory perception.

6.4 Challenges of Manipulating Sensory Cortices

Both lesions and optogenetic manipulations, among other strategies, are useful yet imperfect ways to study the role of different brain regions. Manipulating sensory cortices — such as the visual, auditory, or somatosensory cortex — using optogenetics or lesioning presents several challenges that impact neuroscience research in general and our work in

particular, influencing how we interpret neural function and plasticity.

Lesion studies allow us to understand sensory processing by removing or damaging specific brain areas. However, this approach has several inherent limitations (Slonina et al., 2022) such as: the irreversible damage it entails to the target areas – once a lesion is made, it cannot be undone (Lavond & Steinmetz, 2012), making it difficult to assess transient or reversible functional changes. The variability in lesion size and location – small differences in lesion location can lead to different functional outcomes (T.-Y. Lee et al., 2024; Lomber, 1999; Pai et al., 2011), reducing reproducibility –. Collateral damages – lesions often spill over to nearby regions due to inaccuracies in the method of administration (Koo et al., 2004) or due to broader network effects – plasticity as a response to damage, which can mask or alter expected deficits (Depner et al., 2014; Lomber, 1999), or atrophy of upstream regions that lose their input source (Lomber, 1999), making it difficult to distinguish between primary and secondary effects and to attribute functional loss to the targeted area alone (Slonina et al., 2022). Despite these caveats, lesions remain an important means to study the brain and a way to parallel what is a common tool for human neuroscience with naturally occurring lesions (for instance the work done by Broca, 1863; Inouye, 2000, thus, some methods have been devised to minimise some of these issues, such as targeting lesions with the help of electrophysiological recordings (Nodal, Kacelnik, et al., 2010) or MRI scans (Gil et al., 2024; Severo et al., 2024), as employed for this work as well.

Optogenetics have revolutionised several areas of biology, including neuroscience, by allowing precise temporal control of neuronal activity using light-sensitive proteins (Deisseroth, 2011; Fenno et al., 2011). Recent advances allow complex patterns of stimulation to be enacted on the brain and evolving fibre optics and other optical technologies allow for an enormous degree of freedom and accuracy in this manipulation (Portugues et al., 2013), as well as incredible temporal resolution (Chen et al., 2019; Montagni et al., 2019; Shemesh et al., 2017). Despite its advantages, several key challenges hinder its efficacy in sensory cortex research. Some are technical challenges: Limited light penetration due to

interactions between tissue opacity and utilized wavelengths. Difficulties achieving precise targeting and avoiding off-target effects – these may happen due to inaccurate opsin injection or expression or excessive light penetration – which may undermine the interpretation of any perceived effect. Both of these effects might be addressed by the use of implanted optical fibres, but this then implies tissue penetration, which might disrupt normal neural activity (Montagni et al., 2019; F. Pisanello et al., 2017; M. Pisanello et al., 2018). Furthermore, besides these technical challenges, optogenetics also brings about some biological caveats. The ability to manipulate circuits with high temporal precision, might in itself disrupt natural oscillatory activity and can create artificial neural dynamics that mask true physiological function (Boyden et al., 2005; Malyshev et al., 2015). In fact, optogenetics may induce unnatural activity patterns, for instance, the ability to inhibit a brain region through the direct inactivation of excitatory cells (Mahn et al., 2018). While potentially useful, this might not be a naturalistic manner in which to silence a brain region, making it difficult to separate experimental manipulation from natural cortical reorganization. High-intensity light exposure may also cause cellular damage, and potential toxicity due to prolonged activation of opsins. Long exposure to a light source may also originate overheating, a particularly problematic instance considering temperature may not only kill neurons, but also change their activity patterns (Gotoh et al., 2020; Monteiro et al., 2023; Wiegert et al., 2017).

6.4.1 Limitations of Our Lesion and Optogenetic Data

Although results from our lesion experiment were robust and consistent across animals, some of the lesions were rather confined. If we were dealing with a subtle effect, this might be sufficient to have masked it.

Regarding optogenetic manipulations, we are presenting here a small subset of what was performed. Indeed, reaching this manipulation paradigm required the execution of different manipulations where the LEDs were turned on either slightly prior to, or during, RT. These paradigms suffered greatly with, what we now know to be the proactive

increase, and the animals were not waiting for the sound to be played and seemed to be reacting exclusively to the LED. We instituted the current paradigm of sound playing during Fixation Time to differentiate Reaction to the LED from reaction to the sound, and managed to achieve a behaviour in which they did not immediately abandon the central port at light onset. Interestingly, at first we wondered if the animals might be reacting immediately due to an aversive sensation or percept created by the light, but we theorise that is not the case as they continued to initiate trials just the same and did not present any signs of added stress. Adding to this, at first, considering we expected a modest effect of silencing ACx, we targeted the entirety of ACx, which caused a broader but also more variable expression pattern, with some animals having the expression of the opsin extend to areas beyond ACx. This presented us with a confound – were we causing these shorter RTs due to silencing other areas? The cohort presented in the Thesis had their virus expression targeted to A1, minimising the possibility of this confound taking place.

However, the improved targeting of our injections does not discount possible effects of virus diffusion independently of injection, which we have seen for some animals. This might also cause unbalanced expression between hemispheres, which might produce lateralised effects or potentially mask them, if hemisphere-specific effects exist (Oviedo et al., 2010). We have also noticed, in some animals, that there was preferential expression of the opsin in layers other than layer IV. It is unclear why this occurs, but we believe the opsin is still expressing in layer IV, just at a lower level/intensity than in the other layers. However, this might also contribute to the heterogeneity in the behaviour that we observe (Andrei et al., 2021; Slonina et al., 2022; Talwar et al., 2001). There are also indications that the promoter used in the virus construct we employed (CamKII) might express in interneurons and not exclusively on pyramidal cells, further contributing to unexpected expression and activity. An alternative to the current inactivation method would be to employ an excitatory opsin targeting inhibitory interneurons. This would reduce the firing of other neurons in what is potentially a more natural way. However, this is not without its own set of caveats, and it seems like even

in these more naturalistic conditions the brain tries to compensate for the unbalance created by external manipulations. These compensatory mechanisms might, within milliseconds, correct excitation-inhibition imbalances caused by our manipulations (Moore et al., 2018). Even the notion of targeting different neuronal types is not as straightforward as one might assume, as there are also different subtypes that might organise in functional units, further complicating any sort of generalisation of targeting and silencing techniques (Fishell & Kepecs, 2020; Slonina et al., 2022).

Besides the variability in the expression pattern of the opsin, there is also some, seemingly independent, variability of LED effect across animals. All rats showed an effect, however, it was more pronounced for some animals than for others. This might, once again, be due to viral expression, but we do not, at the time of this writing, have histology data for the cohort analysed in Chapter 5. Also highly variable across animals is the data for unilateral silencing (not shown). Mostly used, so far, in order to assess if both LEDs were in fact affecting each animal, for some animals one LED created a large bias, while for others one alone had a much more subtle effect. Once again, histology might guide our conclusions for this aspect of behaviour.

In spite of these limitations, it is important to point out that all the analysis in this work was performed with no specific data curation, as for bilateral LED ON the data was remarkably consistent, and thus we focused our modelling efforts on averaged data, although we plan on fitting the model to each rat in the future.

6.4.2 Manipulations may be Perceived as Stimuli

It has been demonstrated that animals that are subject to optogenetic manipulations are able to sense these – optoception (Luis-Islas et al., 2021). In fact, animals have been shown to perceive optogenetic manipulations and to show behavioural effects caused solely by this stimulation (Carrillo-Reid et al., 2019; Chong et al., 2020; Luis-Islas et al., 2021; Marshel et al., 2019; Ortiz-Rios et al., 2023; Sun et al., 2021; Weible et al., 2020).

For instance, mice were able to differentiate between several "synthetic" odours created by optogenetically activating neurons in the olfactory bulb (Chong et al., 2020), and have discriminated optogenetic stimuli as true visual percepts (Marshel et al., 2019). While this opens several avenues of research, namely for the study of causal roles of specific neurons, groups of neurons or networks for certain behaviours and perceptions, or therapies to recover sensory perception after an injury, it also creates a large confound for the use of optogenetic manipulations, especially when targeting a sensory area such as a sensory cortex. When optogenetically manipulating an area, let us say, for instance, the motor layers of the superior colliculus -specifically the cells that project to the brainstem and are responsible for orienting movements (Cregg et al., 2020; Usseglio et al., 2020)– we would have a strong hypothesis on what to expect behaviourally when activating these cells – the animal would orient towards the contralateral side to the stimulation, or perhaps rotate for prolonged stimuli. Thinking now on optogenetic inactivation, if these circuits are necessary for turning and orienting, once they are inactivated, the animal would not be able to turn at all, even if cued. Thus, motor circuits have some ease in this respect, as one can easily test whether the manipulation is taking place, because these circuits cause external changes. When it comes to sensory circuits, however, any changes are "internal" and may relate to perception. We are unable to ask animals what exactly they are seeing or hearing, and so our window into their percepts is through behaviour. But it is not necessarily easy to interpret changes in perception due to these manipulations. Perhaps by silencing the auditory cortex we could be adding something akin to extra noise onto the stimulus, or a new, very loud sound, or just complete absence of sound, which can also be quite jarring considering how unnatural it would be. All of these alternatives could be potentially captured as "noise" in the evidence accumulation process. This means that it is difficult to interpret behavioural outcomes of optogenetically manipulating a sensory area, such as the auditory cortex, in terms of how it actually affects the animals' perception, as many changes can result in the same external outcome.

6.4.3 Cortical Impairments due to Manipulations

Besides creating percepts of stimuli that are not really present, a different issue may arise from optogenetically manipulating, or lesioning, sensory areas such as the auditory cortex. In humans, lesions in a sensory cortical area lead to peculiar perception issues, such as cortical blindness, when visual cortex or immediate afferents are affected (Saionz et al., 2022; Zihl, 1980); or cortical deafness ("deaf hearing"), when auditory cortex is affected (Dumanch & Poling, 2019; Engelien et al., 2000; Garde & Cowey, 2000; Graham et al., 1980). In these conditions, patients are technically still able to see or hear, if the remaining sensory pathway is still healthy. However, they are seemingly not able to create percepts from those stimuli, and will, in general, act as if unable to see or hear. However, when faced with, for instance, an object moving towards them, or instructed to perform auditory forced-choice task, they are still capable.

It may be that attempting to silence the Auditory Cortex is creating defective percepts, or removing auditory information, at least in part, from the data the animal can use in order to decide. It might be preventing the formation of (normal) auditory percepts even though the animal is able to still hear. This could be due, for instance, to secondary auditory areas still responding and transmitting forward a faulty or incomplete version of a normal percept. Or to subcortical areas transmitting this information to those higher cortical areas without the ACx. In this case perhaps the information is more "raw" and not in the normal form of a percept. It is somewhat unclear the effect this would have on behaviour, but a possibility is that the animal would, in general, act as if no sound had been played, an effect that has been described before for chemical inactivations of the cortex, where animals in a Go-NoGo task greatly increased their rejections, as if no longer listening to the sound in sessions where they had been injected with muscimol (Steinfeld et al., 2024). Similarly, in a frequency discrimination task, the animals acted as if unable to detect the sounds, but if forced to respond, were still able to discriminate (Tai & Zador, 2008).

A way to possibly disentangle all the potential outcomes of silencing an area is to merge the knowledge of the possible biological effects of these manipulations with computational modelling. This way, one may attempt to replicate behavioural effects or to predict the effect of future manipulations, even potentially targeting specific areas based on intended outcome. The modelling efforts undertaken for this work revealed additional layers of complexity within decision-making dynamics. Our attempts to reproduce behavioural data through adjustments to drift rates, decision bounds, and sensory noise highlighted the multifaceted nature of these processes. While some hypotheses provided partial insights into observed behaviours, the fact that all of them in tandem is what best describes the actual behavioural effect shows the intricacy of the signals taking place in the cortex or influenced by them. Moving forward, future research should focus on refining our understanding of how these components interact within neural circuits during auditory decision-making tasks. Incorporating advanced methodologies such as multi-neuron recordings and real-time imaging techniques could provide deeper insights into the neural dynamics underlying these processes.

6.5 Role of Auditory Cortex in Sound Lateralisation

It is important to mention that sound lateralisation has been classified as a "simpler" task for rats. ILDs are mainly computed subcortically and the comparison between the sound level that reaches both ears is considered to be a simple one. This could be the reason why sound lateralisation has shown less cortical dependency for rats and mice (Kelly, 1980; Kelly & Masterton, 1977) when compared to other species that might make use of more complex temporal and spectral cues for sound lateralisation and localisation (Heffner & Heffner, 1990; Jenkins & Merzenich, 1984; Kavanagh & Kelly, 1987; Nodal, Kacelnik, et al., 2010). Deep deficits have been shown when abstraction from the sound is required for the response, as when the task requires estimation of a source's location(Kavanagh & Kelly, 1987), however, our response is lateralised and most likely does

not require any complex association. As mentioned in previous sections, we believe ACx is not involved in the computations of ILD, the basic sensory processing necessary for the task. We hypothesise ACx might instead bridge the sensory computations that calculate the difference in intensity between the two ears and the computations that are responsible for solving our task – evidence accumulation to bound. Perhaps if the sound source remained on even as the animal responds, as in our initial stages of training, silencing ACx would not have a clear effect. But we believe that this region is important for the creation of percepts used in the process of decision-making, even if not for the process of ILD calculation.

6.6 Future Directions

For the continued study of this perceptual decision-making task, we aim to perfect the modelling work regarding the Auditory cortex. For this Thesis, we manipulated the individual parameters "by hand" in order to qualitatively match our behavioural data, instead of fitting the model, for the sake of time. However, considering we strive for quantitative explanations of behaviour, we will perform this fitting exercise as well, and hopefully better understand the effects of the manipulation.

We believe one can only accurately explain behaviour and its neural underpinnings by making use of tasks and paradigms that allow us to quantify as many characteristics and components of behaviour and decisions. Although experiments might never be enough to fully explain what effects come from directly manipulating the brain, the more data we gather – not only session numbers or trials, but the more we are able to record of these individual decisions and their timings and processes –, the more accurate we will manage to be. Therefore, we plan on adding electrophysiological recordings to our dataset. One might, for instance, expect certain characteristic firing patterns from the areas responsible for the different mechanisms in a DDM, that can only be accessible in this way. At the same time, it would allow us a deeper understanding of the mechanisms of cortical silencing and other manipulations. Neuropixel

probes, with their capability to record from hundreds of cells and in varied patterns, might contribute to a deep understanding of the entire process.

We also plan on branching out into different brain areas in much the same way. Targeting different brain regions that might be implicated in this process, be it more related to the motor end – such as the superior colliculus, with its role in orienting and turning –, or the sensory end – such as the inferior colliculus, widely seen as the first area with ILD encoding at a population level –, applying the same principles as described in this Thesis – behavioural manipulations in conjunction with optogenetics or lesion work – might enable us to accurately describe all the processing steps that are undergone for the performance of our task.

6.7 Final Remarks

This thesis has explored the role of the auditory cortex (ACx) in auditory decision-making, combining behavioural experiments, computational modelling, and neural manipulations. Our findings demonstrate that while ACx is not necessary for sound lateralisation, it plays a crucial role in optimising the decision-making process. Lesion studies revealed that rats could still perform the task with intact accuracy, whereas optogenetic silencing led to significant deficits, suggesting a modulatory rather than essential function for ACx. Furthermore, our work highlights the interplay between proactive and reactive decision strategies, shedding light on how sensory evidence and internal states influence auditory-guided behaviour.

Beyond these findings, this work contributes to auditory neuroscience in a methodological manner as well. The development and validation of Ratphones provide a precise tool for controlled sound delivery in freely moving rodents, addressing limitations of previous methodologies. Additionally, the use of computational modelling allowed for a deeper understanding of the neural mechanisms underlying ILD-based decision-making.

In conclusion, this thesis contributes to a more nuanced understanding of auditory decision-making by highlighting how temporal dynamics, proactive and reactive strategies, and cortical involvement shape perceptual outcomes. By integrating behavioural experiments with computational modelling, we have taken crucial steps in the understanding of the complexities in decision-making processes.

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A

Scientific Publication

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Open Source Tools and Methods

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Ratphones: An Affordable Tool for Highly Controlled Sound Presentation in Freely Moving Rats

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Abstract

Encoding and processing sensory information is key to understanding the environment and to guiding behavior accordingly. Characterizing the behavioral and neural correlates of these processes requires the experimenter to have a high degree of control over stimuli presentation. For auditory stimulation in animals with relatively large heads, this can be accomplished by using headphones. However, it has proven more challenging in smaller species, such as rats and mice, and has been only partially solved using closed-field speakers in anesthetized or head-restrained preparations. To overcome the limitations of such preparations and to deliver sound with high precision to freely moving animals, we have developed a set of miniature headphones for rats. The headphones consist of a small, skull-implantable base attached with magnets to a fully adjustable structure that holds the speakers and keeps them in the same position with respect to the ears.

Key words: behaving animals; headphones; interaural level differences; rodents; sound presentation

Significance Statement

Presenting sensory stimulation reliably is critical in many experimental paradigms. Different methods have been used to accomplish this goal in different sensory modalities, but it has proven difficult to control sound presentation in small species, such as rats and mice. In this work, we present the Ratphones, a set of miniature headphones, and provide all necessary information for building, adjusting, and using the Ratphones to reliably present auditory stimulation to freely moving rats.

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Introduction

Processing sensory inputs is crucial for understanding the environment and adjusting behavior accordingly. In addition to psychophysics, in which sensory evidence is critical, sensory stimuli are used in many paradigms within behavioral neuroscience. For these experiments to be valid and reliable, it is key to ensure a high degree of control over the physical properties of the stimulation that reaches the sensory organs, so that that they can be accurately replicated. Moreover, precise stimulus control is critical for the experimenter to be able to interpret neural variability and its relationship with behavior; only if one can accurately repeat the same stimulus, can a distinction between internal and external neural variability be made.

In visual experiments, this requirement has been fulfilled mostly by using eye-tracking systems in head-fixed (or head-restrained) subjects (Britten et al., 1993), but also by

using head-mounted eye-trackers (Cognolato et al., 2018). Recently, a magnetic eye-tracking system that can be used in both head-fixed and freely moving mice has been developed (Payne and Raymond, 2017). For olfactory and tactile stimulation, researchers have developed high-precision devices for this purpose (Romo et al., 2002; Kepecs et al., 2006). In the auditory modality, there have been two main strategies. On the one hand, in species with relatively large heads, such as monkeys (Schroeder et al., 2001; Fishman and Steinschneider, 2009) or ferrets (Nodal et al., 2010; Keating et al., 2013), sound presentation can be controlled by using headphones. On the other hand, for smaller animals, such as rats and mice, the sound can be reliably delivered by using head-fixed (Joachimsthaler et al., 2014) or anesthetized (Yao et al., 2013) preparations. However, to our knowledge, delivering sound under strictly controlled conditions to freely moving rodents is a challenge that has been only partially solved so far in rats by chronically implanting a plastic structure into which the speakers were screwed before each behavioral session (Otazu et al., 2009).

We have designed the Ratphones, an affordable set of miniature headphones that consists of a small, skull-implantable base attached with magnets to a fully adjustable structure that holds the speakers and keeps them in the same position with respect to the ears. With the Ratphones, the experimenter only needs to implant a small base, which is less disruptive for the animals than implanting the whole structure except for the speakers, and the headphones are attached to this base with magnets, thus avoiding the need to screw (and unscrew) the speakers before (and after) every behavioral session.

These headphones allow the experimenter to control independently the sound delivered to the two ears, which is especially important for studying sound localization, where the interaural level difference (ILD) of the sound is the main cue used by the auditory system to extract azimuth in the horizontal plane (Wesolek et al., 2010). The speakers we chose are good for high frequencies (up to 40 kHz) and can deliver pure tones and narrowband noise. Thus, the Ratphones can be used in most experiments requiring highly controlled sound presentation. However, the speakers are limited in terms of sound intensity [maximum, 79 dB SPL (measured in 0.1 m distance)] and may not be the best option for experiments demanding very high sound intensities.

Design Requirements

The main functional requirements behind this design were to have a precise, reliable, and robust relative positioning between the speaker and the pinna, while at the same time allowing flexibility to adjust this positioning to the variations between base positioning and pinna location on each individual animal. To achieve this, (1) the design contains movable pieces that can be adjusted on the anteroposterior, mediolateral, and dorsoventral axes; and (2) the procedure to configure the Ratphones consists of a first step in which the base is implanted, and a second step in which the pieces are adjusted for each individual animal under anesthesia and glued in their final configuration for each rat.

Another critical design requirement for the Ratphones was to use them in behaving animals, as opposed to anesthetized or head-fixed preparations. Mostly because of this requirement, we decided to use external headphones instead of placing them in the ear. In-ear headphones in principle afford a higher degree of control, as sounds not coming from the speakers are blocked. They also allow pure monaural stimulation (i.e., one is sure that each ear only hears the sound from its corresponding speaker), which, as previously mentioned, is important for controlling ILD. They have, however, the important drawback that they are much more invasive and uncomfortable for the subject, which, especially in a behavioral context where one has to fit them on every behavioral session, is critical. If the animal starts every session stressed and uncomfortable, it will interfere with the behavioral readouts of the sensory measurements the experimenter is trying to perform. A second important drawback for internal headphones, based on human subjective experience, is the sensitivity of this configuration to slight adjustments in the positioning of the earphone (and supporting sound-isolating material) relative to the inner pinna. A bad seal can completely compromise accurate sound delivery, and the rats cannot report on a bad seal. For all these reasons, we decided to use an external design with close placement. Regarding the downsides, we tested explicitly that monaural contamination is small compared with behavioral ILD sensitivity, but we would in general recommend performing experiments in a sound-isolation box, where the possibility of interference because of a lack of the seal provided by an internal design is minimized.

In this work, we provide all necessary information for building, adjusting, and using the Ratphones to reliably present auditory stimulation to freely moving rats. Empirical data obtained with the Ratphones can be found in the study by Pardo-Vazquez et al. (2019).

Materials and Methods

The Ratphones consist of a small, chronically implantable base and a set of movable parts (Fig. 1*A*,*B*) that can be put together to form a structure that holds the headphones in the desired position with respect to the ears (Fig. 1*C*,*D*). The structure is attached to the base using magnets. All parts, except for the magnets and speakers (Table 1), can be 3D printed using the stereolithography files (stls) we provide in https://github.com/JosePardoVazquez/RatHeadphones. These parts have been designed to be printed using stereolithography (Fig. 1*B*,*D*), but can be easily adapted to other 3D-printing methods. In its current form, the speaker box is designed to be used with a specific receiver model (Table 1), but it can be easily redesigned to fit other models without modifying the other parts. Below, we describe the procedure we used for implanting the base and adjusting the headphones. All procedures were reviewed and approved by the animal welfare committee of the Champalimaud Centre for the

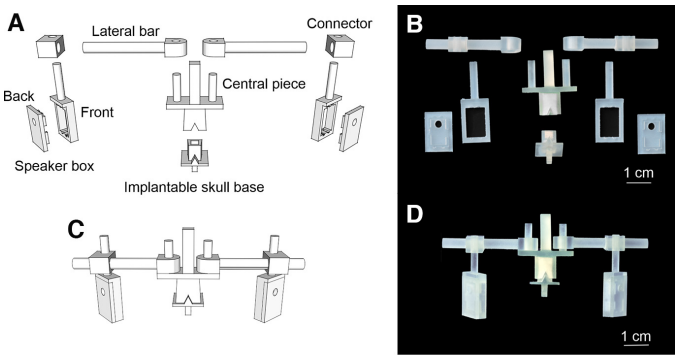

Figure 1. Ratphones 3D design and 3D-printed resin pieces. ***A***, Set of 3D-printable parts. ***B***, Set of resin-printed parts. ***C***, ***D***, Front view of all the parts assembled to form the final structure that holds the speakers, in the 3D model and printed, respectively.

Unknown and approved by the Portuguese Direcção Geral de Veterinária (reference #0421/000/000/2019).

Base implant surgery

Anesthesia was induced by inhalation of isoflurane at a concentration of 5% (oxygen at 2 L per min) and maintained by an injection of ketamine/xylazine (0.1 ml/100 g, i.p.). More isoflurane was occasionally administered for longer surgeries if the animal exhibited signals of pain or discomfort. The animal was shaved and fixed to the stereotaxic frame, and eye ointment was applied to the eyes. Lidocaine (0.2 ml) was injected subcutaneously at the incision site before the incision was made, for local anesthesia. The skin was cleaned using iodine, an incision (~2 cm in length) was practiced along the midline, and the skin was displaced laterally, exposing the surface of the skull. After cleaning the top region of the skull by blunt dissection, four drilling holes were made and titanium screws (length, 3 mm; thread diameter, 1 mm) were attached to the skull, allowing for most of their length to remain outside. Cement was poured on top of these screws, ensuring it reached the space between the screws and the skull for a secure attachment [using a strong dental adhesive, such as Super-Bond (Sun Medical), it might be possible to firmly implant the base without screws; this cement has shown high tolerance, resistance, and durability for chronic implants in different species, including mice (Lohse et al., 2021) and ferrets (Nodal et al., 2010)]. A small cube-shaped magnet (Table 1) was placed inside the resin base (Fig. 2*A*), which was then placed on top of the cement layer, and more cement was added around the lower part of the base until it was covered. The displaced skin was then stitched around the base, only allowing the necessary structure for the attachment of the headphones to remain visible (Fig. 2*B*,*C*). Antibiotics (8 mg/kg, s.c.; cefovecin, Convenia) and analgesics (5 mg/kg, s.c.; carprofen, Rimadyl) were administered after the surgery. The base (with the magnet) weighs ~0.9 g, but together with the cement it weighs ~2.4 g.

Individual adjustment of the Ratphones

This procedure was performed 1 week after the base implant surgery, during which the animal was allowed to recover with free access to water and food. Under anesthesia (induced and maintained with isoflurane at 4% and 2.5%, respectively), the structure with all pieces temporarily assembled, including the speakers and

Table 1: List of parts needed to build a set of Ratphones

Parts	Description
3D-printed pieces (stls files provided)	Implantable skull base
	Central piece
	Lateral bar (×2)
	Speaker box, front (×2)
	Speaker box, back (×2)
	Lateral bar to speaker box connector (×2)
Magnets	B222 (K&J Magnets, Inc.) one-eighth × one-eighth × one-eighth inch thickness; grade N42, nickel-plated magnetized through thickness
	B3301 (K&J Magnets, Inc.) three-sixteenth × three-sixteenth × one-thirty-second inch thickness; grade N42, nickel-plated magnetized thru thickness
Speakers	Knowles active receiver (MOD 2403260 00031; "Petra")
Connector	Connector header through hole 3 position 0.100 inch (2.54 mm)

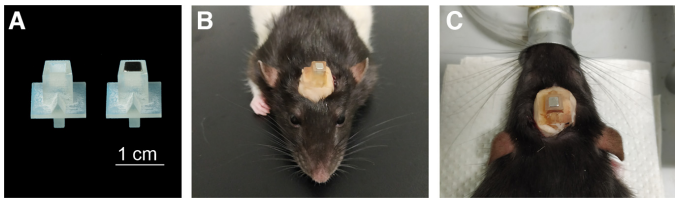

Figure 2. ***A***, Skull base 3D printed in resin, without and with the magnet in place. ***B***, ***C***, Front and rear view of a skull base chronically implanted.

their connections, was placed on the implanted base and the different angles between the pieces were adjusted so that the speakers were placed at ~5 mm from the opening of the ear of the animal. The pieces were then fixed with cyanoacrylate, removed from the animal, and covered with flexible silicone rubber (Sugru), providing extra fixation to the resin pieces and protection to the electrical cables that connect the speakers. Initially, we covered most of the structure of the headphones with flexible silicon rubber (Pardo-Vazquez et al., 2019, their Supplementary Fig. 1), but lately we have been covering only the central piece (Fig. 3), reducing the weight of the Ratphones while keeping a strong attachment in the part that supports more tension when attaching/detaching them. A magnet (Table 1) was glued in the bottom of the structure to attach it to the implanted base during the behavioral sessions. A three-pin male connector (Table 1) was affixed to the part of the 3D-printed structure. Before each behavioral session, a standard three-wire sound cable—soldered to a matching three-pin female connector—was plugged to the male connector. A passive commutator, attached to the ceiling of the behavioral box, was used to avoid tangling. The headphones weigh 7.22 g without flexible silicone rubber and 12.8 g with a full coverage of this material.

We think that it should be possible to scale the Ratphones down to be used in freely moving mice. The 3D parts can be easily scaled; there are miniature neodymium magnets that are strong enough to keep the headphones attached to the base (e.g., a $1.6 \times 1.6 \times 1.6$ mm neodymium cube weighs 0.06 g and can hold 90 g); and there are speakers that are light (0.6 g) and small (diameter, 10 mm) enough to be used in mice.

Behavioral and sound attenuation tests

The Ratphones were used in freely moving rats performing a sound lateralization task (Pardo-Vazquez et al., 2019). The arena consisted in a standard Coulbourn Instruments modular box ($30 \times 25 \times 30$ cm) equipped with three nose-pokes, one of them with a water delivery system. Before each behavioral session, the rat was placed in the box and a set of individualized Ratphones was brought near to the implanted base, until the magnets were attached, without restraining the movement of the animal. The Ratphones were plugged, through a standard sound cable, to a real time processor (RP2 by Tucker-Davis Technologies) that controlled the behavioral task, including presenting the sound and recording the responses of the animal. To avoid tangling, the cable was attached to a passive commutator.

Since we decided to minimize any physical contact between the speakers and the pinnae, it is expected that some residual sound from one speaker will reach the contralateral ear. We addressed this empirically, by playing cosine-ramped (10 ms) broadband noise (5–20 kHz) at 65, 70, and 75 dB SPL from one speaker and recording the sound with the microphone placed by the contralateral ear canal. The noise was independently generated for each presentation using a RP2 module at a sample rate of 50 kHz. The speakers were calibrated using a Brüel & Kjær Free-field one-quarter inch microphone placed in front of the speaker, 5 mm apart. We found that the head plus near-field positioning of the speaker attenuates the sound by ~22 dB (Pardo-Vazquez et al., 2019, data published in their supplementary information). Since the just noticeable difference for lateralization of sound in this task is 2.2 dB (Pardo-Vazquez et al., 2019), this suggests that level differences played through the Ratphones are an accurate approximation of actually experienced level differences (relative to the behavioral accuracy for sound lateralization

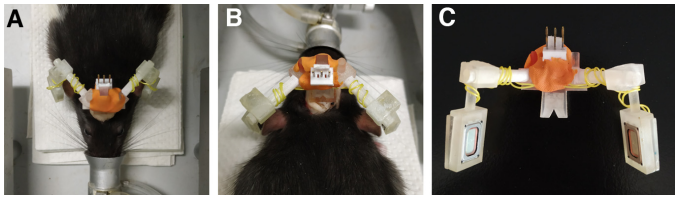

Figure 3. ***A***, ***B***, Front and rear view of a set of Ratphones adjusted under anesthesia. ***C***, Set of Ratphones assembled and glued together, including the speakers, cables, and connector.

in rats), which validates the use of the Ratphones for psychophysical testing of sound lateralization.

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