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Perceptual Decision-Making: Behavioural and Chemogenetics Approaches in Murine Auditory-Based Task

By

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This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

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Abstract

Perceptual decision-making, in recent decades, has become one of the central topics in neuroscience. Combining behaviour with techniques for neural activity monitoring and modulation allows to study structures and mechanisms involved in the process. Elucidating its elements and functional connectivity will not only deepen our knowledge of this matter but also provide with information essential to answer more complex questions related to mechanisms guiding our perception of the world. The view on this topic has also been remodelled in recent years switching from seeing the brain as an observer of events to an active analyst.

In the past various studies were carried out on the topic of perceptual decision-making, basing mainly on visual paradigms in humans and non-human primates. Thanks to developments in technology, genetics and behavioural paradigms, studies in rodents spanning through different sensory modalities are more readily implemented allowing for more in-depth analysis.

In this thesis I developed and used auditory-based detection task to study perceptual decision-making on the example of temporal expectations in mice. Further, I combined this paradigm with chemogenetics to examine the results of modulation of activity in auditory cortex on sound detection in behaviour task. Finally, during electrophysiological recordings from auditory cortex, I looked at effects of modulation of neural activity using chemogenetics. These acute experiments were performed in anaesthetized mice.

The results have proven that mice model is a highly useful tool in the study of perceptual decision-making, allowing for combination of behavioural task with neural activity monitoring and modulation. In the behavioural task the influence of foreperiod duration on reaction time was not confirmed but a correlation between foreperiod presentation ratio and reaction time was observed.. Implementation of chemogenetics in behavioural paradigm influenced hit rate and reaction time dynamics only for some of the higher sound intensities, therefore not providing confirmation of effect of used technique on behaviour. In electrophysiological

recordings, statistically significant changes in levels of neural activity after activation of chemogenetics were reported for both sound- evoked and no-sound related activity in auditory cortex. Altogether, the obtained results do not provide confirmation of presented research theses which presumed the importance of auditory cortex for perceptual decision-making. However, due to the complexity of the matter in question , further studies are needed.

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Abbreviations

2-AR – β_2-adrenergic receptor

A1 – primary auditory cortex

AC – auditory cortex

AL – antero-lateral belt of auditory cortex

AlstR – allatostatin receptor

AM – amplitude modulated

AN – the auditory nerve

AVCN – the anteroventral cochlear nucleus

BOLD – blood oxygen level dependent imaging

ChETA – ChR2 E123T accelerated

ChR2 – Channelrhodopsin-2

CID – chemical induction of dimerization

CNO – clozapine-N-oxide

CNS – central nervous system

CP – choice probability

CSF – cerebrospinal fluid

DCN – the dorsal cochlear nucleus

DDM – Drift-Diffusion model

dlPFC – dorsolateral prefrontal cortex

DREADDs – designer receptors exclusively activated by designer drugs

EEG - electroencephalography

EPR – event-potential related

FEF – frontal eye field

FLEX – flip excision

fMRI – functional magnetic resonance imaging

FOF – frontal orienting fields

GFAP – glial fibrillary acidic protein

GIRK – G-protein inwardly rectifying potassium channels

GPCRs – G-protein coupled receptors

IC – inferior colliculus

IHC – inner hair cells

ITI – intertrial interval

IP – intraperitoneal

KOR – κ-opioid receptor

KORD - κ-opioid-derived DREADD

L2/3 – layer 2/3

LFP – local field potential

LIP – lateral intraparietal cortex

LL – lateral lemniscus

LSO – the lateral superior olivary nucleus

LW – licking window

MEG – magnetoencephalography

MGB – the medial geniculate body

MISTs – molecules for inactivation of synaptic transmission

ML – middle-lateral belt of auditory cortex

MSO – the medial superior olivary complex

MT – middle temporal area

MU – multi-unit

NDMC - N-desmethylclozapine

NpHR - halorhodopsin

NpHR-EYFP

OHC – outer hair cells

PAF – posterior auditory field

PET – positron emission tomography

PPC – posterior parietal cortex

PV^+ – parvalbumin-positive

RASSLs – receptors activated solely by synthetic ligands

RDK – random dot kinetogram

ROC – receiver operating characteristic

RT – reaction time

SBF – striatal beat-frequency model

SC – superior colliculus

SDN – state-dependent networks

SDT – signal detection theory

SOC – the superior olivary complex

SU – single-unit

tDCS – transcranial direct current stimulation

TMS – transcranial magnetic stimulation

vlPFC – ventrolateral prefrontal cortex

1. Literature Review

A crucial ability of an animal to survive in an environment is decision-making, which often needs to be based on imprecise sensory stimuli coming from the external world (Basso and Wurtz, 1997; Brunton et al., 2013; Carandini and Churchland, 2013). To have an insight into neuronal underpinnings of this process, one should be basing on a composite approach. First, a reliable behavioural paradigm should be implemented and when showing high efficacy, neuronal activity should be measured by means of electrophysiological recording or functional imaging while the task is performed. Then, the contribution of different structures and cell types should be evaluated. For the latter purpose techniques allowing neural modulation - such as chemogenetics or optogenetics can be applied.

The aim of this chapter is to review the concept of perceptual decision-making with special interest in the role of early stage cortices in rodent auditory-based, head-fixed behavioural tasks. However, for the better understanding of the subject and its importance, various related topics have been briefly introduced and the history of research on perceptual decision-making has been summarized based on data from various species and sensory modalities. Subsection 1.1. concentrates on the notion of decision and its characteristics, subsection 1.2. describes the anatomy and physiology of the auditory sytem, while subsection 1.3. presents the development of the field based on selected experiments conducted mainly on primates and humans. Subsection 1.4. focuses on the research in rodents with emphasis on behavioural paradigms and possible structures and elements of involved networks. Subsection 1.5. describes techniques used for monitoring and modulation of neurons engaged in perceptual decision-making and finally, subsection 1.6. introduces the objectives of this thesis.

1.1. What is a decision?

A decision is an act of choosing one of the presented options or schemes of action selected on the basis of information and advantages related to feasible results. An important characteristic of this process is its elastic timing not always immediately following acquirement of evidence. Decisions are often preceded by steps such as

reflecting on and preparing action and strategy (Shadlen and Kiani, 2013). In the following subsections, key concepts in the field of decision-making are summarised.

1.1.1. What is decision making?

Although present in numerous areas of life, from a neuroscientific point of view, decision-making is a neurobiology of cognition that incorporates rules of neural processing found at the basis of a plethora of mental functions (Britten et al., 1996; Churchland et al., 2008; Brunton et al., 2013; Shadlen and Kiani, 2013).

Decision-making can be divided into three main categories: value-based, social or perceptual decisions. The former is related to choices between various positive or negative options, for example, the choice of food or money versus punishment and pain Social choices, on the other hand, are most frequently linked to behaviours such as mating, fighting, distributing goods or establishing dominance. Both types of decision, although based on evidence, are strikingly different in their bases and mechanisms from perceptual decision-making, as being based on a consultation with a random number generator, they are highly unpredictable (Shadlen and Kiani, 2013). As the theory behind value and social based decision-making is vastly antithetical to that related to perceptual decision-making, which is the core of this thesis, they won't be further discussed.

Perceptual decision-making is a phenomenon where an animal using information obtained from its senses detects, discriminates and categorizes information from the external world. This process has gained a lot of scientific interest in the last three decades, raising it to one of the core topics in neuroscience. In its course, noisy sensory inputs are eventually converted into discrete motor acts. The key to the research relies on strict control over sensory input available to the animal as well as appropriate and timely reward delivery after correct sensorimotor behaviour has been presented by the animal (Hanks and Summerfield, 2017).

The interest in decision-making and the realisation that its mechanisms may be involved in or related to various other concepts made some new theories appear and extend the notion of decision-making to be something more than just a selection

between available options. In 2011, Shadlen and Kiani proposed to look at decision not exclusively as an action but as an initiation of subsequent decisions. This approach introduces more complex intellectual structures, such as volition and consciousness (Shadlen and Kiani, 2011). They identified volition as a '*conscious will to perform an action*' that, similar to decision-making, is based on evidence and executed once thresholds for evidence accumulation and timing are met. They defined consciousness as a choice to engage in a certain way with the environment and claim that in people, even complex decisions depend on wakefulness but not necessarily awareness, whereas in animals, decisions are normally a selection between variants, and that in this regard consciousness may be guided by non-conscious mechanisms related to decision-making (Shadlen and Kiani, 2011). This example shows that elucidating the mechanisms guiding perceptual decision-making will provide knowledge not only about this crucial phenomenon itself, but may also contribute to better understanding, from a neurobiological point of view, of concepts, so far being elusive and complex, such as consciousness.

1.1.2. Reaction time, accuracy and confidence – 3 core parameters of decision-making

The following subsection introduces a key method used in decision-making studies-psychophysics, together with its crucial parameters. It also introduces Drift-Diffusion Model and Signal Detection Theory, which explain some of the calculations behind decision-making. The aim of this subsection is to present important factors that need to be taken into account during behavioural tests examining perceptual decision-making as well as some of the theory behind the process.

Psychophysics, dating back to 1860 and Gustav Fechner (Fechner, 1860; Carandini and Churchland, 2013) is a quantitative study of the relationship between a physical stimulus and its representation in an animal's perception. It allows for experiments based on stimulus detection or discrimination.

The reason why this method is still broadly used and provides a base for studies of perceptual decision-making is the fact that it allows to establish a clear connection between behaviour and its underlying neural activity. The key to successful

psychophysical experiments is the precise control of a sensorimotor input and a behavioural output shown as a motor response together with applying the most suitable reward which motivates the animal to perform (Gold and Shadlen, 2007).

The detection of a stimulus depends proportionally on its intensity. Despite that, there is always a range in which the stimulus can be either detected or unnoticed. This indicates that there is a variability in behaviour for which the basis may lay in variability of sensory neurons' responses. A cumulative probability curve is used to describe the relationship between stimulus intensity and the level of its detection. It spans from 0% detection for the weakest stimulus intensity to 100% for the most robust. In the middle, at 50% there is a point of intensity for which the stimulus is detected in half of the trials. This point is often, although not exclusively, selected as a detection threshold. The slope of psychometric function is related to the variability of behaviour with shallower slope representing greater variability. The variability of neuronal responses, translated to changes in behaviour, may have diverse sources originating in either external stimulus, sensory periphery or the central nervous system itself. Regardless of the source, the variability will be visualised in psychometric detection function (Barlow, 1972; Parker and Newsome, 1998).

As mentioned in the title of this section, the three main parameters that should be considered in such studies are accuracy, reaction time and confidence.

Accuracy shows the statistics of the outcome of behavioural response. There are 4 possible results: a) hit – when the animal correctly responds to the stimulus b) miss – when the animal fails to correctly respond within provided time window c) false alarm – when the animal performs before stimulus presentation and d) correct rejection – when the animal refrains from action in trials where no stimulus was presented (applies only for 'catch trials' measuring impulsiveness of the animal).

Reaction time is a period of time between stimulus onset and the animal's reaction. This aspect is crucial for the study of temporal expectations (described in subsection 1.3.3). In behaviour, it's crucial to know not only which option to select but also when to select it. As described in section 1.3.3., a decision is made upon accumulation of

sufficient evidence of good quality. However, recent studies imply that apart from evidence accumulation another factor is involved in decision-making. The evidence-independent 'urgency signal' which, although unpredicted by classical models, seems to help in making a decision when available data is weak or blurry (Hanks and Summerfield, 2017). Evidence of this signal has been seen in trials, performed by monkeys, where the coherence of stimuli was 0% but the neurons in the lateral intraparietal cortex (LIP) responded at a rate approaching the threshold for response (Churchland et al., 2008). This evidence-independent signal has been noticed, again in LIP, in a study where monkeys were estimating elapsed time of intertrial interval (ITI), preparing for a response (Janssen and Shadlen, 2005).

Speed and accuracy trade-off is vital for assuring the most satisfying outcome of one's action. As all perceptual decisions are undertaken within a motivational context, often the decision about *what* is as important as the decision about *when,* as the availability of resources changes in time. Mathematical models of calculations underlying decision-making such as the Drift-Diffusion Model (DDM) suggests that a decision is being made when available cumulative information reaches a specific threshold, a fixed criterion value (Wald and Wolfowitz, 1949). Pinpointing this value is key for the process of decision-making as the precision of noisy signals, on which the choice is made, increase with time, therefore longer sampling results in a more accurate choice. On the other hand, a fast choice reduces the time of evidence collection at the risk of jeopardizing a correct decision. Hence, deciding when to decide is basically a way to set a trade-off between speed and accuracy of a decision (Bogacz et al., 2010). The DDM, typically applied to binary choices, assumes that decisions and their latencies are dependent on the rate of acquisition of information necessary to decide between option A and B (this rate is called a drift), the amount of noise present in the signal and threshold representing the minimum amount of information gathered to make a choice. A key value in this process is decision variable- a computation placed between integration of sensory evidence and making a choice. It is a quantity directly related to the likelihood of one option being more probable than the other one (Heekeren et al., 2008). The DDM, a sequential sampling

model, is in accordance with experimental data bringing together speed and accuracy and can explain the distribution of response latencies in both hit and false alarm trials where response period, reward probability and value, as well as signal quality, has been manipulated (Bogacz et al., 2006; Ratcliff and Smith, 2017).

Confidence is a parameter that demands an internal reflection of one's state, known as 'metacognition' (Yeung and Summerfield, 2012). In humans, this subjective estimate of certainty is quite easy to obtain as people can indicate its value on a scale. Although studies of certainty in *homo sapiens* are over a century old, we still do not know its function across species (Fetsch et al., 2014; Meyniel et al., 2015). Notwithstanding, research performed in recent years on monkeys and rodents provided us with data about levels of confidence in experimental animals (Kepecs and Mainen, 2012). The way of measuring the degree of confidence in animals is addition to the paradigm the 'opt out' option meaning obtaining a smaller but certain reward contrary to a bigger but uncertain one (Hampton, 2001). When the quality of the neural signal is low, animals are more prone to select this option at the same time getting higher scores on the trials where they chose a risk (Kiani and Shadlen, 2009). An alternative task introduces a delay after the majority of correct trials, without providing feedback about the outcome, allowing animals at the same time to reset the trial. Animals willing to await reward for a longer period have a higher level of confidence (Lak et al., 2014). These paradigms have proven that rodents and monkeys, similar to humans, have access to an internal estimation of confidence and highlight the importance of a properly tailored behavioural task (Kepecs and Mainen, 2012).

1.2. Auditory system

The auditory system creates a sensory modality characterised by high sensitivity and engaged in multiple tasks. Perception of sounds is divided into several steps. Sound waves after being transformed to discrete patterns of neural activity are further integrated with information coming from other modalities to guide movements,

enhance behaviour or facilitate communication (Fitzpatrick, 2008). The initial transformation of auditory stimuli originating in the environment takes place in external and middle ears, responsible for collecting sound waves and enhancement of their pressure which allow the signal to be transmitted to fluid-filled cochlea located in the inner ear. Subsequently, processing of the signal in the inner ear leads to its components: frequency, amplitude and phase, being in detail transduced by sensory hair cells and further encoded as electrical activity in the auditory nerve fibres. One of the results of decomposition of the signal is tonotopy- representation of sound frequency distributed along the cochlea. The importance of this process is visible in its preservation in central auditory pathways. Central processing is initiated in cochlear nucleus from which the signal is distributed to several parallel central pathways. The output of this structure reaches multiple targets such as superior olivary complex which combines signals from both ears and allows for placing sound source in space. Another structure to which cochlear nucleus projects is inferior colliculus, situated in the midbrain, which serves as an integrative hub and first nuclei where auditory signals interact with the motor system. From inferior colliculus auditory information is conveyed to the thalamus and cortex where further, more complex analysis of the signal occurs. The number of relay points between auditory periphery and cortex is significantly higher than in other sensory systems. This fact points out the complexity of this neural process (Fitzpatrick, 2008).

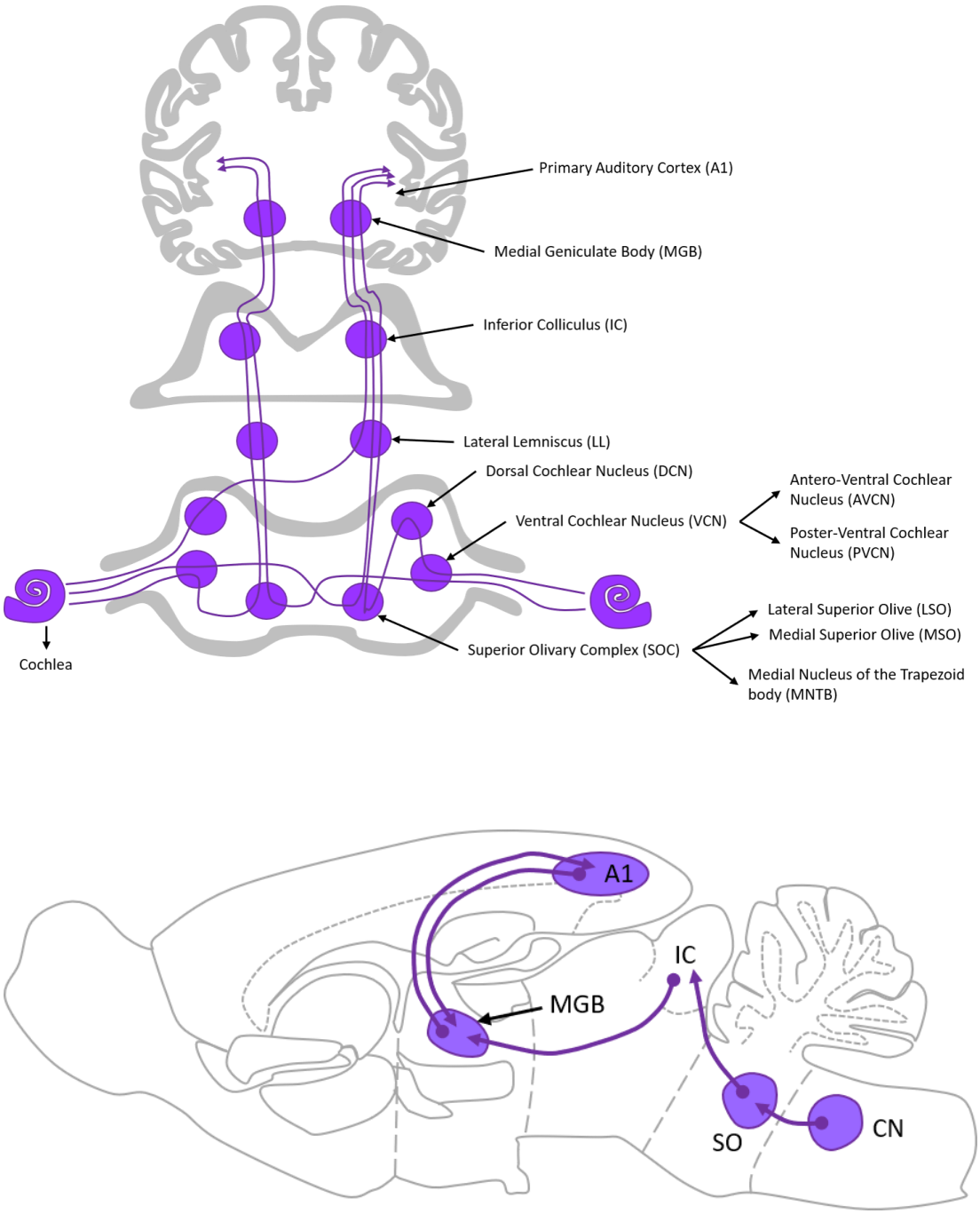


Fig.1.1. Auditory pathway

A: General scheme of auditory pathway (Jayakody,D.M.P. *et al*., 2018 modified)
B: Murine auditory pathway (Mueller, T., 2012 modified)

1.2.1. Anatomy and physiology of ascending auditory pathway

1.2.1.1. The external ear

The auditory pathway begins with the external ear consisting of pinna, concha and auditory meatus (Gardner, M.B. and Gardner, 1973). Its role is to gather sound energy and relay it to the tympanic membrane. Additionally, pinna and concha filter sound frequencies, providing information about the location (elevation) of the sound source.

1.2.1.2. The middle ear

The main function of the middle ear is to tackle the change in medium resistance between the airborne signal in the external ear and the fluid-based environment of inner ear (Fitzpatrick, 2008). Such crossing, from low to high impedance medium, is normally related to a loss in conveyed energy. This issue is resolved by multi-fold augmentation of the pressure measured at the tympanic membrane. To allow for this process, the pressure from the tympanic membrane, of a larger diameter, is transmitted to a much smaller in diameter, oval window. This action is enhanced by the work of ossicles – malleus, incus and stapes- 3 interconnected bones of the middle ear. Other structures that take part in the regulation of the quality of sound transmission are two muscles located in the middle ear: the tensor tympani and the stapedius. Their flexion, having an impact on ossicles protects the inner ear (Henson, 1974).

1.2.1.3. The inner ear

The inner ear presents a higher level of complexity than the aforementioned parts. From an anatomical point of view, the inner ear consists of semi-circular canals, vestibules and cochlea which functionally are divided into the cochlea and vestibular system (Fitzpatrick, 2008). The crucial function of the cochlea, a structure located in the inner ear is to transform the energy from pressure waves into neural activity. Apart from boosting sound waves and transforming them into neural activity, the cochlea is in charge of mechanical frequency analysis leading to extraction of simpler elements from acoustical waveforms. The cochlea, taking its name from Latin and meaning snail, is a coiled structure filled with fluid (Hawkins and Schacht, 2005).

At the basal end of this tubular structure, both oval and round windows are located. The cochlea is divided along its length by a partition – a flexible entity that supports the basilar membrane and the tectorial membrane. On each side of the cochlear partition, chambers filled with fluid are located. Apart from scala vestibule and scala tympani, a third chamber, scala media, is located within the partition itself. The partition terminates before the apical end and creates an opening- helicotrema that by joining scala vestibule with scala tympani allows for mixing of perilymph, the fluid present in both channels. A mechanism crucial for cochlear functioning is the way in which basilar membrane vibrates in response to sound. Both the vibrations along the membrane as well as rates of discharge of auditory nerve fibres that terminate along its length are tuned, responding stronger to sounds of a particular frequency (Fitzpatrick, 2008).

Frequency tuning originates partially from the fact that that basilar membrane is uneven, with its apical end being wider and more flexible than basal end. This mechanism was explained by Georg von Békésy (Von Békésy, 1960), who proved that changes in flexibility and width of a membrane's maximal vibration at different positions are what translate to a function of stimulus frequency. Békésy further discovered that sound initiates a travelling wave of the same frequency in the cochlea which subsequently propagates from the base in the direction of the apex of the basilar membrane, enhancing amplitude and decreasing velocity until a point of maximum displacement is reached. This point is dependent on sound frequency. Points related to high frequency are located at the base of the basilar membrane whereas low frequencies are gathered at the apex, creating a topographical map of frequency. Complex sounds form vibrations equal to the superposition of those originating from individual tones being elements of complex sounds. This spectral decomposition is an important element of cochlear function.

The movement of a travelling wave triggers sensory transduction by displacing the cells of the organ of Corti, hair cells that are located on the basilar membrane. In the organ of Corti, two types of hair cells are present. Outer hair cells (OHC) form three

rows while one row is made of inner hair cells (IHC). Every hair cell has multiple tiny projections, stereocilia (Fitzpatrick, 2008). The organ of Corti is pivotal to the process of converting mechanical energy into electrical signals that propagate through the auditory nerve to the central auditory system. Sound waves provoke displacement of the basilar membrane and create a travelling wave that propagates from the base to the apex of the cochlea (Von Békésy, 1960). This travelling wave provokes lateral movement of the tectorial membrane over the stereocilia which are placed on top of hair cells (Russell, 1987). The hair cells are receptor cells (Dallos and Cheatham, 1976) and their role is to convert mechanical energy into electrical energy. Due to the lateral movement of the tectorial membrane, created mechanical fluctuations provoke opening of potassium channels which leads to an influx of potassium ions and depolarization of hair cells (Zdebik et al., 2009). Further, voltage-gated calcium channels get activated which results in neurotransmitter release from the basal end of the hair cells. Subsequently, auditory nerve endings get stimulated, ending the peripheral stage of auditory processing.

1.2.1.4. Auditory nerve

The function of the auditory nerve (AN) is to transfer the acoustic information from the organ of Corti to the central nervous system. This process takes place basing on two types of primary afferents - spiral ganglion neurons (type I and type II) which neurons form part of the AN (Heil and Peterson, 2015).

The vast majority of primary afferents (90-95%) of the AN consists of bipolar, type-I spiral ganglion neurons. Each of large diameter peripheral processes of type-I afferents is connected to a single IHC through a compact bouton. Each synapse contains a structure called a ribbon which extends into a cytoplasm at the active zone and ties up synaptic vesicles. A single IHC has connection with several boutons and depending on species can have up to around 30 ribbons (Meyer et al., 2009).

The peripheral process of type-I afferent is myelinated, with the exception of the near-synapse area. Myelinated is also the central process, which bifurcates and innervates neurons in both ventral and dorsal divisions of the cochlear nucleus in the

brainstem. The myelination of large cell bodies in the spiral ganglion depends, on the other hand, on species where cats, differently from humans have their cell bodies myelinated (Rattay et al., 2013). According to biophysical modelling, spike conduction is slower in the same region when it lacks myelination (Rattay et al., 2013).

The remaining 5-10% of primary afferents is built by unipolar type-II spiral ganglion neurons. Their peripheral processes of small diameter are unmyelinated, same as cell bodies and central processes that connect to various cell types within the cochlear nucleus (Benson and Brown, 2004). Having as a start point the cell bodies in the spiral ganglion, the peripheral processes run radially, parallel to type-I afferents, to later turn in the direction of the cochlear base. These processes run for several hundred micrometres next to the OHCs base and each of them obtain synaptic contacts from various OHCs (Weisz et al., 2012).

1.2.1.5. Central nervous system. Subcortical structures

In the central auditory system, various parallel pathways coexist and they converge and diverge along different stages. Some pathways deal with temporal data with a high degree of fidelity, whereas other have as objective analysis of activity patterns measured as neurons population's mean response rates. These two sorts of pathways diverge already at the level of auditory nerve input to the cochlear nucleus. The fusion of information starts in the inferior colliculus (IC) and continues subsequently in other structures allowing for refinement based on signals originating in both pathways. Neurons embedded in this process will create, as a final outcome, representation of the auditory object coming from the external world (Fitzpatrick, 2008).

1.2.1.5.1. Cochlear nucleus

The auditory nerve is the origin of division into two pathways. At the entrance to brainstem auditory nerve fibres bifurcate creating anterior and posterior branches of the nerve. The anterior branch, responsible for sound localization, innervates the anterior cochlear nucleus and initiates the ventral auditory stream of the brainstem. The dorsal auditory stream of the brainstem which serves the complex stimulus analysis is connected to the posterior branch which innervates dorsal cochlear

nucleus (DCN) and, in passing, the posteroventral cochlear nucleus (PVCN). The cochlear nucleus is divided into three substructures. The anteroventral cochlear nucleus (AVCN), which belongs to ventral stream and two divisions of dorsal stream of brainstem: the PVCN and DCN. Posteroventral cochlear nucleus has its contribution to the ventral auditory stream as well (Feldman and Harrison, 1969).

While entering the cochlear nucleus, the auditory nerve fibres still maintain the spatial distribution originating from their initial sites in the cochlea. This makes the incoming fibres tonotopically arranged, spatially ordering their characteristic or best frequencies. Each of the divisions of the cochlear nucleus is also tonotopically organised. This organizational feature is further preserved in higher order structures of the central auditory pathway.

The precision of temporal information embedded in neural firing is maintained and further increased in the AVCN of the ventral auditory stream of the brainstem. From spherical bushy cells of AVCN, the signal travels to the medial superior olivary complex where sounds are localised by means of comparison of relative timings and intensities between two ears (Rouiller, 1997). They target the ipsilateral lateral superior olive as well, where the comparison of stimulus intensities between two ears takes place.

The AVCN is made of two types of cells: spherical and globular bushy. Both types receive a direct signal from the AN but globular bushy cells obtain input from more AN fibres.

The firing in spherical bushy cells matches that of the input fibres with a temporal accuracy of 1:1. However, with an increase of stimulus intensity, the sensitivity of cells is reduced due to inhibitory inputs from other areas of the nucleus and to trigger an action potential under these circumstances, a larger number of simultaneously arriving inputs is necessary (Typlt et al., 2012). As a result of temporal averaging over numerous inputs, the temporal accuracy of action potentials is enhanced. This leads to a more precise representation of the mean firing in the auditory nerve. The averaging is even more potent in globular bushy cells, due to the higher number of

synaptic inputs. Altogether, the temporal accuracy of AVCN's output is enhanced and surpasses the one obtained at the level of individual input auditory nerve (Joris et al., 1994).

1.2.1.5.2. Superior olivary complex

The next hub after cochlear nucleus is the superior olivary complex (SOC) (Lee and Murray Sherman, 2010). Although the majority of fibres ascending to SOC is contralateral, some ascend ipsilaterally. The ascending fibres pass through the lateral lemniscus (LL) (Schwartz, 1992) and further converge in the midbrain, into the IC.

The SOC is anatomically divided into three nuclei: the medial superior olivary complex, the lateral superior olivary nucleus and the medial nucleus of trapezoid body with each of them being connected to distinct parts of the ventral cochlear nucleus (Schwartz, 1992; Rouiller, 1997).

The medial superior olive is a flat structure, made of bipolar cells, that receives direct inputs from AVCN. The cells undergo optimal activation under coincidence of both ipsilateral and contralateral excitatory drives. As a result of combined physiological effects, cells respond optimally in cases where contralateral ear was first to receive auditory stimulus (Golding and Oertel, 2012).

The lateral superior olive is a flat surface of cells that are folded in a complex way that is species-dependent. It has two major inputs: direct excitatory input from the ipsilateral AVCN, mostly from spherical bushy cells, and an inhibitory input from the AVCN's globular bushy cells that crosses the medial nucleus of trapezoid body. A vast majority of cells in the LSO receives ipsilateral excitatory stimuli, whereas inhibitory ones are contralateral. Due to the fact that the thresholds and tuning for both ipsi- and contralateral effects are similar, sound lateralization takes place in a frequency-specific way.

The principal, uncrossed output of the MSO projects to the ipsilateral IC. The main output of the LSO is crossed and similar to MSO responds, in the majority, to sounds on the opposite side of the head. Both timing-related localization signals from MSO

and intensity-based localization signals from the LSO add up at the level of the inferior colliculus. (Lee and Murray Sherman, 2010).

1.2.1.5.3 Inferior colliculus

The inferior colliculus is a crucial hub in the auditory pathway (Aitkin and Phillips, 1984). Its inputs come from the cochlear nucleus (Oliver, 1987), the SOC (Saldaña et al., 2009) and lateral lemniscus (Winer, 2005). The tract of fibres ascending to IC forms lateral lemniscus which consists of two nuclei. The dorsal nucleus of the lateral lemniscus enhances the accuracy, contrast and dynamic range of the localization information with respect to the superior olive. The ventral lateral lemniscus is related to monoaural pattern identification and functionally belongs to the dorsal auditory stream of the brainstem. Anatomically, IC consists of various nuclei (Faye-Lund and Osen, 1985). Basing on studies in rats, they were divided into three sub-structures with characteristic spatial organization of neural processes (Faye-Lund and Osen, 1985). Resembling picture emerges from studies in cats (Oliver, 1987), monkey (Fitzpatrick, 1975) and humans (Geniec and Morest, 1971). The central nucleus receives the majority of the ascending inputs, the two remaining sub-structures form the external nucleus and the dorsal cortex. The core nucleus of the IC is the central nucleus. This sub-structure is characterised by laminar organization (Faye-Lund and Osen, 1985) responsible for spatial representation of neurons tuned to distinct frequencies. The external nucleus and dorsal cortex function as feedback loops, as well as multisensory part of the auditory pathway. The auditory signal is further transmitted to auditory thalamus (Aitkin and Phillips, 1984).

1.2.1.5.4. The auditory thalamus

The auditory thalamus is a relay centre of the auditory pathway. This structure comprises several elements: the medial geniculate body (MGB) which forms the principal division of the thalamus, the posterior nucleus of the thalamus and the auditory section of the reticular nucleus. The MGB is the main recipient of the primary afferents from the IC (Aitkin and Phillips, 1984; Lee and Murray Sherman, 2010). Similar to IC, also in MGB a lamination representing the spatial arrangement of frequencies is present (Cetas et al., 2001). The MGB itself contains different areas:

ventral, medial and dorsal segments playing role in diverse aspects of auditory processing. Posterior and reticular nuclei are also innervated by inferior colliculus (Calford and Aitkin, 1983). The projections originating in the thalamus are further relayed to various areas of the auditory cortex. This stage is bidirectional (Lee and Murray Sherman, 2010).

In recent years, new structures in auditory thalamus have been pinpointed as important for sound processing. Murine MGBv seems not to be homogenous in structure but instead composed of various compartments that in frequency-related way send topological projections to diverse sites in auditory cortex (Horie et al., 2013). This would imply presence of parallel projections in the lemniscal thalamocortical pathways, as was previously suggested in cats (Lee et al., 2004). However, for now, there are not enough evidence to equal the topography of murine MGv with tonotopy. Nonetheless, some data has already shown that tonotopic gradients in MGBv and AC are connected through topological projections (Hackett et al., 2011).The existence of parallel pathways in lemniscal pathway suggest that multiple tonotopy could be based on numerous topological thalamocortical inputs.

1.2.1.6. Central nervous system. Auditory cortex

In mammals, the auditory ascending pathway is of great importance as it directs auditory information from environment to auditory cortex. This pathway, denominated lemniscal, starts with ear and ends up in AC after passing through several hubs such as central nucleus of inferior colliculus in midbrain and ventral division of medial geniculate body in thalamus (Lee and Murray Sherman, 2010).

The auditory cortex is the final target of the afferent auditory information. The first significant advances in understanding the importance of auditory cortex were made by Santiago Ramón y Cajal (Moore et al., 2007). Since then this structure was investigated in various species, with technological developments enabling more detailed and more diverse studies. Initial electrophysiological studies in cats (Genis et al., 1974) were further extended by imaging studies in humans and non-human

primates (Wessinger et al., 1997; Rauschecker, 2015). In recent years, research done in rodents is gaining popularity as well (Tsukano, 2017).

Functional specialization in mammalian brain is frequently related to location (Kanwisher, 2010). Basing on this approach, also auditory cortex consists of several subregions that vary in their functions related to sound processing. Although this area is built by numerous subdivisions, showing some variability between species, a basic division can be made between primary (A1 and AAF) and secondary (belt) areas, further surrounded by a lateral parabelt (Kaas and Hackett, 1998). The A1, positioned in the temporal lobe on the superior temporal gyrus receives input from vMGB and therefore contains a precise tonotopic map. As other primary sensory cortices, A1 has a topographical map of the cochlea. The belt areas possess less accurate tonotopic map. They receive broader input from the belt areas of MGB complex as well as from the primary auditory cortex.

The number of identified primary core areas is different between species with three described in monkey (Kaas and Hackett, 1998), five in cat (Reale and Imig, 1980), two in mouse (Stiebler et al., 1997) and ferrets (Kelly et al., 1986) and three (Sally and Kelly, 1988) or possibly more (Polley et al., 2007) in rats . Although one-dimensional gradient of characteristic frequency is preserved across species, there are differences between subregions (Sally and Kelly, 1988). In the aspect of laminar cytoarchitecture, auditory cortex is similar to other cortical areas in displaying the typical six-layered organization (Rose, 1949). Those layers are characterised by specific neural architecture and unique functional connectivity. They can be divided into supragranular, granular and infragranular (Kaur et al., 2005). Different parts of thalamus target specific layers of auditory cortex (Huang and Winer, 2000).

The most frequent neuronal type in auditory cortex is formed by excitatory glutamatergic neurons (80%) followed by inhibitory GABAergic interneurons (20%) (DeFelipe, 1993), which are further divided into numerous subtypes with various distribution across layers (Bayraktar et al., 1997).

Murine auditory cortex was first described in late nineties of previous century, by looking at characteristic frequency (frequency for which neuron has the lowest excitatory threshold) (Stiebler et al., 1997). Based on those studies AC was divided into 5 subregions, two of which: anterior auditory field and primary auditory cortex, are tonotopic. The remaining three are: secondary auditory field, ultrasonic field and dorsoposterior field. In AAF an A1 characteristic frequency is lower than 40 kHz, whereas in dorsolateral corner of AC where ultrasonic field is placed, tonotopy deals with values over 40kHz that correspond to sounds used for vocal communication in this species (Guo et al., 2012; Tsukano, 2017).

1.2.2. Importance of layers and cell-types in cortical circuitry

A key role of the cortex is to create a perception basing on incoming information. To decipher this process, it's indispensable to bear in mind the unique input coming both from different cortical layers and cell types (Adesnik and Naka, 2018). There are two main approaches concerning the creation of sensory-based percepts. A hierarchical, synthetic process and a process rooted in statistical inference. The hierarchical approach is based on the integration of sensory data filtered from various inputs that as the endpoint provide an output neural activity conveying information about stimulus features. This process allows, in theory, for sophisticated computations leading to scene analysis and object identification (Hubel and Wiesel, 1962). Meanwhile, the idea of statistical inference is based on a generative model of the external environment, where incoming sensory data is constantly compared, at different stages of processing, against the internal model of the outside world (Bastos et al., 2012).

The hierarchical model got backed up in the primary visual cortex (Hubel and Wiesel, 1959) and inferotemporal cortex (Gross, 1972) with the firmest example being orientation tuning in V1 (Hubel and Wiesel, 1962). However, very scarce data was obtained on de novo transformation during propagation of the signal through cortical layers of a single sensory area. Processes such as orientation tuning or direction selectivity were restricted to layer 4 (Hubel and Wiesel, 1962), while in other sensory domains data was even less informative. Therefore, the hierarchical model not only

fails to prove that within a single cortical area the circuitry expanding through layers can create feature selectivity de novo (Adesnik and Naka, 2018) but also it cannot justify the extensive amount of feedback connections from higher to lower order cortical areas (Felleman and Van Essen, 1991).

The probabilistic inference model, on the contrary, fits in the top-down control. It assumes that neurons originating from different layers have different impact on computing the chance of specific neural pattern to encode a given sensory stimulus (Bastos et al., 2012). It's based on the idea of constant comparison between afferent inputs from various computational stages and internal model relayed by top-down projections. This model combines recent events with previous experience to convey predictions from higher to lower stages. The information, being transferred through feedback connections through layers not only of a single area but also in between areas is analysed by neurons in earlier stages that oppose predictions against errors marked as a deviation from the bottom-up, ascending sensory information (Bastos et al., 2012). Although this concept, highlighting probable different functions of various layers and cell-types, is promising, not much data has been gathered so far (Homann et al., 2017). Nonetheless, in recent years the interest has been shifted from layers to cell-types distributed along specific cortical layers. The main difference between those concepts is that the first one implies that analysis on just one layer is sufficient to reveal specific computational mechanisms, whereas second demands simultaneous analysis of activity across multiple layers (Harris and Shepherd, 2015). At the present stage of knowledge, it seems that apart from rare exceptions the basic cortical computational units are spread both between layers and cortical areas (Adesnik and Naka, 2018).

1.2.2.1. Conventional outlook on cortical circuits

The conventional approach towards cortical circuits surged as the effect of anatomical and connectivity studies performed on rodents, cats and primates (Gilbert, 1983). Here, the flow of information is directed from thalamus to L4 and then L2/3, basing on feedforward loops, to sub-sequentially pass through L5 from which the information reaches other cortical and subcortical areas (Rodney et al.,

1991; Binzegger et al., 2004). In this model, the long-range connectivity patterns are based on laminar distribution. Layer 4 is assumed to communicate mostly locally (Binzegger et al., 2004). The principal neurons of L2/3 are said to target exclusively other cortical areas and striatum, while L5 apart from intratelencephalic cells (same as in L2/3) contains additionally pyramidal tract cells which project to several subcortical areas. Layer 6 harbours both intratelencephalic cells and corticothalamic cells that project back to the thalamus (Adesnik and Naka, 2018). In the corticocortical pathways, which play different roles (Felleman and Van Essen, 1991; Gamanut et al., 2018) laminar organization is crucial (Souza and Burkhalter, 2017). The pattern of translaminar connectivity is quite distinct and complex. The primary sensory thalamus sends output to cells across all cortical layers (Petreanu et al., 2009). L4 neurons are both densely interconnected within layer and project to all other layers but at the same time, they do not send feedback to the thalamus (Binzegger et al., 2004). Layer 2/3 display scarce feedback to L4, while it's connection with all types of pyramidal neurons in L5 is strong (Adesnik and Scanziani, 2010). The intratelencephalic cells of L5 connect onto pyramidal tract cells and those connect to other cells of the same type while providing scarce feedback to preceding layers (Yamawaki and Shepherd, 2015). In cats (Binzegger et al., 2004) and monkeys (Briggs and Callaway, 2001), neurons of L6 receive main input from L5 as well as superficial layers and they project back to L5. Meanwhile, corticothalamic cells of rodent L6 project mainly to L5 but also L4 (Kim et al., 2014) while they receive strong long-range inputs (Kinnischtzke et al., 2016). Despite clear differences in cortical circuits observed between different species, it seems plausible that different animals, as well as different brain areas, share common characteristics guiding laminar connectivity (Adesnik and Naka, 2018).

To decipher functional input of specific cortical layers and cell-types anatomical studies have to be accompanied by studies based on perturbation of function of particular cell assembles. However, even after the surge of precise opto- and chemogenetics-based techniques for neural manipulation, this matter still presents numerous caveats as the exact extent of a layer is hard to specify due to branching

processes of a cell (Larkum et al., 2018). Additionally, cutting-edge techniques such as optogenetics, allowing for laminar control of cell functions bring data contradicting at various stages the conventional model of laminar circuitry (Olsen et al., 2013). The question of laminar distribution and connectivity itself may not be crucial as shown in a murine model with a disorganised laminar pattern, where the animal did not present sensory deficits (Guy and Staiger, 2017). The core of the matter may lay instead in specific cell-types that distributed throughout layers, together form basic computational units (Adesnik and Naka, 2018). Nonetheless, taking into account the high degree of conservation of cortical lamination among different animal species (Striedter, 2005), that is of significant importance to synaptic connectivity, it's feasible that lamination was indeed developed and preserved as efficient in various computational aspects (Adesnik and Naka, 2018). Overall, it's plausible that in specific cases of particular computations or behaviours a single cortical layer or a subclass of neurons would be enough to perform a task due to its afferent input and long-range output. The fact that principle neurons from all layers receive bottom-up thalamic inputs and that majority receives as well top-down cortical input while sending long-range projections out of cortex, gives anatomical support to this idea. A strong long-range input could enable a function that omits local circuits (Adesnik and Naka, 2018). Evidence for that was seen in mice (Matyas et al., 2010).

1.2.3. Features of neuronal tuning and its modulation

Neuronal tuning results in the selective representation of particular information or feature. Receptive fields are entities that delineate stimulus features provoking the strongest response. They show the tuning properties of sensory neurons. Representing the spectral analysis originating in the inner ear, auditory neurons are described by their sensitivity to sound frequency. The spectrotemporal receptive field, used often in auditory neurons response analysis (Aertsen and Johannesma, 1981) indicates that auditory cortical neurons are characterised by a higher level of complexity than neurons belonging to inferior colliculus (Atencio et al., 2012). That is probably due to fact that it is the cortex where various features of sound, such as frequency, amplitude modulation over time, spectral bandwidth or spatial location,

get integrated (Sloas et al., 2016). Various articles have so far reported the importance of dynamic changes in the response properties of neurons in auditory cortex (Schneider and Woolley, 2011; David and Shamma, 2013).

As sounds in the environment constantly change, neurons need to adapt fast by managing features such as stimulus probability or contrast and adapt sensitivity according to behavioural demands (David, 2018). As a result, depending on a feature change, the response can selectively change or stay constant (Rabinowitz et al., 2013). Dynamic coding in auditory nerve (Wen et al., 2009) is based on adaptation to mean sound level. As those structures send input to auditory cortex, incorporating IC adaptation to input of AC enhances efficacy of model of cortical neurons (Willmore et al., 2016). In ferrets, neurons of A1 compensate for sound contrast (variance of the sound level distribution) (Rabinowitz et al., 2012). Similar noise robustness was proven in cortical neurons of other mammals (Rabinowitz et al., 2013).

The features of AC neurons' receptive fields originate from inputs from the thalamus and are further shaped by the interplay between excitatory and inhibitory neurons. The confirmation of crucial role of ascending inputs in shaping of the functional organization of the auditory pathway is evident in the presence of tonotopic maps throughout processing stages, which begins with decomposition of sounds into individual frequencies along the inner ear cochlea (Saenz and Langers, 2014). Using techniques of fine spatial resolution, it was hinted that spatial organization in A1 is different across layers and that frequency tuning in layer 4, which obtain main input from thalamus, is more homogenous than in superficial layers (Winkowski and Kanold, 2013). Although some questions remain unresolved, there is a probability that heterogeneity in the frequency selectivity of ascending inputs delivers data for context-dependent modulation of cortical response characteristics.

The contribution of local communication between excitatory and inhibitory neurons to the features of auditory cortical responses has been documented various times (Li et al., 2014). Parvalbumin and somatostatin-expressing interneurons of layer 2/3 play role in regulation of frequency selectivity of A1 neurons (Kato et al., 2017), while

manipulation of PV^+ neurons activity leads to modulation of behavioural outputs mice trained to discriminate between sound frequencies (Aizenberg et al., 2015). Additionally, local interneurons shape the way in which A1 responses vary with respect to recent stimulus history (Phillips et al., 2017). Available data suggest that interplay between varying cell types heavily contributes to context-dependent alterations in auditory processing throughout the auditory cortex. Therefore, adaptive coding of auditory signals not only exists on subcortical levels but also can occur due to circuit interactions in the cortex.

It's highly probable that in various animal species, different coexisting systems for behaviourally meaningful sound features are present to varying levels. However, we still possess incomplete knowledge on how those systems map onto the tonotopic organization of various auditory areas including A1. An important factor to be kept in mind, as previously mentioned, is layer-specificity of auditory cortex in regard to frequency representation (Winkowski and Kanold, 2013), together with other response characteristics (Morrill and Hasenstaub, 2018). Additionally, neural population activity present in putative cortical columns may be responsible for representing auditory information (Schaefer et al., 2017; See et al., 2018).

1.3. Perceptual decision-making in humans and primates

Deciphering neural basis of decision-making is one of the biggest and most ambitious goals of modern neuroscience.

To investigate perceptual decision-making, at least two factors need to be taken into consideration: 1) a behavioural paradigm allowing for precise control of input stimuli and output reactions of the animal/subject and 2) ability to localize structures, neuronal cell-types and circuits (of short and long range) involved in the process. With the appropriate tools and preliminary knowledge, we can then investigate the functional connectivity within the involved system and examine the mechanisms

underlying this process on multiple levels. This task is laden with several caveats. First, the complexity of the behavioural paradigm depends on examined species with the biggest difference between humans and other animals being that we are capable of being guided by instructions. Furthermore, the differences between species and even strains make some paradigms more appropriate for some animals than others (e.g. visual vs auditory stimulus; type of motor reaction). Second, there are restrictions in the usage of some techniques in particular species. In humans, the most common approaches are the non-invasive ones like EEG (electroencephalography), MEG (magnetoencephalography), fMRI (functional magnetic resonance imaging) or PET (positron emission tomography). Studies in primates are highly dependent on electrophysiology and single-unit recordings, whereas experiments in rodents (to which section 1.3. is dedicated), thanks to genetic manipulations, allow for the use of techniques such as chemogenetics, optogenetics, GCaMP and two-photon imaging. Last but not least, there is an issue of translating the results between species in the area of anatomy and functional connections. Although there are several anatomical similarities between different animals, it's quite obvious that the level of complexity of the brain of a rodent, a primate and a human is different. Nonetheless, this fact, rather than creating more chaos, is allowing for bridging the gaps in our knowledge, adding up pieces of information obtained from different paradigms and techniques. Nowadays, thanks to a plethora of experimental techniques, we can perform experiments which bring us closer to understanding the mechanisms of perceptual decision- making.

1.3.1. Perceptual decision-making in humans

The study of perceptual decision-making in humans can be traced back to the 1960s when event-potential related (EPR) technique was explored in search of processing stages of computation occurring between stimulus presentation and behavioural output (Walter et al., 1964; Hillyard and Kutas, 1983). This method has good temporal resolution and gained popularity before sequential sampling models spread out in the neuroscientific community (Link and Heath, 1975). One of the first major components implicated in decision-making was the P300, found in the centroparietal

cortex. It occurs only after task-related events which were provoking decision (Sutton et al., 1965). It was stronger in hit than miss trials (Hillyard et al., 1971) and its timing was closely related to reaction time (Kutas et al., 1977). Despite the fact that those properties fit well into bound accumulation process, no link was made at the time with sequential sampling models and therefore the main characteristics of decision variable weren't tackled at the time.

The classical view on perceptual decision-making assumed that it's a hierarchical process where events occur as in a chain reaction. Nevertheless, more recent discoveries make us look differently at this question, opening a possibility that some of the components of this process, in reality, appear simultaneously. Four major steps towards making a choice which, although separate, can still influence each other are: 1) gathering and comparison of available sensory evidence 2) estimation of the level of difficulty and uncertainty (provoked, for example, by quality of signal) as well as implementation of additional attention means 3) involvement of motor structures based on decision variables and 4) performance control and strategy readjustment (Heekeren et al., 2008).

As mentioned before, studies on perceptual decision-making in humans pair behavioural paradigms with, in the vast majority, non-invasive methods of recording of neural activity which allow the investigation of possible brain structures involved and the level of their engagement. They also shed light on questions such as the role of rhythmic neural activity present in those structures, namely neural oscillations. The next subsections focus on two main approaches used to examine decision-making in humans: imaging based and EEG/MEG based. Additionally, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are mentioned as alternative/complementary methods. The objective is to look at the advantages and limitations of the techniques, as well as at humans as a model for elucidating mechanisms guiding perceptual decision-making.

1.3.1.1. Techniques used for the study of perceptual decision-making in humans

Nowadays, we can utilize an arsenal of non-invasive techniques that, alongside behavioural paradigms, can provide us with data about neural underpinnings of decision-making. The most common categories of techniques used for this purpose are: functional imaging-based approaches, EEG/MEG-based approaches and computational models (Kelly and O'Connell, 2015). Depending on the structure of the experiment and the questions asked, other techniques such as TMS or tDCS can be also be applied (Tegenthoff et al., 2005; Berkay et al., 2018; Müri and Nyffeler.T., 2018).

1.3.1.1.1. Imaging-based techniques

Current neural-imaging approaches allow to tract dependencies between behaviour and neural activity on a trial-by-trial basis (Heekeren et al., 2004). They are appropriate for measurement and modification of sensory evidence representation and its distinction from decision variables. Those characteristics make it feasible, up to a point, to compare this data with studies in monkeys. Imaging-based methods have been used to tackle the decision-making process in various sensory domains, such as visual (Epstein and Kanwisher, 1998; Ishai et al., 1999; Heekeren et al., 2004, 2006), auditory (Binder et al., 2004) or somatosensory (Pleger et al., 2006; Preuschhof et al., 2006).

Application of imaging techniques, such as fMRI, allow the tracking of structures involved with millimetre-level precision. It can operate on a system level and therefore, covering a larger area, provides more general data about brain functioning which, depending on the question asked, may be considered as an advantage over single-unit recordings. On the other hand, the main limitation of fMRI is its temporal resolution which is dependent on slow BOLD (blood-oxygenation-level-dependent) signals. It is, therefore, incapable of detecting fast changes in neural activity (Heekeren et al., 2008).

Early fMRI studies, based on visual cues and event-related designs, were searching for structures responsible for evidence gathering (Josephs et al., 1997). Later, they allowed to locate areas related to decision-making (Heekeren et al., 2004). Studies

using near-threshold visual stimuli showed that the level of activation in the sensory cortex predicts the behavioural outcome, as the level of activation in false alarm trials was greater than in those finished with a miss (Ress and Heeger, 2003). Another interesting study revealed that face-recognition specific areas (ventral occipital cortex) presented elevated activity not only in response to presented faces but also to objects that were misinterpreted as such (Summerfield et al., 2006). Above examples demonstrate that fMRI studies are appropriate for the search of structures involved in evidence accumulation. Unfortunately, it's usefulness for pinpointing structures involved in decision variable encoding is weaker due to poor temporal resolution. Various caveats related to this technique boosted the search of parameters and methods of analysis that would bring the most reliable results. Initial studies aiming at localizing decision-making-related structures using fMRI assumed that the BOLD signal should be greater on easy trials as they provide more evidence. Those studies highlighted the role of the left dorsolateral prefrontal cortex (DLPF) in the process (Heekeren et al., 2004, 2006). However, this view did not get the full support of the community. In a different paper (Liu and Pleskac, 2011) contrary assumptions were made. This discrepancy may be explained with the idea that BOLD parameters are dependent on the dynamics of a decision variable after reaching the threshold. If the signal remains high in an epoch after crossing the threshold, the resulting BOLD signal is strong. On the other hand, the fast decline of the signal after reaching the threshold would lead to a decrease in BOLD (Liu and Pleskac, 2011). However, this approach may be confusing as it has been shown that some of the structures not related to the decision-making stage also show increased activity with higher evidence strength (Tiffany C Ho; Scott Brown, 2009; Filimon et al., 2013). This inconsistency in results led to the introduction of additional criteria in the hunt for decision-making-related structures. Some groups looked at earlier onset in trials with stronger evidence (Tiffany C Ho; Scott Brown, 2009), others were expecting higher activation in correct trials in a somatosensory task (Pleger et al., 2006). An alternative method of analysis is based on paradigms where sensory evidence grows with the passage of a trial, and therefore allows for identification of areas where BOLD signal

increases over time (Ploran et al., 2007). The differences in obtained results, described in the above paragraph, are the cause of chaos in the literature related to decision-making. A good example of that is the dlPFC (dorsolateral prefrontal cortex) area, announced by Heekeren (Heekeren et al., 2004) as being crucial for perceptual decision-making. Other groups not only failed to replicate this result but also increased the complexity of the matter by naming other structures involved in the process - insula (Tiffany C Ho; Scott Brown, 2009; Liu and Pleskac, 2011) and intraparietal sulcus (Kayser et al., 2010a; Liu and Pleskac, 2011). Although this situation adds complexity to the matter, at the same time it points into new directions of investigation.

Study of perceptual decision-making using fMRI in auditory-based paradigms seems less explored comparing to visual-based tasks. An example of an auditory-based task is one where subjects had to detect speech sounds masked by a noise of varying intensity. It revealed an area adjacent to primary auditory cortex involved in the representation of sensory evidence important for decision-making (Binder et al., 2004).

The utility of imaging-based techniques, such as fMRI, for locating structures involved in perceptual decision-making is unquestionable. Nonetheless, the limitations of the technique, especially in the temporal domain, require in-depth analysis for correct interpretation of results. For acquiring a fuller picture it's also recommended to pair this technique or compare its results with methods characterised by high temporal resolution such as EEG or MEG. Additionally, fMRI being a non-invasive method operating on a larger area provides information that does not include full dynamics of local circuits. However, data gathered in such experiments may be used as a guideline for invasive experiments tackling the topic on a finer scale.

1.3.1.1.2. EEG & MEG based techniques

Another non-invasive category of studies in humans are experiments based on EEG, a technique detecting neural activity from the scalp, and MEG, a method based on detecting changes in the magnetic field caused by electrical activity of neural

populations. Contrary to fMRI, both techniques show high temporal resolution. Their limitations are related to the spatial domain. As every behavioural task elicits a plethora of neural signals underlying: sensory stimuli processing, decision-making and motor activity preparation and execution, functions that are located in various structures, separating those signals using EEG or MEG techniques is quite demanding (Kelly and O'Connell, 2015). An approach trying to overcome this issue, in a paradigm based on visual modality, applied machine learning to discriminate between signals corresponding to adequate stimuli categories (Philiastides and Sajda, 2006).

In the auditory domain, a study based on MEG in two-alternative forced-choice task, where participants needed to state identity (same/different) and location (same/different) of subsequent syllables, demonstrated changes in the power of gamma in the left inferior frontal cortex in response to identity and in the right parietal cortex in response to location. These results identify structures involved in sensory evidence processing (Kaiser et al., 2007).

Another achievement of EEG/MEG-based studies in the auditory domain was linking endogenous oscillation frequencies with temporal resolution of perception, and therefore accuracy of behavioural outcomes in detection tasks (Baltus and Herrmann, 2016). In humans, EEG oscillations in the range of 30-80Hz are involved in a plethora of cognitive tasks. The recent hypothesis on their role in cognition is that they may serve as pace-makers for other neural functions (Buzsáki, 2006). It has been shown that an action potential reaching a neuron oscillating at sub-threshold levels will more likely elicit a response if it appears within a peak of oscillation (Lampl and Yarom, 1993). This further translates to when it's more probable to detect a sub-threshold stimulus (Neuling et al., 2012). To create an auditory object, both the measurement of changes in auditory input and storage of past events in memory are crucial. Results of some studies advocate the idea that gamma oscillations process auditory input in a way that aids further processing in higher order structures (Baltus and Herrmann, 2016).

Similar to imaging-based techniques, EEG/MEG based techniques are suitable for investigating possible structures involved in decision-making and the role of their neural oscillations in the process. The advantage of these approaches is their temporal accuracy. However, they're not highly efficient in dealing with the spatial distribution of the signal. Again, considering the limitations of the approach it's worth implementing it in the experiment together with methods of fine spatial discrimination as well as validate the results against adequate computational models.

1.3.1.2. Summary on human studies

In conclusion, both imaging and EEG/MEG-based studies can contribute to our knowledge about perceptual decision-making. Nonetheless, due to the limitations of those approaches, careful analysis is advisable as well as comparing the results between studies exploiting different techniques.

Humans are, of course, a crucial model for studying perceptual decision-making if we want to gain a full insight into this process in our species, especially that they permit usage of elaborate behavioural tests. However, obvious ethical and technological limitations don't allow to approach the process on all levels and with fine resolution. Moreover, the majority of novel techniques allowing modulation of neural activity is used in animal models. Bearing those issues in mind, the following subsections present the results of studies in primates and rodents, showing pros and cons of both animal models and techniques implemented for their study

1.3.2. Perceptual decision-making in primates

As mentioned previously, although studies in humans allow for the implementation of more complex behavioural paradigms (containing instructions) the technical and ethical limitations restrict usage of various techniques that can provide us with data about structures and mechanisms responsible for perceptual decision-making on different levels of the process. Therefore, there is a strong need for the use of animal models to both compare the results with human studies and get a deeper insight into the process. Although not all results obtained from monkey studies can be directly translated to humans (because of differences in anatomy and some divergence in underlying mechanisms), data from animal experiments allows for a more detailed

view as more advanced and precise methods can be used, at the price of implementing invasive approaches. Studies in monkeys, allowing for electrophysiological recordings from single neurons of behaving animals, resulted in great progress in the research of perceptual decision-making. As neurons are the basic units of information processing in the brain, the ability to track characteristics of single cells involved in cognitive processes is necessary to fully evaluate pooled data gathered from multiple neurons (Parker and Newsome, 1998). Results from primates compared with other data, both experimental and originating from computational models, show some convergence between species but also provides us with novel theories and broadens our understanding of the topic (Hanks and Summerfield, 2017). Below, examples of studies in primates contributing to our knowledge in the field of perceptual decision-making are presented.

The beginnings of research into perceptual decision-making can be traced back to William Newsome who, in the eighties, studied visual modality in macaque monkeys using random dot kinetogram (RDK, a screen with randomly moving dots) and rewarded animals for making a saccade to a spatial target that was associated with the direction of motion of a majority of the dots (Newsome and Pare, 1988; Newsome et al., 1989; Celebrini and Newsome, 1994).

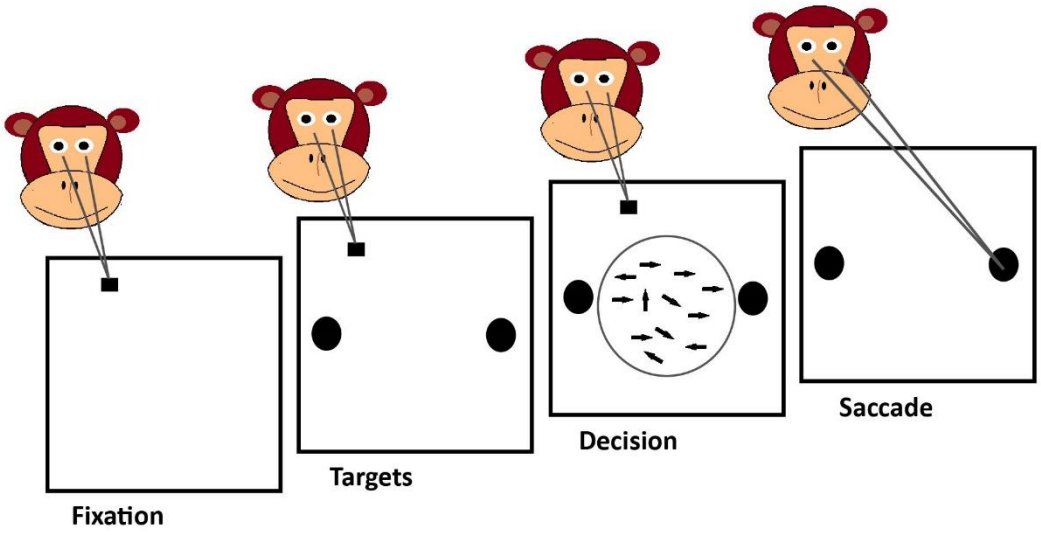


Fig.1.2. The random dot motion discrimination task

Fixation- animal has to fix gaze on a particular point of screen and hold it until it makes a decision **Targets**- possible targets are presented **Decision**- animal is presented with RDK, a set of points that move in various directions with a fraction of points presenting a coherent movement towards one of the targets. **Saccade** – animal has to move its gaze towards the target of coherent motions of dots. Correct choice results in reward delivery

This fairly simple perceptual task (involving only one type of stimulus and not relying on working memory), provided a plethora of information related to evidence accumulation and decision variables. During the task, a monkey watches a group of dots presented on the screen and needs to decide which of the two possible directions its net motion moves. The difficulty of the trials is regulated by the level of coherence, the fraction of dots travelling in the same direction. The animal indicates its decision with a saccade (fast eye movement) in the selected direction. As the dots traverse the screen only for a short period of time, the decision has to be based on the accumulation of momentary evidence. This characteristic relates this task more to cognition than perception issues (Shadlen and Kiani, 2011). Neurons in the middle temporal area (MT) have been indicated as a source of evidence on which direction decision is being made (Gold and Shadlen, 2007). Their strength and variability analysis, based on sound detection theory, provided data for mechanisms guiding behavioural accuracy (Britten et al., 1992; Shadlen et al., 1996). However, MT

neurons characteristics such as activity increase correlating with presented dots coherence, high level of variability revealed in noisy samples gathered over short periods of time and responsiveness of short latency dependent on the presence of the stimulus, clearly indicated that there is no accumulation of evidence or holding information in the memory. Above traits of MT neurons clearly show that this structure is not involved in either decision formation or its outcome (Shadlen and Kiani, 2011). In this situation, other structures had to be investigated as potential encoding structures for decision values. Due to the fact that RDM task can be seen as a question of movement selection (as direction decision is related to a saccade), structures involved in eye movement preparation were taken into account. Most studies focused on the lateral intraparietal area (LIP), dlPFC, frontal eye field (FEF) and superior colliculus (SC) (Gold and Shadlen, 2007). Neurons of LIP, located in the association cortex, receive input from the visual cortex and send output signals to structures involved in either eye movements or spatial attention. This position puts LIP in a perfect place to transform momentary evidence into decision variables. On each trial, the firing rate of LIP neurons represents combined values of momentary evidence gathered in the visual cortex. This process continues until the threshold is reached, which stops the decision process. After that, no more evidence is taken into account. The moment of reaching the threshold represents additionally reaction time, action execution (Shadlen and Kiani, 2011). An interesting observation is that LIP neurons encode the probability of making the right choice, that is the level of confidence (Yang and Shadlen, 2007; Kiani and Shadlen, 2009). This data suggests that LIP, rather than being directly involved in stimuli or action coding, is responsible for encoding probability. In summary, neurons of the lateral intraparietal cortex involved in the process of decision-making possess the following properties: they gather evidence from various sources over time, there is a threshold (being either a set period of time or the amount of collected evidence) which once reached, stops the decision process, the firing rate represents the level of confidence and, although they are affected by sensory stimuli and contribute to motor output, they're not strictly connected to either (Shadlen and Kiani, 2011).

The use of RDK paradigm in the visual modality, combined with electrophysiological research, initiated the application of paradigms suitable for primates in studying the neural bases of perceptual decision-making. This crucial step allowed the pairing of a neural response, up to cell resolution, with a provoking it stimulus, opening the era of an intense search for structures and mechanisms responsible for various aspects of perceptual decision-making spanning through all sensory modalities.

1.3.2.1. Auditory-based perceptual decision-making in primates

In monkeys, there is multiple evidence implying the crucial role of the ventral auditory pathway for auditory-based decision-making, especially when it comes to decisions about the identity of the stimulus (Romanski and Averbeck, 2009; Bizley and Cohen, 2013). However, while distinct auditory cortical areas have been identified like the visual system, the full impact of each area on different stages of auditory decision-making is not fully unravelled yet. In macaques, there are no less than 3 core, 7 belt and 2 parabelt cortical fields already discriminated (Kaas and Hackett, 1998). Early stages of the pathway, including the core regions as well as the anterolateral (AL) and middle-lateral (ML) of the belt region, are responsible for encoding features related to stimulus identity, e.g. stimulus frequency (Tsunada et al., 2016). Signals from those belt structures can later on either directly or indirectly travel to higher order structures, like the ventrolateral prefrontal cortex (vlPFC) which are capable of encoding behavioural choices and their outcomes. Nonetheless, the precise role of early vs higher order structures in auditory decision-making has been neither well delimitated nor well established so far (Tsunada et al., 2016). In a study by Tsunada *et al*., the belt structures AL and ML were put under close investigation (Tsunada et al., 2016). The results of a frequency discrimination task have shown that both structures contain frequency-tuned neurons. However, the results obtained after electrical microstimulation pointed at a more direct and causal role of the anterolateral belt region in auditory-based decision formation. Importantly, it has shown that two structures of the ventrolateral pathway, although demonstrating resembling evoked responses, have different impacts on perceptual decision-making. From the perspective of the underlying mechanism involved in perceptual decision-

making, the even more important accomplishment of this paper than naming anatomical structures, was to emphasize the importance of correctly evaluating the time window in which, on every trial, an animal gathers sensory evidence. Underestimation of the importance of the time window for decision-making led previously to confusion related to the role of MT neurons in visual-based decision-making. The early studies by Barlow (Barlow, 1972) predicated that behaviour emerges due to the activity of a very limited population of neurons whereas subsequent studies on the matter saw the bases of action in the activity of multiple cells (Britten et al., 1996). A way to deal with this discrepancy of experimental outcomes was to look closer at the timing aspect of the paradigms and precisely match neural activity with perceptual decisions. In older paradigms, neural activity was monitored during a 2 second viewing interval, whereas more recent studies indicate that monkeys make a decision as soon as after only a few hundredths of a millisecond (Roitman and Shadlen, 2002; Kiani et al., 2008). Likewise, Cohen & Newsome (Cohen and Newsome, 2009), by applying shorter stimuli, managed to improve the prediction of a pooling model. This study gave also a new perspective on the measurements of neuronal sensitivity basing on a MT example. It showed that in reaction time experiments, as stimuli are presented for shorter periods, estimated neural sensitivity is lower. On longer stimulus presentation, neural sensitivity seems higher as some of the noise is averaged out over time. This study allowed for better alignment of neuronal and psychophysical data.

1.3.2.1.1. The role of primary auditory cortex

Evidence gathered in recent years, from various animal models, is changing the way we see the primary auditory cortex. From an early stage decoder of stimulus features, it got promoted to a hub which is able to deal with much more complex calculations, including multisensory integration (Hackett and Schroeder, 2009; Kayser et al., 2010b) and processing task-related information (Jaramillo and Zador, 2011). Those changes could be the basis for improvements in behavioural performance.

Firing rates in the auditory cortex during both spontaneous and evoked activity are modulated by the behavioural state as confirmed in both rhesus monkeys (Miller et

al., 1972) and rodents (Otazu et al., 2009; Jaramillo and Zador, 2011). There is some inconsistency in the reports about spontaneous and evoked responses between passive/active listening, but it can be, up to a point, attributed to different behavioural tasks (Sutter and Shamma, 2011).

The question of the influence of non-auditory factors such as attention on activity in A1, also at the single unit level, remains open, although some data has been already collected. Studies in monkeys showed that in a task where animals discriminated amplitude modulated (AM) from unmodulated sounds, their results were better during active engagement than passive listening. Interestingly, the effect was visible not only in firing rate but also in vector strength, an indicator of a neuron's ability to deal with the temporal dynamics of the stimulus. The engagement in task resulted also in an increase in the spontaneous activity, but the activity provoked by unmodulated sounds was decreasing. Active AM sound discrimination was based both on firing-rate and phase-locking, indicating that the accuracy of both features can be modulated by behaviour (Niwa et al., 2012a). The improvement was noticed not only in single unit but also in multiunit activity. Further analysis showed that it was not the effect of the general firing rate increase, but a precise, stimulus-dependent change. Due to the increase in firing rate for modulated sounds, the difference in response between modulated and unmodulated sounds got bigger, making them easier to discriminate. Interestingly, despite the fact that both spontaneous and evoked firing rates were higher during behavioural engagement than passive listening, relative to spontaneous activity evoked responses were higher for a passive listening condition. However, discriminability was still good as there was a significant difference in the strength of responses towards modulated and unmodulated sound (Niwa et al., 2012a). Similar results, in ferrets, were obtained for spectral contrast in sounds (Atiani et al., 2009).

Comparing firing rate of both SU and MU in A1 just before animal's response with their firing rate during stimulus presentation in AM discrimination task, Niwa et al. (Niwa et al., 2012b) noticed that in the latter case firing rates were lower. They

concluded that there can be 3 explanations of this effect: attentional effect, the involvement of higher decision areas which may either take information from A1, or provide feedback. Recording from single units in this sort of paradigm, apart from looking into the type of mechanism used for sound discrimination, allows the study of the influence of non-auditory signals in the auditory cortex. This paradigm, based on near-threshold discrimination, allows to check if responses to identical stimuli are correlated with a behavioural choice. The role of A1 in perceptual decision-making has not been established yet but there is evidence that its activity may be related to non-auditory factors (Ghazanfar and Schroeder, 2006; Lakatos et al., 2007). In Niwa's study (Niwa et al., 2012b) increase in activity was observed on hit trials in AM-sensitive neurons. Studies using choice probability (CP) analysis (quantification of the correlation between neural activity and an animal's response with trial to trial resolution) in V2 (Nienborg and Cumming, 2014) and A1 (Niwa et al., 2012b) registered CP change of 2-3% for all units. Although at first this change may not appear important, quantitative analysis made by Shadlen et al. (Shadlen et al., 1996) showed that those changes pooled over larger amounts of neurons may be held responsible for changes in behaviour. This activity might be further processed by higher order structures. Additionally, the enduring activity taking place after stimulus presentation and shortly before a behavioural response may underline somatosensory or motor inputs to A1 (Niwa et al., 2012b). Definitely an important factor of analysis in this case is the time window. Depending on its alignment, it may factor in reward-related information. The results presented in the above-mentioned study indicated that somatosensory or motor-related activity, rather than having chaotic distribution, is related to those neurons which contain the most information about the task (AM discrimination).

The effect of non-auditory factors can differ depending on the cortical area, method of recording (spatial resolution, single units vs LFP) and the ability to generate activity vs modulate the activity elicited by an auditory stimulus. The reason why it is not easy to examine possible choice-related signals in A1 is that the techniques are often based on larger spatial scales. Moreover, the effect may be modulatory (Kayser et al.,

2009). Although there are examples of direct influence on single neurons in A1 coming from non-auditory inputs, they are not numerous and, as they are originating from different animal models and utilise diverse paradigms, it's hard to get full insight into the process (Brosch, 2005; Bizley et al., 2007).

Above examples show that our current definition of the role of A1 is far from being complete. The realisation that the primary sensory cortex may have its involvement in more complex processing leaves the door open for further investigation. The data obtained from studies in monkeys is not always consistent with studies in other animal models. The differences may originate not only from animal models but also from behavioural paradigms, examined parameters and type of implemented analysis. Therefore, there is a strong need to perform more well-tailored experiments that could shed more light on the issue of functionality of primary sensory cortices.

1.3.2.2. Summary

History of the investigation of perceptual decision-making in non-human primates spans over the last couple of decades. As it allows invasive techniques, contrary to studies in humans, it provided more detailed data about neural dynamics in this process and was crucial for establishing core concepts. However, although experiments in monkeys allow for developed behavioural paradigms combined with electrophysiological insight, they also present various limitations. This type of investigation is time-consuming (learning behavioural paradigms), costly and is subject to ethical questions. In addition, experiments in monkeys are run on small groups of animals (often just 2) and the current state of technological development does not enable immediate usage of all cutting-edge techniques in primates.

Therefore, in recent years it's the rodent model that gained more popularity in elucidating perceptual decision-making.

1.4. Perceptual decision-making in rodents

In recent years, the advances in modification of murine genetics, together with creation of multiple cutting-edge techniques enabling precise recording and manipulation of rodent neural activity, led to more widespread usage of those

mammals for researching various brain functions. This animal model has numerous advantages. As far as mice are concerned, not only over a century of their contribution to research has proven they're easy in housekeeping, handling and breeding, but also due to the creation of multiple inbreed lines they allow for precise investigation of genetic factors and usage of wide range of innovative techniques (Beck et al., 2000; Bućan and Abel, 2002; Busse et al., 2011; Carandini and Churchland, 2013; Burgess et al., 2016; Whissell et al., 2016). Thanks to those advances, a plethora of proteins have been investigated, highlighting influence of single molecules on various aspects of brain function in healthy individuals as well as in pathological states. Additionally, the anatomy of a rodent brain is well studied (Oh et al., 2014; Kuan et al., 2015), and the lack of salient gyri and sulci in its cortical structure enables detailed study of function of neocortex (Schwarz et al., 2010). In this section, types of behavioural paradigms are summarized. Furthermore, important structures involved in the process are presented and other factors that may influence perceptual decision-making are described.

1.4.1. Rodents in perceptual decision-making: head-fixed based paradigms

Rodent models have been used for decades to study behaviour and its neural correlates. The main emphasis was on paradigms relying on freely moving animals. Although this approach is valid for multiple uses, it's necessary to underscore the aptitude of head-fixed rodent models for merging behavioural studies requiring precise stimulus control with recent techniques allowing for investigation on cellular and subcellular levels.

Head-fixing allows for micrometre precision on a sub-millisecond scale (Carvell and Simons, 1990). It guarantees mechanical stability pivotal for neural activity visualisation using calcium or voltage-sensitive dyes in awake animals (Dombeck et al., 2007; Ferezou et al., 2007; Greenberg et al., 2008) and decreases contamination of electrophysiological signal with artifacts which mainly occur due to the animals' movements.

Of course, head-fixed models present some limitations, with the most salient one being a narrow repertoire of possibly implemented tasks. Fortunately, in recent

years, even those caveats are finding new solutions (Sanders and Kepecs, 2012; Burgess et al., 2016; Giovannucci et al., 2017; Toda et al., 2017; Han et al., 2018; Li et al., 2018), allowing for usage of yet more sophisticated behavioural tasks. Nevertheless, depending on the goal of the study various task designs should be taken into consideration. In the studies that assume usage of intracellular recordings or two-photon imagining, undoubtedly head-fixation is a technical advantage. Various approaches can be implemented in such a case (Schwarz et al., 2010). Apart from simple apparatus, some tasks combine various mechanisms like levers/trackball for response selection and a spout for reward obtaining (Sanders and Kepecs, 2012). In other cases, even combining electrophysiological recordings, it's preferable to allow animals free movement. Here, a three-port setup approach can be used. It has been successfully applied in both rat and murine studies (Busse et al., 2011; Raposo et al., 2012). An interesting fusion of those approaches, which opens new possibilities, is implementation of tasks where a rodent walking on a treadmill needs to make a decision based on stimuli provided by virtual reality. This method was reported in both rats (Holscher et al., 2005) and mice (Harvey et al., 2012; Pinto et al., 2018; Radvansky and Dombeck, 2018).

1.4.1.1. Task design

As mentioned, to evaluate the importance of neural circuits and mechanisms related to decision-making based on perceptual cues, precise assessment of sensory input is necessary. Over decades, various paradigms were invented. Apart from technological side they vary in terms of advantages and limitations. Even more importantly, depending on task complexity and characteristics they may be tackling the same question from different sides and therefore obtain different, sometimes contradictory results (Sousa et al., 2006; Hanell and Marklund, 2014). One of the issues is identifying if the selected paradigm deals only with low-level computations or does it elicit responses in higher order structures. Below is presented a general overview of paradigms used in head-fixed rodents.

1.4.1.2. Go/No-Go Task

The simplest paradigm used in rodents to probe perceptual decisions is a Go/No-go task, which dates back to the seventies of 20th century (Blough and Blough, 1977). The animal is asked to perform a certain response when a given stimulus or its particular feature is presented, but restrain from it in the absence of target stimulus or feature (Schwarz et al., 2010b; Histed et al., 2012; Miguelez Fernández et al., 2018). The main advantage of this design is that it's quickly learned by rodents. On the other hand, this paradigm is highly vulnerable to changes in the animals' motivation and impulsivity. Decrease in motivation provokes a lack of response, even though the animal correctly detected the stimulus, whereas an increase in impulsivity increments the number of trials where a response is not guided by stimulus parameters but rather an internal, non-sensory driven state (Dolzani et al., 2013).

One of the methods to overcome these obstacles is to use reference stimuli and catch trials. The role of reference stimuli is to consistently examine the level of the animal's motivation. When it's high, in detection tasks a strong suprathreshold stimuli should yield close to 100% of correct GO trials. Catch trials, during which no stimulus is presented, serve to calculate false-alarm rate when animal's responses are sensory unfounded. Randomly occurring licks may be a strategy for an increase in the chance of reward obtaining. In detection tasks, false-alarm rate should oscillate between 10-20% (Schwarz et al., 2010). Applying SDT where rates of both correct detections and false alarms may be calculated allows for calculation of detectability *d'* which is independent of criterion and hence not influenced by its changes (Carandini and Churchland, 2013).

During discrimination tasks, implementation of those methods may be more demanding. Still, it's of high importance to reduce salience of impulsive licking. Recommended approaches in such a case are longer inter-trial intervals or introduction of 'blackout time' where animal is punished with a longer time-out before a new trial if a lick was performed out of the response window (Schwarz et al., 2010). Nonetheless, those measures need to be implemented with caution as they may lead to a decrease in motivation or attention.

1.4.1.3. Two-alternative choice task (yes/no task)

A modification of Go/No-Go task is the two-alternative choice task previously known as yes/no task. In each trial a single stimulus is presented but the animal needs to perform one out of two possible responses, normally implemented by means of symmetrical apparatus (Schwarz et al., 2010; Resulaj and Rinberg, 2015; Burgess et al., 2016). Therefore, differently to Go/No-Go task, although the animal signalises the presence of a stimulus or its attribute, it does so by choosing one out of two options. In contrast to go/no-go tasks this paradigm is independent of changes in motivation as the animal needs to present a decision in each trial. SDT may be implemented for analysis. As the animal needs to report choice in every trial, it's easier to quantify which responses were real decisions. One of the limitations of this paradigm is the fact that it's still vulnerable to changes in decision criterion(Carandini and Churchland, 2013).

1.4.1.4. Two-alternative forced choice task

Another paradigm used in studies of decision-making is two-alternative forced choice task, used either for detection or discrimination. Here, a pair of items is presented during each trial and the goal is to select the target stimuli (Harvey et al., 2012; Histed et al., 2012; Sanders and Kepecs, 2012; Mayrhofer et al., 2013). Two-alternative forced choice task, differently than in previous paradigms, is independent of decision criterion as there is no bias for presence or absence of stimulus attribute (Carandini and Churchland, 2013).

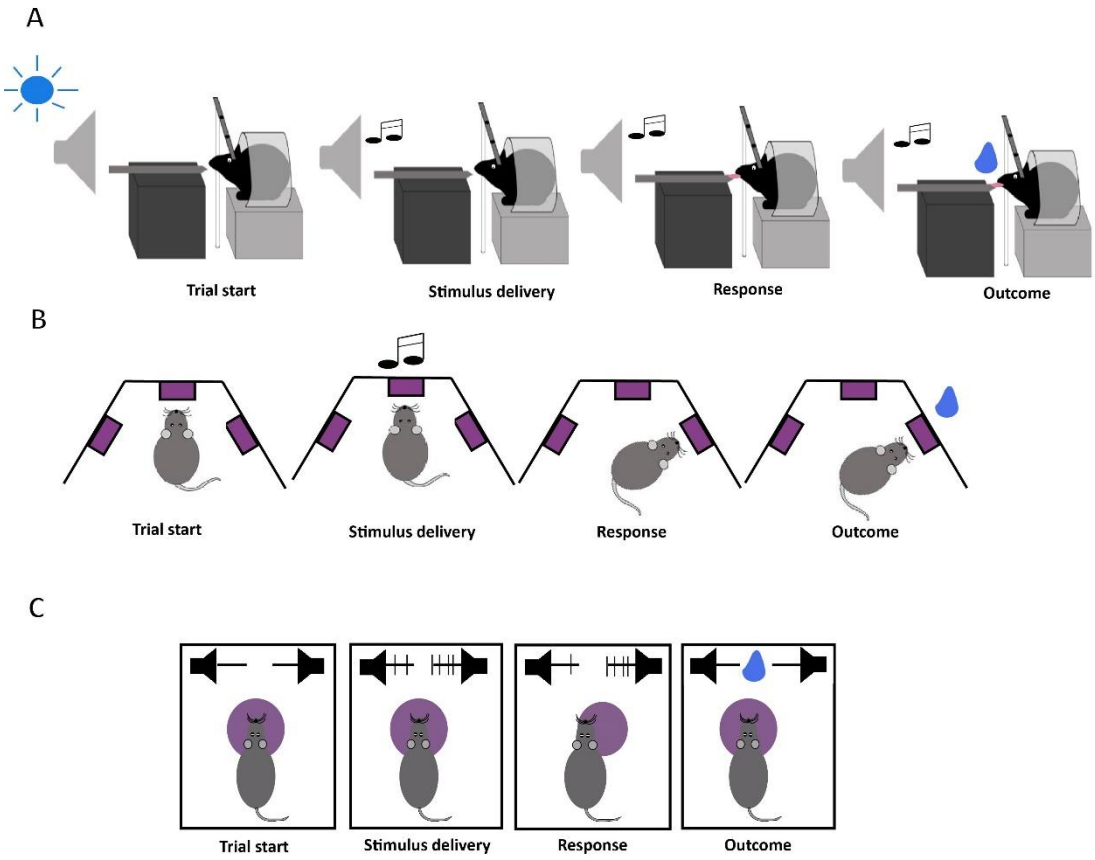


Fig.1.3. Examples of behavioural tasks in rodents

A- Go/No-Go task. Head-fixed conditions

Cue indicates start of a trial. Stimulus is presented. To obtain the reward animal has to perform an action during the presentation of the stimulus

B- Two- alternative choice task. Freely moving animal

Animal starts trial by poking the central port. During stimulus presentation animal has to decide which out of two possible stimuli was presented (e.g. low/high frequency). To obtain reward the animal has to choose port linked to a specific stimulus

C- Two-alternative forced choice task. Head-fixed conditions

To start the trial animal has to be placed in the centre of the tracking-ball. Two stimuli are delivered simultaneously from 2 speakers (e.g. higher/lower frequency). Animal has to move tracking-ball in the direction of the target stimuli. On correct trials reward is delivered

1.4.2. Behavioural paradigms - summary

Aforementioned examples prove that rodents are a valuable animal model for studying perceptual decision-making. Head-fixed paradigms, successfully used in different sensory systems, are constantly being refined and creative approaches allow for implementation of cutting-edge techniques for monitoring and modulation of neural activity during the behavioural task. The available task designs have both their advantages and limitations and those should be taken into consideration while choosing the appropriate design for goals of experiment. There are also general parameters which appear in all of types of described tasks and which values are important for the outcome (training duration, reward application).

In order to collect viable results, it's vital to assess factors such as motivation, response bias or perceptual criterion. Also, it's worth to bear in mind the significance of parameters such as training duration or choice of either reward or punishment as the motivational factor of the task (Hanell and Marklund, 2014).

Behavioural training in rodents is significantly shorter than in primates (Carandini and Churchland, 2013) but it still demonstrates various caveats. Prolonged training period may lead to overtraining resulting in occurrence of uncontrolled plasticity, whereas too short training often results in high levels of variability in the animal's performance between sessions as well as often presence of errors which are hard to interpret (Carandini and Churchland, 2013). Choice between reward/punishment motivation is a trade-off between lower stress levels and shorter training period (Carandini and Churchland, 2013).

1.4.3. Temporal expectations

1.4.3.1. Overview

Our understanding of processes taking place in the brain still have various gaps. Fortunately, with numerous cutting-edge techniques emerging in recent years and an intense focus on mechanisms underlying diverse brain functions, we are able to create and test new hypotheses providing us with deeper insight. However, one of the effects of such actions is that some of our main hypotheses and based on them models need serious modifications. A clear example of this is that for many years we

used to consider the brain a rather passive representative of the outside world. This view, after gathering some new data, switched to the notion of an interpreter. At present, acquired knowledge tends to indicate the role of the brain as a predictor that shows a proactive attitude in dealing with the surrounding world. By evaluating data from past experiences spanning over a broad range of timescales, the brain prognosticates upcoming possible events relevant to behavioural aims and, combining this with information about the motivational state of an animal, it shapes responses of perceptual and motor systems. It has been proven that encoding expectations about position, identity or other basic characteristics of an event leads to reconstruction of neural processing at several stages (Nobre, 2004). Those predictions incorporate not only information about content but also about timing of an event (Nobre et al., 2007). Time is an indispensable characteristic of the vast majority of events that occur around us. Its importance relates to all sensory modalities but seems to be especially prominent in the auditory domain (Nobre, 2004; Schroeder and Lakatos, 2009). Humans, as well as other animals need the ability of measuring elapsed time not only to improve their performance but most importantly in order to survive. In recent years we are witnessing growing interest in the mechanisms underpinning time-related information processing in the brain, both for subsecond and suprasecond scales. Due to the fact that brain circuits and neural mechanisms seems to be heavily pending on time scales and sensorimotor character of particular behaviours, many techniques as well as animal and human models have been engaged in exploring this matter. The temporal aspect of events is of high importance for any behaviour, no matter the time range. It has been hypothesized that mechanisms responsible for processing of time-related information span over scales covering 12 orders of magnitude. One way to divide them is along ranges of: microsecond scale, millisecond scale, seconds-minutes scale and circadian rhythm (Merchant and Lafuente, 2014). Timing of periods which duration last between approximately 200- 1000 ms plays important role in a broad set of behaviours. Also, coordination of precise movements occurs in this range (Merchant et al., 2013). Various animals present efficient domination of estimation of time onsets and offsets

according to the signals coming from the environment. As processing of time in this range is crucial for so many diverse events, the underlying mechanisms are highly complex and still far from being understood in detail. Elapsed time is also processed on the seconds-to-minutes scale, which shows dependency on consciousness and cognitive control. Here we can place activities like decision-making (Brody et al., 2003) or sequential motor performance (Bortoletto et al., 2011). Of note, processing at this stage works together with attention and memory which can modulate the final outcome.

1.4.3.2. Timing – main concepts

The current strong interest of neuroscientists in the topic of time encoding is not an interest in a novel topic, but rather a resumption of studies initiated as early as in the late nineteenth century (Fraisse, 1984). The attention to the neural underpinnings of timing is, however, a more contemporary matter which originates around 1980. Although many hypotheses concerning time encoding were formulated, we still can't claim with certainty that any of them is fully correct (Merchant and Lafuente, 2014).

The notion of timing in the frame of neuroscientific approach can be divided on many levels. The basis for that lies in the hypothesis that, most probably, timing related to intervals of different lengths (milliseconds-minutes) is supported by different brain structures and operates using distinct mechanisms. On the whole, the general question around which many theories were created is if the brain possess one ubiquitous mechanism to deal with time independently of task requirements, or does it distribute information locally according to the function of a given area (Macar et al., 2002; Ivry and Spencer, 2004; Michael D. Mauk and Dean V. Buonomano, 2004). This dilemma provokes more detailed inquiries such as: Do different structures process time within different time scales (Ivry, 1996; Lewis and Miall, 2003)? Is hemispheric lateralization involved in time processing (Coull and Nobre, 1998; Lewis and Miall, 2006)? Is timing related to perceptual or motor information encoded by the same mechanisms (Schubotz and Von Cramon, 2001; Lewis and Miall, 2003)?

One way to divide concepts dealing with timing, and related to them mechanisms, is by evaluating if there is a task requirement of presenting an evident assessment of duration. This leads us to the concepts of explicit and implicit timing.

In explicit timing, where either stimulus duration or interstimulus period duration is estimated, the subject can either compare intervals or express the difference using sustained, delayed or periodic motor act. Explicit timing is involved in tasks that require exact estimates of elapsed time (Coull and Nobre, 2008).

Implicit timing on the other hand is a by-product in tasks where temporal information is not crucial. Nevertheless, data about time is encoded as one of the features of sensory stimuli or motor responses. Mechanisms based on time-related features may get activated thanks to extraction of information included in motor execution or stimulus presentation rate. Implicit timing coded as a by-product of motor output is defined as emergent timing (Zelaznik et al., 2002; Spencer et al., 2007), whereas when encoded as predictability of perceptual input it's called temporal expectation.

1.4.3.3. Temporal expectations and hazard functions

The response of an organism to a sensory stimulus that is awaited tends to be faster and more accurate than if it appears at unforeseen intervals (Niemi and Näätänen, 1981). Such a response is called temporal expectation, implicit timing (Praamstra et al., 2006), anticipation of event timing (Janssen and Shadlen, 2005) or future oriented attending (Barnes and Jones, 2000).

Temporal expectations undergo constant dynamic processing partially due to their interaction with other factors important for ongoing and planned behaviours so that the preparedness of the system for coping with sensory stimulation would increase.

Temporal expectation is narrowly related to hazard function which is the conditional probability of an event occurring at a specified time given that it has not yet occurred (Luce, 1986). In practice, this translates to an increase in our certainty of an event taking place proportional to the passage of time which can lead to either faster response (Niemi and Näätänen, 1981) or even quicker perception (Vangkilde et al., 2012).

The impact of hazard rates on numerous kinds of behaviours is well documented. It affects both motor-related behaviours: anticipatory saccades (Kingstone and Klein, 1993), manual responses (Nickerson, 1965) as well as perceptual judgements: enhancing thresholds for orientation, luminescence and stereoscopic discriminations (Lasley and Cohn, 1981; Westheimer and Ley, 1996). At the same time it has been demonstrated that hazard-rate manipulations have an influence on the speed of encoding items into visual short-term memory (Bundesen, 1990; Bundesen et al., 2011) rather than time-related threshold for perception.

In a study using manipulations of fixed foreperiods together with temporal cueing paradigm, it was shown that temporal expectations shape the duration of stages like target encoding and response preparation but don't modulate significantly the response threshold (Jepma et al., 2012). Those results show considerable discrepancy with those published by (Cravo et al., 2013) Cravo et al. in 2013 and by Rohenkohl et al. in 2011 (Cravo et al., 2011). These differences clearly demonstrate that further investigation with implementation of various protocols for mnemonic, perceptual and motor requirements is needed. Nevertheless, it's already legitimate to state that changes imposed on hazard rates lead to alterations in information processing especially in tasks demanding perceptual discrimination or speeded responses (Nobre and Rohenkohl, 2014).

Foreperiod, which is the notion standing for time gap between warning and imperative signals is a crucial determinant of response speed. It has been shown that limiting changes in foreperiod variability lead to acceleration of detection and discrimination responses (Woodrow, 1914; Klemmer, 1957). This has been noticed among others during smooth-pursuit behaviour (Barnes et al., 2005) or in tests measuring saccade latencies (Kingstone and Klein, 1993). With stimuli taking place at isochronous, anticipated moments, perceptual judgements are magnified (Jones et al., 2002; Doherty et al., 2005) and the accuracy declines with stimuli occurring too early or too late comparing with the prediction (Barnes and Jones, 2000). To further investigate the impact of different foreperiods types on reaction parameters Coull et

al. (Coull et al., 2016) looked at differences between prior probability (based on fixed foreperiods) and posterior probability (evolving, based on hazard function). In the cued reaction time task participants' RTs were measured on trials with one out of 4 either predictable or variable foreperiods. The fMRI confirmed that the responses were faster in prior probability condition, confirming again the known fact that fixed foreperiods speed up the response. However, for longer foreperiods RTs were faster only in posterior, and not prior probability condition, showing the benefit of hazard function only in evolving temporal predictability. The applied fMRI analysis allowed to associate those results with some of the cortical structures. The authors showed that the left inferior parietal cortex was activated in both prior and posterior temporal probability designs, whereas right inferior frontal cortex can be associated only with the evolving dynamic present in trials with foreperiod guided by hazard function (Coull et al., 2016). The differences between fixed and hazard-function dependent foreperiods have also been seen in other studies (Mento et al., 2015; Mento and Valenza, 2016) where, using EEG, they observed activation of left-lateralised premotor-parietal circuits after fixed cues, whereas prefrontal activity was predominantly visible after evolving foreperiods. The above-mentioned study by Coull at al. showed, similarly to other studies, that in fixed foreperiods comparing to variable ones, RTs are slower at longer periods of anticipation for the target stimulus (Klemmer, 1957; Niemi and Näätänen, 1981). Already, Gibbon et al. (Gibbon, 1977) observed that longer duration of awaiting the stimuli introduces greater variability of reactions. Due to that, even in fixed foreperiods when they are longer, the accuracy of estimation of stimulus onset will be dropping leading to more variable RTs (Piras and Coull, 2011).

The whole-brain fMRI analysis used in the study by Coull et all (Coull et al., 2016) gave an insightful view on the role of the parietal cortex in managing various foreperiods. They observed that fixed foreperiods activated both the left and right superior parietal cortices together with left inferior parietal cortex. Additionally, in contrast to inferior parietal cortex and intraparietal sulcus, where activation was left-lateralised, in superior parietal cortex activation was bilateral. Symmetrical activation of this

structure was previously observed for fixed contrary to evolving cues (Coull et al., 2013). This data implicates that the role of the left inferior parietal cortex is important for temporal prediction regardless of the type of foreperiod involved (fixed or evolving) (Coull et al., 2016). It has been known already from studies in primates that parietal activity indicates sensory evidence accumulation prior to behavioural response (Roitman and Shadlen, 2002; Kiani et al., 2008) and that this activity reflects hazard function (Janssen and Shadlen, 2005). As far as the right inferior frontal cortex is concerned, it seems that this structure is active during dynamic updating of temporal predictions related to hazard-function based foreperiods (Coull et al., 2016). This data is in agreement with previous studies (Stuss et al., 2005; Vallesi et al., 2007; Bueti et al., 2010).

The fact that rhythmic, anticipated stimuli enhances perceptual judgements still after two periodic cycles of temporal interval (Jones et al., 2002; Doherty et al., 2005) makes it legitimate to suspect that temporal expectations are supported by oscillatory mechanisms (Jones, 2004). However, despite the fact that temporal predictions are often based on regularities present in occurrence of various stimuli, they not necessarily equal to entrainment to periodic sensory input. In addition, while examining the behavioural outcomes it remains unclear if improvements in accuracy and reaction time are supported to the same extent by temporal predictions and periodic stimulation. Enhancement in efficiency of sensory systems, seen as changes in activity of selected neural populations, due to entrainment of neural oscillations to rhythmic stimulation, have been observed in various studies (Large and Jones, 1999; Schroeder and Lakatos, 2009; Cravo et al., 2013). In parallel to that, other experiments gave examples of temporal predictions arising from non-rhythmic inputs (Cope et al., 2012), which creates a possibility that the mechanism behind temporal predictions in some cases is not supported by oscillatory entrainment. In addition, it's possible that temporal predictions are merged with content-based predictions while the effects of rhythmic stimulation may not be included in this equation (Nobre et al., 2012). In various experiments investigating temporal predictions, both reaction times (Lakatos et al., 2008; Lange and Röder, 2010) and perceptual accuracy (Jones et al.,

2002; Lawrance et al., 2014) were taken into account. To check if both parameters are guided by the same mechanism, Morrilon et al. (Morillon et al., 2016) used a paradigm based on near-threshold detection of auditory signals embedded in streams of sounds. They confirmed that both accuracy and reaction time improve when stimuli are delivered in rhythmic pattern (Jaramillo and Zador, 2011; Cravo et al., 2013) which suits the notion of oscillatory entrainment that was proposed as a factor enhancing sensory processing (Schroeder and Lakatos, 2009). Their results indicated that although reaction time is influenced by periodic stimulation, the accuracy of responses depends on temporal predictions regardless of their rhythmicity (Morillon et al., 2016). They raised a hypothesis that there may be a mechanism guiding temporal predictions that is not based on oscillatory entrainment and that could bind together seemingly diverse results, including temporal predictions based on symbolic cues (Nobre, 2001), hazard functions (Cui et al., 2009) or isochronous streams of events (Lakatos et al., 2008). They proposed an attention-based mechanism related to sensorimotor synchronization (Repp, 2005; Repp and Su, 2013) capable of aligning the phase of ongoing activity with optimal phase of signal processing (Schroeder and Lakatos, 2009). Apart from that, they pointed in the direction of motor system as an important element in the mechanism guiding sensory processing enhancement (Fujioka et al., 2012). In the case of temporal expectations, inferior parietal and premotor cortices are action-related areas which take part in coding both exogenous and endogenous temporal expectations. It's worth stressing that action circuits have a role in both motor and perceptual tasks, yet there may be some level of specialization in different parts of the circuit. For example, the ventral premotor area deals with perceptual discrimination of stimuli (Schubotz and Von Cramon, 2001; Schubotz et al., 2003; Grahn and Brett, 2007), while the dorsal part is involved when predictable stimuli demand a speeded motor response (Jones et al., 2002; Praamstra et al., 2006).

The fact that action-related structures get activated by perceptual temporal expectations, although may seem confusing at the beginning, actually demonstrates

that one of the crucial tasks of temporal expectation is to improve up-coming motor performance.

Electrophysiological data from primates provides evidence for the impact temporal expectations have on neural firing patterns in motor areas. A synchronization in firing can be seen in the primary motor cortex in the vicinity of the anticipated time of the go-signal (Riehle, 1997).

Also, experiments carried out in humans show that temporal expectation influences motor-related activity. As mentioned previously, action-associated networks, such as premotor and inferior parietal areas have been proven to take part in temporal expectation (Coull et al., 2000) thanks to neuroimaging studies. Also, lesion studies and TMS contribute to our understanding of the matter, showing the involvement of the right prefrontal cortex in foreperiod effects (Vallesi et al., 2007).

On the other hand, there are also studies showing that as far as temporal expectations are concerned, cognitive and perceptual stages of processing can be altered autonomously, without motor surveillance (Correa et al., 2005).

However, above studies were mainly tested on human subjects, meaning more complex behavioural paradigms were implemented, which could combine activity underlying various processes. As the design of paradigm, chosen parameters, animal model and techniques used for investigation all contribute to obtained results, further studies are needed so that we could gain a more complete understanding of mechanisms guiding temporal prediction (of different categories).

1.4.3.4. Time encoding mechanisms - major hypothesis

To date, several diverse models concerning encoding the time by the brain were presented. The two mostly vital issues appears to be 1) whether time is represented by centralized (Ivry, 1996; Ivry and Spencer, 2004; Buhusi and Meck, 2005) or distributed neural system and 2) does a system designed especially to process time-related information (Ivry and Schlerf, 2008; Buonomano and Laje, 2011) exists?

The hypothesis of dedicated timing mechanisms is opposed by the conviction that time is encoded intrinsically within the circuits which at the same time, and by no means less importantly, process other features of stimuli (Karmarkar and Buonomano, 2007). That means that although neural processes do occur in time, they may not be coding it. If this is a case, what could have been perceived as a mechanism devoted to dedicated timing, in reality, could be nothing more than processing other stimuli features. Also, it's noteworthy that time spanning through various scales could be encoded in different manners (Buhusi and Meck, 2005).

1.4.3.5. Temporal expectations – studies in rodents

Among other structures, the auditory cortex is an area vulnerable to acoustic regularities which help to improve performance. It is already known that, for example, cortical neurons show stronger response to oddball stimuli even when it's not indispensable for task completion (Ulanovsky et al., 2003). Also, expectations of alteration in task-relevant sound frequency lead to modulation in auditory cortex single neurons activity (Fritz et al., 2003). Yet, our knowledge about the influence of temporal expectation on the auditory system originates mainly from human studies, which obviously creates many limitations. Zador et al. in an article from 2011 tried to look for changes in neuronal activity caused by temporal expectations. Using behavioural study in rats, they have shown that temporal expectations result in changes in neural activity at the level of the primary sensory cortex. In addition, what is extremely important is that those changes are reflected in better performance during a behavioural test in an auditory two-alternative choice task. In this task, rats had to both detect the target – the carrier frequency of a frequency-modulated target sound which detection was hindered by pure tone distractors and discriminate the target frequency from 2 variants. The outcome of animal's actions (both its reaction time and accuracy) in this case is determined by its expectation about timing of target presentation. In the same study the importance of the auditory cortex for the task was evaluated. By temporal and reversible inactivation of the auditory cortex by muscimol, a GABAA receptor agonist, it was confirmed that its role is of high significance for the task as its inactivation leads to a considerable decrease in

performance. The basis of performance modulation by temporal expectations were studied using electrophysiological recordings from single neurons of the primary auditory cortex. The results show that responses to tones that directly precede a target are enhanced. This phenomenon was also observed for LFP. Further investigation showed that an increase in evoked response for expected targets occurred only when the target matched a neuron's preferred frequency (Jaramillo and Zador, 2011). In earlier studies, it has been already shown that an increase in motor preparedness leads to enhanced speed (Nobre, 2001) but improved accuracy is typically associated with perceptually demanding tasks (Nobre et al., 2007). The Jaramillo and Zador study shows that valid temporal expectation results in improved sound processing, which could also be related to increased motor preparedness (Jaramillo and Zador, 2011).

1.4.4. Role of attention on decision-making

Auditory attention is another important factor when it comes to effectiveness of performance. It can be divided into bottom-up attention responsible for choosing salient signals from environment (Niwa et al., 2012a) and top-down, a task-dependant attention that enhance accuracy and reaction time of responses. It directs cortical processing towards goal-directed actions (Scott et al., 2007).

A study in cats (Zhong et al., 2017), using tone-detection task demonstrated that, while the primary auditory cortex was not significantly influenced by attention, responses in the posterior auditory field and amygdala were enhanced (improving performance), whereas those in striatum were suppressed. Moreover, firing rates of A1 and PAF in the task decreased during licking response. The above results suggest that influence of attention may be diverse in structures, or parts of a structure, performing different functions (Zhong et al., 2017). These results go along with previous studies in animals which also showed diversity of results depending on the investigated structure. Although many studies showed increased neural activity related to animal's engagement in a task (Scott et al., 2007; Niwa et al., 2012a), other demonstrated suppression (Otazu et al., 2009) while the older studies have not

recorded any differences between active and passive listening (Hocherman et al., 1976).

In attention, as in any other aspect related to perceptual decision-making, behavioural design is one of the key factors. Depending on it, during tone-detection task neural responses could be either enhanced or decreased, as shown in cats (Dong et al., 2013; Zhao et al., 2015). Studies in ferrets pointed out the role of yet another factor - behavioural context (reward vs punishment) which can also provoke neural dynamics of opposite sign (David et al., 2012).

1.4.5. Brain regions implicated in (auditory) decision-making in rodents

1.4.5.1. Higher brain regions implicated in (auditory) decision-making in rodents

While looking for structures involved in decision-making in rodents, experiments are often based on previous work in non-human primates. Although it may be a good starting point, many things have to be kept under consideration. Apart from the difference in selected modality (monkey studies are predominantly based on paradigms exploring vision), the manner of dealing with electrophysiological signal is also different. Whereas in monkeys, neurons are often pre-screened for selectivity, and target is placed in the centre of response field, in rodents screening is performed post-recording and the signal is obtained from multiple cells simultaneously (Hanks and Summerfield, 2017).

In rats, the posterior parietal cortex (PPC) and frontal orienting fields (FOFs), which are respective counterparts of monkey's PPC and FEF, display a signal-dependent build-up of neural signal during accumulation of decision evidence in auditory tasks (Brody and Hanks, 2016). Contrary to experiments in monkeys, which emphasize similarities between PPC and FEF in decision coding, rodent-based experiments revealed significant differences between parietal and frontal tuning curves, representing evidence accumulation (Hanks et al., 2015). Those tuning curves, showing impact of single cell response on cumulative decision variables, were created by linking the state of accumulator on a given point of a trial (in applied frequency direction discrimination task, the stream of stimuli was known for each trial) with neural signals. In rodents, while dependency between accumulator values and spike

count is almost linear in PPC during evidence accumulation, neurons in frontal cortex appear to encode the signal in more categorical way (Hanks et al., 2015). This suggest that FOF may present more step-up dynamic than PPC.

Data collected both from mice (Li et al., 2016), in the anterior lateral motor cortex, and rats (Hanks et al., 2015), in FOF, that displays strong choice-related signal indicates that prefrontal cortex might be a part of circuitry responsible for perceptual decision-making. On the other hand, in various rodent studies, PPC failed to impact significantly on decision-making (Erlich et al., 2015) even after its pharmacological inactivation or optogenetic modulation (Raposo et al., 2014). Similarly to studies in macaques, those results position PPC in an auxiliary, not causal role, alluding that this structure probably uses evidence accumulation to help in the process of decision-making, but only alongside other structures (Znamenskiy and Zador, 2013).

1.4.5.2. The involvement of subcortical brain regions in (auditory) perceptual decision-making

Auditory stimuli, after reaching the cortex, is processed and sent to various areas in both the cortex and subcortical structures. Those perception and response related structures, connected with primary auditory cortex by mostly non-overlapping pyramidal neuron populations, include the secondary auditory cortex, parietal cortex inferior colliculus and striatum. The striatum is fed with topographically organised signals from the cortex and may be playing an important role in controlling motor responses by sensory cortex (Znamenskiy and Zador, 2013). Indeed, a study of auditory discrimination learning in rodents where, using optogenetics, the authors marked corticostriatal connections of spatially non-overlapping low and high-frequency projections that underwent selective potentiation in the learning process, indicated selective strengthening of corticostriatal synapses as possible process in which motor responses are selected on the basis of sensory representations (Xiong et al., 2015). Prefrontal and motor cortices send input to striatal areas related to a variety of cognitive functions such as decision-making (Ding and Gold, 2010), learning (Pasupathy and Miller, 2005) or action selection (Kimchi and Laubach, 2009). It is also known that the striatum, its ventral part, encodes relation between stimulus and reward (Goldstein et al., 2012). Striatum has been shown to influence the superior

colliculus, a structure involved in decision-making in two-alternative choice task (Felsen and Mainen, 2008). Encoding of dependency between stimulus and response may be taking place due to the plasticity of corticostriatal connections (Kreitzer and Malenka, 2008). A study in Mongolian gerbils, in auditory-cued discriminative Go/No-Go avoidance task, demonstrated the significance of strengthening of the functional coupling between the auditory cortex and ventral striatum for goal-directed learning (Schulz et al., 2016).

The auditory cortex projects to a particular region of the striatum (McGeorge and Faull, 1989) which is one of its major long-range destinations. The role of striatal areas directly connected to sensory cortical areas is not yet fully described. Znamenskiy and Zador (Znamenskiy and Zador, 2013) showed however, that in an auditory frequency discrimination task, corticostriatal neurons in the auditory cortex relay signal on which basis rats make behavioural choices. As those cells are not numerous, yet their inactivation with optogenetics had impact on behaviour, it seems that they're of importance for auditory discrimination. As known from monkey studies, more neurons are involved in transmission of task-relevant signal than is responsible for psychophysical performance (Britten et al., 1992).

1.4.5.3. Role of primary sensory cortices in perceptual decision-making in rodents and other animals

For many years, processing sensory inputs was seen as step-by-step, hierarchical process where early stage cortices would transform information concerning stimuli features, whereas a more abstract picture would emerge only after involvement of higher order areas. This view represents the classical bottom-up idea of information processing (Felleman and Van Essen, 1991). Nonetheless, we are gathering more and more evidence on the engagement of higher-order factors at the stage of early sensory cortices proving that, in reality, the flow of information is not unidirectional (Kondo and Kashino, 2009; Schneider and Mooney, 2015). Involvement of agents such as attention, temporal expectation, prior experience and task-related motivation seem to interfere with the neural activity already at early stages as shown in studies from various species (Brosch, 2005; Fritz et al., 2005; Janssen and Shadlen,

2005; Jaramillo and Zador, 2011; Snyder et al., 2012; Francis et al., 2018; Irvine, 2018). It has been shown that the behavioural state of animal/human and level of their engagement in the task modulates the activity of the auditory cortex (Otazu et al., 2009). In A1 it has been observed (in ferrets and cats) that engagement in a task leads to modulation of neural activity compared to passive listening - missed/correct rejection trials (Fritz et al., 2003; Lee and Middlebrooks, 2011). Moreover, spatial tuning of A1 neurons has been observed, possibly based on suppression of non-preferred locations, (Lee and Middlebrooks, 2011), while Fritz (Fritz et al., 2003) observed facilitative changes (increased excitation/reduced inhibition around target frequency) in spectro-temporal receptive fields during tone detection.

The dynamic interchange of information between different levels of processing chain can be seen even in the salience of a stimulus which, apart from its physical values, is analysed for its relevance for current or an upcoming behavioural process (Veale et al., 2017).

Additionally, various data was gathered on the involvement of early stages cortices in multisensory processing of information. Previous theories were placing multisensory integration in higher order cortices. Currently, it seems that, for example auditory signals can modulate activity of the primary visual cortex (Ghazanfar et al., 2005; Ghazanfar and Schroeder, 2006; Petro et al., 2017).

Detection of sound is crucial in a plethora of aspects, allowing proper functioning in environment. It has been shown that auditory attention can positively modulate responses to task-related stimuli already at the level of A1 by either suppression or facilitation of responses to a behaviourally valid stimulus (Fritz et al., 2003; Atiani et al., 2009; Kato et al., 2015; Kuchibhotla et al., 2017). This modulation can originate from various factors that are not intrinsic features of stimulus, such as: motivation, attention, task difficulty or temporal expectation (Fritz et al., 2003; Atiani et al., 2009; Jaramillo and Zador, 2011). Although attention can explain the differences between engagement in task and passive listening, it’s not enough to decipher the process of decision-making, which is the ultimate stage of every behavioural task. For now, it

also stays unresolved how auditory attention contributes to decision-making on the level of A1 (Bizley and Cohen, 2013; Tsunada et al., 2016). It is known that in A1, in layer 2/3 (L2/3) cells are characterised by heterogeneity in their responses to sound, as well as frequency selectivity (Sakata and Harris, 2009; Rothschild et al., 2010; Kanold et al., 2014; Maor et al., 2016; Francis et al., 2018). It is suspected that this variability is caused by diverse connectivity both in L2/3 and coming from other layers (Atzori et al., 2001; Atencio and Schreiner, 2010; Meng et al., 2017). This local variability potentially can translate to differences in coding various task-related factors. To investigate this matter on the level of L2/3 of the murine auditory cortex, Francis et al. (Francis et al., 2018) used in vivo two-photon imaging in tone detection task. They established that the majority of L2/3 neurons increased their activity in response to target tones during hit trials, although a fraction showed a decrease in their activity. The activity was not correlated to frequency tuning. Further analysis showed that the task-related responses (but not motor responses related to licking) were modulating activity of small subsets of neurons, 4 or 5 neurons, characterized by sparse connectivity. The overall conclusion was that contrary to attention which modulates large neural populations, perceptual decisions are encoded by sparsely distributed, small groups of neurons. Additional analysis revealed that neural activity in A1 may also encode the timing of perceptual choice. Comparison between rewarded trials and those ending in punishment showed that latencies of response were longer during hit trials. Again, they demonstrated that this data was related to perceptual choice, not motor activity (Francis et al., 2018). Encoding of timing of perceptual response was also shown in the primary visual cortex (Levy et al., 2017).

The influence on dynamic in A1 by non-auditory factors such as trial outcome or size of reward was shown in several studies (Brosch et al., 2011; David et al., 2012; Weis et al., 2013). They indicate that the behavioural choice may be encoded in the auditory cortex alongside attention-related effects. Also, in the aforementioned study by Francis et al. (Francis et al., 2018), they found that up to 20% of individual cells carried choice related information, a result confirmed in 9/10 mice which shown that decision-cost encoding is strongly represented in A1 L2/3.

The primary auditory cortex is not regarded anymore as merely a sophisticated centre of analysis of auditory features but, thanks to recognition of various forms of plasticity present in this structure (Irvine, 2017), it's seen as a part of a network that brings together auditory and non-auditory features necessary for behavioural task completion. What can influence the processing in sensory cortices related to various modalities is perceptual learning- training in tasks based on detection, discrimination or identification of a sensory stimuli. Perceptual learning, differently to associative learning, is based on improvement in perceptual performance manifested as an increase in accuracy, decrease in reaction time or reduction in threshold necessary for task performance (Irvine, 2018). Auditory perceptual learning has been studied in humans (Wright et al., 2010) and primates (Recanzone et al., 1993). In rats, it was shown that perceptual learning influences the size of the area representing target frequency (Polley et al., 2006). Such changes weren't observed for sound intensity. Differences in results based on same stimulus but different detection task indicates the influence of top-down factors on perceptual learning. The influence of factors such as task demands, or animal's strategy of responses has been confirmed in both classical and instrumental conditioning (Irvine, 2018). It can therefore be expected that in perceptual learning, different task demands will have an effect on neural dynamics (Wright et al., 2010). Perceptual learning brings together sensory based information, attention, reward and decision-making related activity for the refinement of functionality of brain networks (Dosher and Lu, 1999).

Primary sensory cortices seem to be implicated in more complex and diverse functions than previously expected. Although this discovery is fairly recent, already an important body of evidence shows that those regions are involved in processing information related to perceptual decision-making. However, the number of factors involved, as well as differences between different paradigms and animal models/subjects used for experiments, that yield not always similar results, shows that the investigation needs to be continued.

1.4.6. Summary

Despite a broad body of evidence that over last decades we collected on perceptual decision- making, in majority basing on human and primate studies, there are still numerous questions unanswered. This crucial issue may be moved forward thanks to implementation of studies in rodents. The battery of existing behavioural tasks together with advanced techniques allowing for neural activity modulation are shedding light on this matter. Using this valuable model, we may also tackle the problem of involvement of different structures, especially primary sensory cortices which role is still largely unknown, in this complicated process. It has already been proven that rodents are a good model for examining temporal expectations, attention, prior experience, task related motivation or processing of multisensory signals– factors deeply influencing perceptual decision-making. The realisation that primary sensory cortices are in reality involved in more advanced information processing than previously thought, was important for deepening the studies of many processes, including perceptual decision-making. Combining rodent models with the study of functions of primary sensory cortices is certainly going to provide us with better insight into this process that is vital for survival of animals.

1.5. Techniques used to monitor and manipulate neural activity in rodents

1.5.1. Electrophysiology - Recording neural activity

1.5.1.1. Overview

To fully understand rules guiding behavior, one must be able to decipher the neural activity of the brain that is encoding and controlling it. The capability to collect data depicting electrical activity of both single neurons and large neural assemblies for decades has been a pivotal means of deepening our knowledge about the neural basis of behavior. The technique enabling this is electrophysiology and it has come a long way since its early foundations, being constantly modified and enhanced, allowing for collection of more precise data and studying not only properties of a singular cell, but also dynamics of cell populations in selected structures as well as coherence in between different brain areas.

The origins of electrophysiology date back to the 17th century and neuromuscular preparation from frog's leg, where muscle contraction was observed after nerve stimulation. This work was carried out by a Dutch scientist, Swammerdam, and further developed, in more detailed way, by an Italian scientist, Galvani, who first demonstrated propagation of action potential. He created the theory of electrical excitation, describing the fact that neural tissue is by default in a rest state which changes with occurrence of external stimuli that generates electrical signals. He was also claiming that "animal electricity" originates from disequilibrium in accumulation of positive and negative charges on different sides of muscular or neural membranes. Galvani's huge contribution to the field boosted the interest of other scientists and the technique has been expanding throughout the following centuries (Cobb, 2002; Verkhratsky et al., 2006).

Over the past few decades, advances in technology allowed for appearance of a plethora of techniques enabling both *in vitro* and *in vivo* recordings from not only single cells but also large cell assemblies.

In vivo extracellular recordings allow for investigation of both small and large levels of brain organization. Placing an electrode next to a cell results in obtaining data about activity of a single neuron, whereas electrodes placed within a small tissue volume allow for analysis of neural population dynamics, captured as local field potentials (LFPs) arising from superposition of synaptic currents originating from a group of neurons. The amplitude of voltage of LFPs decreases with the increase of distance between the source and recording site. Also, the amplitude and dynamic in time pattern of LFPs relies on the weight of the contribution of diverse current sources and are further altered by different properties of brain tissue (Buzsáki et al., 2012).

1.5.1.2. Electrodes and probes

As it has been suggested various times, the cortex architecture comprises of repetitive elements such as columns, barrels or lamellae. They have been proven to show comparable anatomical and functional properties (Mountcastle, 1997).

Although new approaches like optical imaging, i.e. two-photon calcium imaging (Dombeck et al., 2007; Andermann et al., 2010), are appearing, electrophysiology is still a core method when it comes to monitoring of neural activity, especially in deeper layers and subcortical structures, exploration of which still presents a hurdle for various other techniques (Csicsvari et al., 2003). In the past, simultaneous recordings from numerous cells were mainly made using multiple wire electrodes. Although this method permitted for isolation of single cells, it had many limitations, mainly artifacts occurring during animals' movements and possibility of mechanical stimulation of a neuron, which influences its properties. Also, this method resulted in considerable tissue damage (Chicurel, 2001).

A step forward was an introduction of tetrodes. Tetrodes are four spaced wires. Their main advantage is that as their tips are separated in space, allowing for a precise triangulation of distance (a spike is a function of distance between electrode and neuron) in volume, single cells may be isolated with high accuracy. Additionally, tetrodes, comparing to previously used electrodes, produce less tissue damage, give higher yield of units and are characterized by better mechanical stability. Moreover, as the tip of the electrode does not need to be localised next to the cell, long-term recordings in behaving animals are feasible (Buzsáki, 2004). Of course, what needs to be bared in mind is that although in theory a single electrode can record signal from ~1000 neurons, in practice many of them may be silent, damaged or their response may be too low in amplitude. Also, only a fraction of neurons can be reliably separated. That leads to a conclusion that what is needed is a probe which, while yielding numerous recording sites, would not cause significant damage to the tissue (Buzsáki, 2004).

The next generation probes trying to fulfil those requirements are silicon probes, based on a micro-electro-mechanical system. Their advantage over wire electrodes is that, although they produce similar level of tissue damage, they offer a rise in the number of recording sites (Kipke et al., 2008). Moreover, having all the qualities of tetrodes, they are smaller in size. A very important aspect is that recording sites can

be arranged over longer distance, enabling parallel recordings from different cortical layers. Implementation of a multi-shank silicon probe may provide a recording from even a hundred neurons, which signals can be separated during analysis (Csicsvari et al., 2003). Rapid development seen over recent years in the area of production and control of probes resulted in providing researchers with a battery of devices which size, shape and architecture can be tailored to meet requirements of a particular study. Currently available probes can be tailored to the neural density and local circuit structure of a selected brain area (Buzsáki et al., 2015).

1.5.1.2.1. Electrophysiological data analysis – pre-processing and spike sorting

In the analysis, neurons can be separated due to their spatial location. There are four major steps leading from neural activity recording to the identification of spikes associated with a particular cell: filtering, spike detection, feature extraction and clustering (Quiroga, 2012).

The analysis starts with high-pass filtering and thresholding which function is to reduce background noise and enhance the quality of data (Hazan et al., 2006). Then, detection of spikes takes place, usually by amplitude thresholding. A spike is described by a vector comprised of a sequence of voltages in time (Hazan et al., 2006). Feature extraction is another step. It aims to maintain only those feature that may be helpful for classification (amplitude, width and energy of spikes; extracting principal component; selecting wavelets coefficients) (Quiroga, 2012). Finally, during clustering, spikes are assigned to the neurons that produced them, forming clusters representing activity of different putative neurons. Of course, activity of each neuron is inseparable from the dynamic of the circuit (neural network) that encodes various behaviors. This said, the final aim of spikes analysis is to unravel those dependencies (Hazan et al., 2006). Assigning spikes to the neurons that produced them is pivotal for any electrophysiological analysis carried out on single unit recordings. While designing an experiment, it's worth thinking about a trade-off between the amount of detected neurons and the expected level of mistakes in analysis. In both single unit and population recordings, occurrence of both false-positive and false-negative

errors is not rare. The former mistake, based on assigning spike to a neuron that was not responsible for its production, leads to incorrect reasoning concerning information encoding. The latter, where spikes produced by a neuron in question are omitted, tends to have milder consequences, but only when the error is random. While recording from a neuronal assembly, both errors often co-occur which may result in invalid judgements about population coding and patterns of correlation. Also, if a cell subtype is not correctly identified, this again may lead to incorrect evaluation of population dynamics (Harris et al., 2016).

1.5.2. Manipulation of brain activity and related to it behaviour

In the last decade new, cutting-edge technologies allowed for very precise manipulation of selected brain structures and cell types.

Among those technologies, two have been proven to be exceptionally efficient and diverse, finding application in multiple areas of neurobiological research. Optogenetics, a technique using microbial opsins, which are molecules activated by light, and DREADDs (designer receptors exclusively activated by designer drugs), a second generation of designer GPCRs (G protein-coupled receptors) – one of branches in chemogenetics. Chemogenetics is an approach where proteins are engineered so that they could interact with otherwise inert chemical actuators of small molecular size (Sternson and Roth, 2014).

1.5.2.1. Short history of neural remote control.

One of the earliest endeavours to remotely manipulate neural activity was the application of photocaged capsaicin to activate the TRPV1 vanilloid nociceptor (Zemelman et al., 2003).

In this experiment, allowing for precise on/off kinetics of activity of cultured hippocampal neurons, light was used to uncage a caged capsaicin derivate. Those studies were then further carried on to map neural circuits and manipulate behaviour of a fruit fly, *D. melanogaster,* using TRPV1, $P2X_2$ and respective ligands (Lima and Miesenböck, 2005).

The next step, control of neural activity in both anesthetized and awake, freely moving mice, was accomplished using Cre-flox based expression of TRPV1 (Arenkiel et al., 2008). As TRPV1 presents various drawbacks, such as excitotoxicity of its ligand capsaicin in higher doses, changes in neurotransmission without agonist application and endogenous expression in central nervous system, another receptor from this family was tested. Crawford *et al*. (Crawford et al., 2009) showed that a mentol-gated non-selective cation channel, TRPM8, possessed characteristics lacking in TRPV1 and therefore is a more reliable tool for neural activation.

Caveats appeared as well during the quest for finding means of reversible silencing of neurons. Techniques already being in place, such as lesions and knock-outs, were highly unsatisfactory as they were neither reversible nor cell-type specific. In an attempt to overcome this issue, in 2002, Lechner et al. utilized allatostatin receptor, AlstR derived from *D. melanogaster* which he expressed in cultured mammalian neurons (Lerchner et al., 2007). This receptor couples to G-proteins G_i or G_0 and, through modulation of GIRK channel activity, leads to neural silencing. It's ligand, allatostatin, is strong and selective. Together with the fact that somatostatin and opioid receptor- ligands of mammalian receptors –are not applicable for AlstR, this characteristic make AlstR-allastatin system a useful tool for neural silencing. This system was further successfully used in mice, decreasing activity of its V1 motor neurons (Gosgnach et al., 2006), as well as in ferrets, rats and monkeys (Tan et al., 2006).

Although applicable in various species, this system is laden with drawbacks. Poor temporal resolution dependant on washing out the ligand and the need for use of invasive methods are the main ones.

Another early example of strategies for neural silencing was molecules for inactivation of synaptic transmission (MISTs) presented in 2005 by Karpova *et al.* (Karpova et al., 2005). This technique is based on reversible inactivation of neurotransmitter release by means of chemical induction of dimerization (CID) of modified synaptic proteins (Clackson, 1997). In this process, dimerizers, small

molecules, bind concurrently to two same (homodimerizers) or different (hetorodimerizers) protein modules creating homo-MIST or hetero-MIST. MISTs based on the vesicle proteins VAMP2/Synaptobrevin and Synaptophysin were proved to be capable of inducing short and reversible block of synaptic transmission in both cultured neurons and brain slices. When tested *in vivo* in transgenic mice expressing MIST exclusively in Purkinje neurons, it led to decreased performance in the rotarod test (Karpova et al., 2005).

MISTs don't change neuron's excitability like methods based on membrane channels, and are faster than those employing transcriptional induction. Strong inactivation of both excitatory and inhibitory synaptic transmission takes place within 20 to 30min. The reversal of the process was shown over periods of 1h *in vitro* and 1 day *in vivo* (Karpova et al., 2005). As in the former case, although this method was proved to be useful in remote, neurobiological studies, temporal resolution highly restricts its usage and the permeability of the dimerizers through the blood-brain barrier is unclear (Rogan and Roth, 2011).

Abovementioned approaches, though applicable under certain conditions, still did not meet criteria for non-invasive remote and bidirectional control of neural activity on a precise timescale. In the race to create a highly efficient method for remote control of neural activity, two new players appeared and those techniques are now in world-wide use yielding high-quality results and paving paths for new avenues of research.

Designer Receptors Exclusively Activated by Designer Drugs, 2nd generation of designer GPCRs, and optogenetics, using light sensitive molecules of bacterial origin are currently cutting-edge techniques for remote neural control.

1.5.2.2. Designer G Protein-Coupled Receptors -from RASSLs to DREADDs

Numerous cell surface receptors create the family of G protein-coupled receptors, characterized by a seven-transmembrane domain structure with an extracellular amino terminus and a cytoplasmic carboxyl terminus, that react when presented with a variety of ligands, just to name: peptide hormones, lipids, odorants, biogenic

amines or photons. (Spiegel et al., 1993; Baldwin, 1994; Strader et al., 1994). Pathways related to G-protein coupled receptors are present in a plethora of biological functions and their faulty processing is implicated in numerous central nervous system disorders. As GPCRs can be quite easily modulated by endogenous ligands (initiating conformational change that activates heterotrimeric-αβγ G-protein positioned on inner surface of plasma membrane) (Conklin and Bourne, 1993) and additionally display constitutive- ligand independent- activity, over last couple of decades there has been intense research aiming to develop orthologous pair of receptors and its ligands that would overcome disadvantages of natural GPCRs and provide high spatio-temporal resolution (Nichols and Roth, 2009; Dong et al., 2010; Rogan and Roth, 2011).

This work was initiated by Strader and his group, who carried out mutagenesis of β_2-adrenergic receptor (2-AR). Despite the fact that this method couldn't be used in vivo as it resulted in being too weak, this study opened the research on rational design of drugs and genetically engineered receptors (Strader et al., 1991; Rogan and Roth, 2011).

In subsequent experiments, multiple mutations of GPCRs have been carried out leading to the creation of a family of selectively activated designer receptors. Their 1st generation, RASSLs, was based on rational design, whereas the 2nd generation, DREADDs, depend on direct molecular evolution in yeasts and is currently being widely used.

After Strader's accomplishments, the next step in designer GPCRs creation was work carried out by Coward *et al.* From 4 major classes of GPCRs, they chose $G\alpha_i$ and used G_i-coupled human κ opioid receptor. The G_i-signalling pathway provokes diminished intracellular cAMP levels through adenylyl cyclase inhibition (Coward et al., 1998). The effect of this pathway activity is dependent on type of cell in which it was initiated. In the brain it results in neurotransmission modulation (Wickman and Clapham, 1995).

Coward *et al.* created RASSLs, receptors activated solely by synthetic ligands which were developed basing on mutating amino acid residues taking part in binding endogenous ligand without interfering with residues necessary for binding of synthetic small molecule drugs. The advantage of usage of peptide receptor in developing designer receptors is that peptide ligands interact with extracellular loops of the receptor while small molecules interact with regions closer to the transmembrane domains. They selected human κ opioid receptor as a starting point. This receptor is activated by peptide hormones and a group of enkephalins and, being important for the pharmaceutical industry, it has undergone intense mutagenesis allowing for selection of the most appropriate mutations for RASSLs development (Coward et al., 1998). They created two prototypes: Ro1 and Ro2. The former, RASSL based on opioid receptor, no. 1, is a chimeric receptor that possess the second extracellular loop (EL2) of the δ opioid receptor, whereas the latter as well as δ residues contains an amino acid substitution at the top of the sixth transmembrane region. They confirmed that expression of Ro1 as well as Ro2 resulted in reduction in binding affinity (2000-fold in Ro2) and signalling to over 20 endogenous peptide ligands, at the same time keeping high level of response to small, synthetic molecule agonists. The above study showed additionally that RASSLs have potential for usage in *in vivo* studies (Coward et al., 1998).

This potential was further tested by Redfern *et al.,* who created transgenic mice expressing R_o1 in heart muscle cells in *tet-off* manner. Application of spiradoline, a highly selective κ-opioid agonist, resulted in dose-dependent reduction in heart rate in transgenic mice while not causing any effect in naïve animals (Redfern et al., 1999). However, in the same experiment, questions discussing its constitutive activity have been raised, as animals that weren't given *dox* for a period of 3 weeks died of developed cardiomyopathy and/or systolic dysfunction (Redfern et al., 2000). Sinus rhythm was however successfully restored with an injection of pertussis toxin. This data showed altogether that although R_o1 uses exclusively G_i for signalling, this signalling is still carried out even in absence of synthetic ligands.

R_o1 expression has resulted in a plethora of pathological conditions spanning across different tissues. In astrocytes, it led to development of hydrocephalus (Sweger et al., 2007) whereas its expression in osteoblasts resulted in osteopenia (Peng et al., 2008). The central nervous system is susceptible to R_o1 expression as well, as shown in a study where mice expressing this receptor, in *tet-off* system under control of human GFAP promoter and on a KOR(-/-) background, when not provided with dox developed fatal hydrocephalus (Sweger et al., 2007).

Despite the fact that abovementioned studies depict RASSLs as useful tool in modelling various human pathologies, the fact that the timing and magnitude of their effects cannot be precisely controlled, and more importantly that they display high level of constitutive activity and don't show selectivity for ligands, prevented them from becoming a widely used tool in neuroscientific research.

1.5.2.3. DREADDs -2nd generation of G Protein-Coupled Receptors

DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) are one of the latest advances in the field of chemogenetics. They consist of engineered receptors and selective, artificial ligands that, in brain tissue, are capable of transiently activating or inactivating targeted areas. The technique may be implemented through multiple strategies for gene transfer and, apart from introducing changes limited in time, it also allows for numerous repetitions of the experiment, if needed (Smith et al., 2016).

As previously mentioned, targeting natural GPCRs with selective ligands is difficult and inefficient as those proteins are expressed in various cell types building diverse tissues, so the metabolic effects of targeting GPCRs may be very broad (Wess, 2016). This problem was dealt with by designing DREADDs, which are mutants of muscarinic acetylcholine receptors that, with high efficacy are being activated by a designer ligand, an inert metabolite of clozapine- CNO (clozapine-N-oxide), a widely used atypical antipsychotic drug. CNO, according to most studies, is inert for endogenous receptors (Armbruster et al., 2007) and crosses the blood-brain barrier which enables its application via intraperitoneal injections or addition to drinking water (Wess et al.,

2013). Despite studies showing that CNO can back metabolize into clozapine in guinea pigs and humans, it was believed not to have any effect in mice and rats (Jann et al., 1994). However, a study from 2016 published by MacLaren *et al.* revealed dose-dependent effects of CNO on behaviour of naïve Long-Evans rats (MacLaren et al., 2016). Regardless of this fact, the authors are not recommending generalization of this result to other rat strains or mice. Even more recently, new concerns were raised about CNO metabolism and its ability to cross brain-blood barrier (Gomez, 2017). Although, despite those results, DREADDs are still a valuable technique, precautions must be taken during data analysis.

Recently, new classes of DREADDs are emerging that use different compounds, as in case of KORD (k-opioid-derived DREADD), an inhibitory DREADD for which the ligand is salvinorin B (Marchant et al., 2016). GPCRs, upon biding with its ligand activate heterotrimeric G proteins which were characterized within four main groups: Gq, Gi, Gs, and G12. Out of those, the first three are commonly activated by DREADDs. So far, activation of the G12 protein family by DREADDs has not been reported (Dobrzanski and Kossut, 2017). Latest evidence shows also that GPCRs may activate G protein- independent signaling related to β-arrestin- dependent signaling pathways (Wess, 2016).

1.5.2.3.1. Types of DREADDs

Gq-DREADDS augment neuronal firing and are responsible for activation of Gq signaling in both neurons and non-neuronal cells. Gq-coupled DREADDs originated on the basis of 3 different human muscarinic receptors. hM1Dq, hM3Dq, and hM5Dq were created, and currently hM3Dq is the one being most widely used (Roth, 2016). The ligand for hM3Dq is CNO which according to numerous articles does not seem to provoke any pharmacological or behavioral disruption in either mice (Alexander et al., 2009) or rats (Ferguson et al., 2011, 2013) when applied in suggested doses (0.1 – 3 mg/kg). A crucial advantage of CNO is that it rapidly penetrates CNS. It's easily distributed in the tissue, and in mice it shows at least 60min activity after ip injection (Bender et al., 1994).

The second group of DREADDs are G_i-coupled, which diminish neural activity. They are represented by hM2Di, hM4Di, and KORD. Both hM2Di and hM4Di use CNO as a ligand (Armbruster et al., 2007). The most widely used inhibitory DREADD is hM4Di which can decrease neural activity by induction of hyperpolarization by Gb/g-mediated activation of G-protein inwardly rectifying potassium channels (GIRKs) (Armbruster et al., 2007; Vardy et al., 2015) or by inhibition of the presynaptic release of neurotransmitters (Vardy et al., 2015).

The last group is G_s-DREADDs and currently it has only one representative. Contrary to formerly described groups, G_s –DREADD shows low degree of activity in transfected cells (Guettier et al., 2009).

1.5.2.4. Chemogenetics-summary

To sum up, chemogenetics is a highly useful tool for deciphering the role of neural circuits and specific cell types in both healthy organisms and under pathological states such as Parkinson's disease (Aldrin-Kirk et al., 2016), epilepsy (Kätzel et al., 2014), schizophrenia (Nguyen et al., 2014), depressive and anxiety-like behaviours (Soumier and Sibille, 2014) as well as narcolepsy (Hasegawa et al., 2014).

The most advanced version of chemogenetics, DREADDs, enables implementation of spatially and temporally specified and reversible manipulation of brain circuits or neuronal subtypes that results in either gain or loss of function at the same time being quite easily accessible from technological aspect.

1.5.2.5. Optogenetics

Optogenetics emerged from the necessity to regulate defined events in specific cell types at precise time in an intact system. These factors are of utter importance as the vast majority of events is significant only in the context of other phenomena taking place in other parts of the tissue, organism and also environment (Karl Deisseroth, 2010).

This technique, together with other single-component tools which enable perturbation within the nervous system, opened the way for new, versatile investigations about mechanisms underlying its functionality (Yizhar et al., 2011).

Already at the end of the ’70, Francis Crick speculated that light may be a key to overcome the limitations in neural systems control approaches that are encountered in ”old-fashioned” methods, such as electrophysiology or drug delivery, which lack required precision and time-resolution (Karl Deisseroth, 2010).

Optogenetics is an instrument consisting of optics and genetics that together allow for gain or loss of function during specific phenomena occurring in particular cell types of a tissue (Karl Deisseroth, 2010). The technique needs three components: 1) engineered elements that can be delivered to appropriate cell types 2) techniques for delivery of light 3) technology for combining control elements with readouts such as fluorescent activity indicators, electrical recordings, methods for validation of behavioural performance (Yizhar et al., 2011).

1.5.2.6. Optogenetics-summary

The optogenetics strategies for both activation and inhibition of neural activity has been vividly used in the last decade to elucidate the role of neural circuits and cell subtypes in a plethora of behaviours spanning across both healthy and pathological states. Usefulness of this technique has been proven in multiple species such as Drosophila (Bellmann et al., 2010), zebrafish (Douglass et al., 2008), and mice (Adamantidis et al., 2007).

The field of optogenetics is still exuberantly developing and merging with other techniques to increase efficiency and diversity of possible studies. Recently, apart from temporal precision and applicability for freely moving mammals, the single-component feature of microbial opsins has allowed for generalizable targeting. Thanks to combining the wide range of mouse lines selectively expressing Cre-recombinase in specific cell types with novel Cre-recombinase-dependent opsin-expressing viruses, such complex enterprises as controlling defined cells in freely moving mice are now possible.

1.5.2.7. Methods of expression of systems modulating neural activity

As mentioned, there are diverse methods of expressing DREADDS in targeted cells. They can be either delivered *via* viral transduction where promoter-specific adeno-

associated viruses are implemented (Ferguson et al., 2011) or by means of animal transgenesis where mice lines express hM3Dq under the control of either tetracycline (tet-off) promotor (Alexander et al., 2009) or Cre-mediated recombination (Teissier et al., 2015).

Often, a combination of abovementioned strategies is used in the form of FLEX switch, which allows Cre-mediated cell-type-specific expression in all cell types for which Cre-driver line is available (Roth, 2016). On the basis of FLEX switch, Cre-On DIO system was developed and initially, used by the Deisseroth group in the field of optogenetics for selective opsin delivery (Cardin et al., 2009). In 2011, this system was for the first time utilized as a tool in DREADDs technology (Krashes et al., 2011) and since then has been broadly used in numerous studies. In Cre-On DIO system, a recombination is performed between spots marked by lox sequences. An enzyme originating from a bacteriophage, Cre-recombinase, is capable of recognizing those sequences and performing recombination of reversely orientated sequence placed between them, leading to change of its orientation that results in commencement of transgene transcription (Sohal et al., 2009).

The increasing popularity of this system arises from its characteristics such as readily accessible rodent Cre-dependent driver lines, efficient transgene expression and importantly– high level of selectivity resulting in expression restricted to Cre-expressing cells (Sohal et al., 2009).

1.5.2.8. DREADD vs Optogenetics

Both optogenetics and DREADDs are techniques used in recent years with an aim of mapping neural circuitry or modulating behaviour via activation or inhibition of particular cell-types in selected brain areas (Wess, 2016). In some experiments, they were used alongside each other to produce strong evidence for presence of specific neural pathways responsible for multiple physiological functions (Rogan and Roth, 2011).

The major difference between those two approaches is time resolution. DREADD provokes prolonged changes in activity, lasting even over a couple of hours (Roth,

2016) whereas effects of optogenetics are much more ephemeral, spanning over millisecond timescale. Optogenetics, although more complicated in preparation has the advantage of delivering data which is reasonably easy to interpret as neural changes originate from activation of ion channels or pumps by a light pulse. DREADDs, on the other hand, may have more complex influence as it's believed to modify activity of downstream signalling pathways as well as ion channels and additionally seems to be involved in β-arrestin-dependent signalling (G-protein independent). Both techniques are highly useful tools in deciphering neural basis of behaviour and they can be used either separately, depending on temporal requirements of the experiment or concurrently to yield more detailed results.

1.6. Objectives and Hypotheses

Although perceptual decision-making has been studied for decades now, the majority of work comes from humans and primates. While they allow for usage of more sophisticated behavioural tasks, rodents are fairly capable of performing in a plethora of paradigms, additionally enabling usage of cutting-edge techniques and genetic modifications. This advantage makes them a perfect model for elucidating mechanism guiding decision-making.

Advanced mental functions such as perceptual decision-making itself, were traditionally investigated from the level of higher cortices, while sensory cortices, including auditory cortex, were seen as simple hubs for managing stimuli processing. The current stage of knowledge, acquired due to significant progress in research technical capabilities, enumerates many more complex operations in which those structures could be involved.

Overall, to investigate such a complex process several steps must be taken. Therefore, this thesis was centred around three main aims:

1) Development of a behavioural paradigm to study perceptual decision-making in mice using auditory modality. Further, testing and refinement of the paradigm,

executed by examining the influence of foreperiods on reaction times in temporal expectations task. An appropriate behavioural task creates a core framework for further research.

2) Investigation of the necessity of auditory cortex for the aforementioned task, tested by incorporation of chemogenetics. Application of this method allows for a comparison of behavioural outcomes between baseline condition and results after modulation of the functioning of the structure in question.

3) Search for neural bases of changes in behaviour, carried out by neural recordings in anaesthetised mice, during sound presentation before and after modulating auditory cortex activity using chemogenetics. Looking into neural underpinnings of a process is crucial for its full comprehension.

Each of the presented aims was related to a study hypothesis:

1) In temporal expectations task, it was presumed that an increase in the foreperiod would result in a decrease of reaction times.

2) In behavioural task combined with chemogenetics, it was assumed that CNO injections, aiming at decreasing the activity of auditory cortex, would result in a decrease in hit rate and increase in reaction times.

3) In the experiments in which neural activity was monitored and modulated it was expected, that CNO injection would decrease the level of recorded multiunit activity.

Obtained results did not provide confirmation for presented hypotheses. However, the limitations of used methods were found and discussed, pointing at possible modifications of used techniques as well as future additional studies. The importance of matters such as perceptual decision-making and involvement of primary sensory cortices in this process require further research.

Chapter 2. Materials and Methods

2.1. Overview

In this chapter animals, materials, methods and equipment utilized in experiments presented in Chapters 3-5are presented. Section 2.2. describes animals' characteristics as well as enumerates used materials, Section 2.3. illustrates surgical procedures, stages of behavioural training are described in Section 2.4, whereas techniques applied for in vivo electrophysiological recordings under anaesthesia are presented in Section 2.5. Anatomical position of investigated areas is presented in Section 2.6. where histological procedures are delineated and Section 2.7. explains methods used for both behavioural and electrophysiological data analyses.

2.2. Animals and materials

2.2.1. Animals

For behavioural experiments aiming to develop Noise Detection Task (NDT) 35 (out of 48 animals that underwent surgery) wild type (C57BL/6J) males aged 8-10 weeks were used. Their weight ranged between 23.6 and 39.3 g.

For behavioural and chemogenetic experiments 15 parvalbumin positive, PV+/+(PV-IRES-Cre (JAX008069) males, aged 8-10 weeks were used. Their weight ranged between 23.1 and 27.0. Previously, 7 PV^+ animals (out of 10 that underwent surgery) were used in DREADDs optimisation process that aimed at selection of injection coordinates, virus volume and minimal time of incubation.

The animals were housed in Biological Procedures Unit, SIPBS. They were kept in 12h:12h light-darkness cycle (normal (first 4 animals) or reverse, which allowed testing during their wakefulness period), under standard temperature and humidity conditions with food available *ad libitum* and water access restricted along training process (see subsection 2.4.4.). All the experiments were conducted in accordance with the guidelines of Home Office under Project Licence 70/8883. Below, a table compiling animals' parameters is presented. Murine strain, number of subjects,

gender, age in weeks on the day of surgery and weight on the day of surgery are shown.

Mouse line	No of Animals	Gender	Age (min-max) weeks	Weight(min-max) g
Wild type	48	male	8-10	22.1-39.3
PV-Cre	15	male	8-10	23.1-29.8

Table.2.1. Animals' parameters

2.2.2. Materials

In the table (Table.2.2.) below, materials and equipment used in experiments described in chapters 3-5 are enumerated.

	NAME	COMPANY
GENERAL	Wild type mouse (C57/B6) PV-IRES-Cre Mouse	Local animal facility Jackson Laboratory
SURGERY	Anaesthesia Machine Isoflurane Stereotaxic frame (SR-5R-HT) Heating pad Micro4 Microsyringe Pump Controller Nanoliter Injector 2000 Pipette puller (PC-10)	EZ Anesthesia Zoetis Narishige WPI WPI WPI Narishige

	Glass Capillaries for Nanoliter 2000	WPI
	Drill (Volvere Max)	NSK
	Lidocaine	Henry Schein
	Rimadyl (Carprofen)	Zoestis
	Betadine	Medlock Medical
	Ocular lubricant (Lacri-Lube)	Allergan
	Acrylic resin	Kemdent
	Silicon elastomers (Kwik-Sil, Kwik-Cast)	WPI
	Tools	WPI/Thor Labs/Dumont
	Bone screws	Fine Science Tools
	DREADD (pAAV5-hSyn-DIO-hM3D (Gq)-mCherry)	University of North Carolina
	Fast Green FCF	Sigma Aldrich
	Stereo Microscope (SZ51)	Olympus
	Light (PL2000)	Photonic
BEHAVIOUR	Sound Attenuating Chamber	Med Associates, Inc
	Restrain tube	Local animal facility
	Licometer	RS Components
	Programmable Syringe pump (Aladdin)	WPI
	Electrostatic Speaker	Tucker Davis Technologies
	Electrostatic Speaker Driver	Tucker Davis Technologies
	Tubbing	Tygon
	Syringe	BD Plastipak
	Hydrogel	clearH2o

	Milk	Cow&Gate
	Saccharin	Acros Organics
	DREADD (pAAV5-hSyn-DIO-hM3D (Gq)-mCherry)	University of North Carolina
	Multifunction Data Acquisition (DAQ)	National Instruments
CRANIOTOMY AND ELECTROPHYSIOLOGICAL RECORDING	32channels silicon probe	Neuronexus
	Headstage	Plexon
	Heating pad	WPI
	Recording chamber	Industrial Acoustic Co.
	Multifunction Data Acquisition (DAQ)	National Instruments
	Electrostatic Speaker	Tucker Davis Technologies
	Electrostatic Speaker Driver	Tucker Davis Technologies
	Dil Dye	Life Technologies
	Agar	Life Technologies
PERFUSION AND HISTOLOGY	Urethane	Acros Organics
	Euthatal + lidocaine	Henry Schein
	Butterfly needle	Burton Medical Equipment
	Microtome	Leica
	Epifluorescent upright microscope (E600)	Nikon
	PFA	Acros Organics
	Sucrose	Thermo Fisher Scientific
	Goat serum	Sigma
	Anti-PV	Sigma-Aldrich
	Ds-Red	Takara

	Alexa488	Abcam
	Alexa 594	Abcam
	Slide glass	Thermo Fisher Scientific
	Cover slip glass	Thermo Fisher Scientific
	DPX mountant	Oxoid
	Phosphate Buffered Saline	Oxoid
	Gelatine	Thermo Fisher Scientific
	Tritonx-100	Sigma Aldrich
	24 well culture plates	Techno Plastic Products

Table.2.2. Materials and equipment

Materials and equipment used are enumerated and divided into type/ stage of experiment.

2.3. Surgical procedures

Prior to all experiments, surgical procedures were performed to adjust the animal for used techniques. Section 2.3.1. describes surgery for behavioural experiments whereas Section 2.3.2. enumerates additional modifications necessary for chemogenetic experiments and electrophysiological recordings.

2.3.1. Surgery for behavioural experiments

The animal was anaesthetised (first in induction chamber, then within stereotaxic frame) using 5% Isoflurane (IsoFlo, Zoetis). The depth of anaesthesia was being adjusted during the procedure depending on animal's condition using isoflurane anaesthesia machine (E-Z Anesthesia System). It was put on a heating pad operated by WPI ATC1000 temperature controller and immobilized (Fig.2.1.) in the stereotaxic frame (Narishige, model SR-5R-HT). Lidocaine (100µl) and Rimadyl (5mg/kg) subcutaneous injections were applied to reduce pain during and after surgery. Ocular lubricant (Lacri-Lube, Allergan) was utilised for eyes protection. The head was shaved,

skin cleaned and disinfected with antiseptic solution and 70% ethanol and the skull was exposed. Bregma was identified and levelled against Lambda. Four (or 3 in case of behavioural experiments only) anchor screws (FST) were placed in the skull (2 rostrally and 2 caudally). Acrylic resin (Kemdent) was applied to secure the head-post. Two nuts were attached on the top of two back screws using dental cement as a head-post for future head-fixing. The head was cleaned and the animal was woken up from the anaesthesia. The weight of animal with head post was measured. Its weight and general conditions were monitored 3 days post-surgery.

Fig.2.1. presents the surgery room with part of the equipment. During the procedure the table was covered with autoclaved cloths. All equipment and tools were autoclaved / cleaned with antiseptic spray.

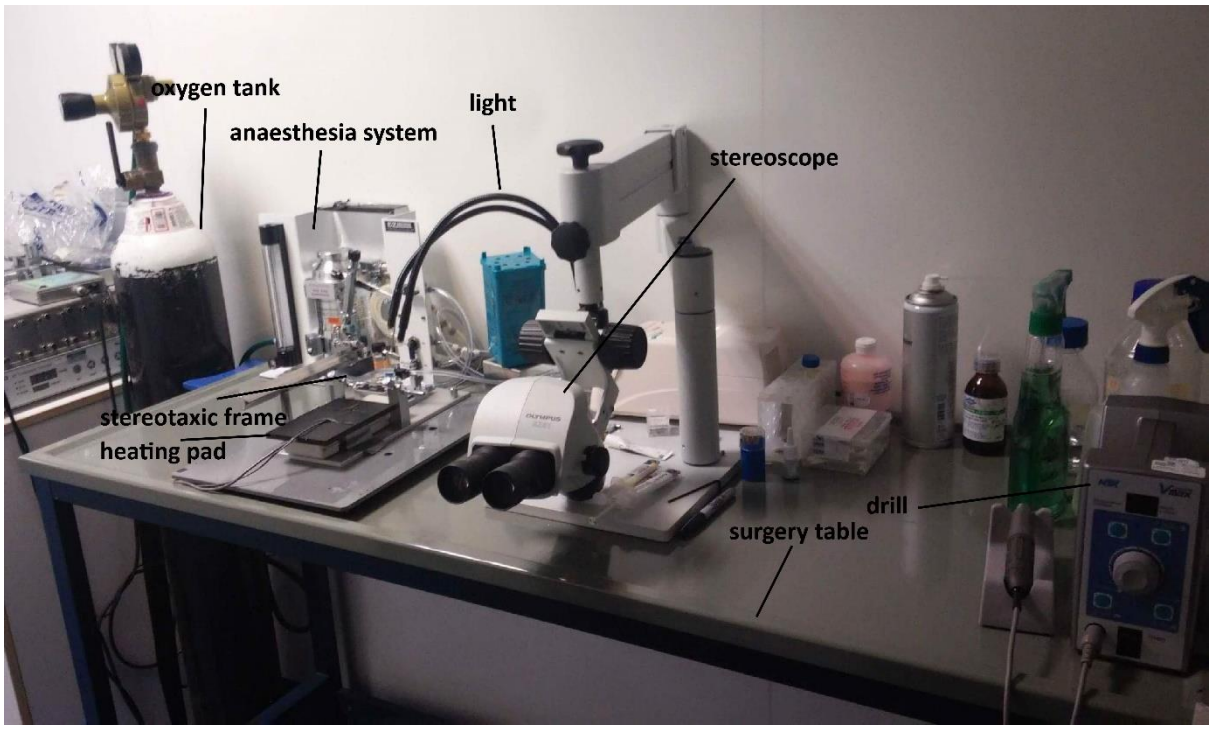


Fig.2.1. Surgery room and equipment

2.3.2. Surgery for chemogenetic and electrophysiological experiments

The modification to the previously described method was injection of DREADD (pAAV5-hSyn-DIO-hM3D (Gq)-mCherry) virus (University of North Carolina; vector

core and titer:$4.5x10^{12}$) using Nanoliter 2000 injector (WPI) operated by Micro Syringe Pump Controller Micro 4 (WPI). After measuring the area for future silicon probe recordings and securing it with silicone elastomers (Kwik-Sil, Kwik-Cast)4 drills were made for viral injections (Bregma: -2.7AP, -4.4DL; -3.2AP, -4.4DL; -2.7AP, +4.4DL; -3.2AP, +4.4DL) (Fig.2.2A.). The virus, previously mixed with 0.5% fast green dye (Sigma-Aldrich) and sucked into glass capillary for Nanoliter (WPI, 504949) made using pipette puller (Narishige, PC-10) and placed in microinjector was then injected with 30µL/min speed. In each of 4 sites 150nL were injected (see subsection 2.4.4.) at the depth of 500µm (variants of 50nL,100nL and 250nL per site were previously tested). Before withdrawing the pipette 10min waiting time was allowed to prevent bleeding and spillage. Then, the pipette was slowly retracted. In animals that were to undergo electrophysiological recordings with a silicon probe, the skull over the auditory cortex was exposed, marked ($2mm^2$; Bregma: AP -2mm, DL -4mm) and covered using Kwik-Sil and Kwik-Cast silicon elastomers (WPI). Holes for 4 screws (Fig.2.2. B) were drilled (2 anchor screws and connector consisting of ground and EEG, Fig.2.2.C). After placing all screws connector's connectivity was checked with a voltometer. After the viral injections surgery continued as described in previous paragraph.

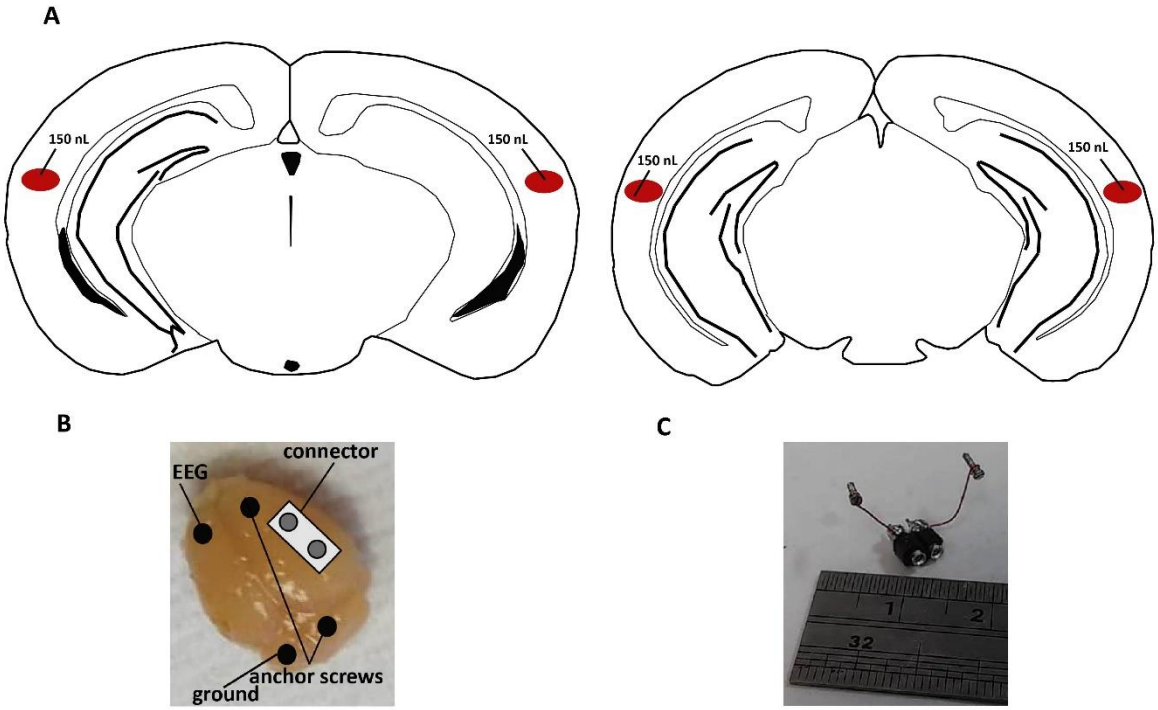


Fig.2.2. Positions of virus injection sites, screws and connector

A: viral vector injections sites (Bregma -2.7AP; -3.2AP)

B: position of screws and connector **C:** Connector

2.4. Behavioural experiments

Previously to habituation and head-fixed training initiation animals were allowed at least 5 days post-surgery recovery period.

Before the experiment started animals were habituated to the experimenter for 3 days. On the 2nd day habituation to restrain tube took place followed by habituation to the experimental box and head-fixed condition on the 3rd day.

The experimental procedure comprised of three types of sessions which number and duration depended on the progress in animal's behaviour: Pretraining1, Pretraining2 and Noise Detection Task (NDT). The experiments were performed daily, on working

days, in the afternoons (depending on batch between 13-20 but see subsection 2.4.4.).

2.4.1. Behavioural equipment

During all stages of training the same equipment, in configurations appropriate for each stage, was used. Detailed information is presented in below sections.

In every session the animal was placed in a restrain tube (8.5cm long with 5cm closed; width 4.5cm, high 5cm) and head-fixed to a bar (using head-post prepared during surgery) inside of sound attenuation box (Med. Associates, Inc.) as shown in picture and scheme in Fig.2.3.A and B. In front of the animal a spout (made from a pipette tip) was placed through which, after crossing a lickometer beam (RS Components) with the tongue (making a lick), it could get liquid reward. The spout was connected by tubing (Tygon) to a syringe loaded with the reward. Amount of the reward was regulated by Programmable Syringe Pump (World Precision Instruments). Inside the chamber a speaker (Tucker-Davis Technologies electrostatic speaker) was placed in front of the animal in the constant distance of 15 cm. It provided sound signals used during Pretraining 2 and NDT sessions. Sound, light presentation and reward delivery (see below) were controlled by DAQ system (National Instruments) connected to a program (S. Sakata) written in LabView.

A **B**

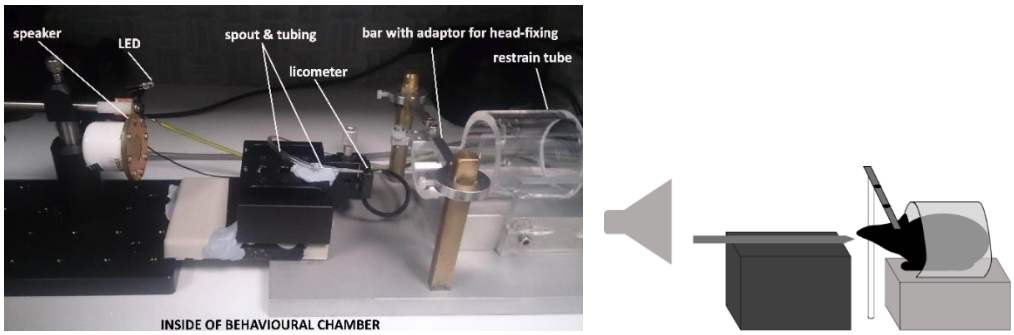


Fig.2.3. Behavioural box with equipment

A: Behavioural chamber with bar for head-fixing and restrain tube for animal, speaker, light source (LED), licometer and spout **B:** Scheme of the inside of the box with animal

2.4.2. Pretraining

Pretraining 1 and Pretraining 2 were meant to shape the animal's behaviour in a way that its success rate and reaction time would allow for implementation of Noise Detection Task. During those stages the animal should gradually improve on its behaviour. The schemes presenting design of both Pretraining stages are presented in Fig.2.4 (Fig.2.4. A-D).

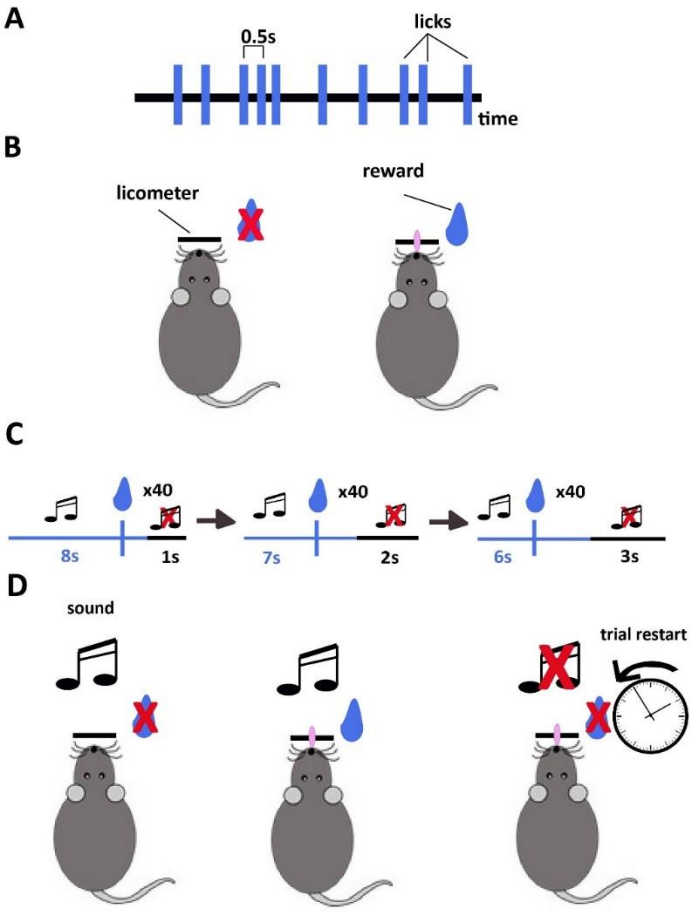


Fig.2.4. Pretraining1 and Pretraining2 schemes.

A: Timeline of Pretraining1 session. Reward could be obtained at any time after making a lick. Minimum time between 2 licks necessary to obtain reward was set to 0.5s.

B: To obtain reward animal needed to make a lick

C: Timeline of initial stage of Pretraining2. Animal was obtaining reward after making a lick during sound presentation. After each successful 40 licks licking window was shortened while ITI extended by a second.

D: Reward could be obtained only by making a lick during sound presentation. Lick made during ITI was restarting the trial.

2.4.2.1. Pretraining 1

This stage was taking 1 up to 4 days. During daily sessions animal was placed in the experimental, sound attenuation box as described in detail in Section 2.4.1. Sessions

lasted between 15 and 40 minutes depending on animal's behaviour. In those sessions animal was obtaining reward every time it made a lick (crossed the beam of licometer with tongue) regardless of the timing of its activity (Fig.2.5.). Applied reward was 10% infant milk (CowGate) dissolved in 0.1 % saccharine solution (see subsection 2.4.4.). The amount of reward applied with every lick was 2 - 4 µl, depending on animal but constant among sessions. When animal accomplished to make at least 100 licks during two consecutive sessions it was ready for Pretraining 2 (apart from rare occasions where after just one robust session-300/400 licks animal was moved to Pretraining2).

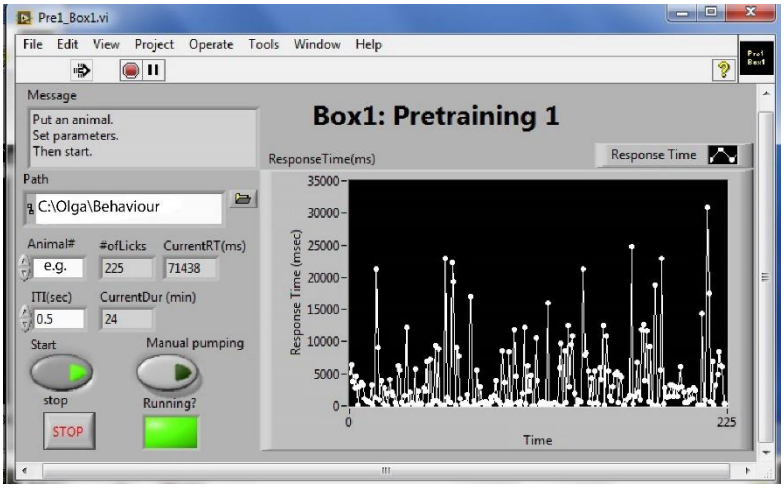


Fig.2.5. Screen from example session of Pretraining1.

Minimum set time between licks (ITI) was set to 0.5s

Number of licks was traced. Response time was used to calculate lick rate/min

2.4.2.2. Pretraining 2

Duration of this stage heavily depended on the speed of animal's progress. As previously, animal was put in the same conditions. The difference comparing to Pretraining 1 lays in that the reward could be obtained only at specific moments which duration varied from session to session. The time window for the reward was marked with white noise of around 62dB. During the first session it lasted for 8 seconds separated by one second of silence. A lick made during silence period

restarted the clock, making the animal wait longer for the reward. When the animal made sufficient amount of licks (40 for vast majority of animals, thresholds for first 9 animals varied between 20 and 50 on different sessions), the system upgraded to the next level of difficulty which was 7s of sound to 2s of silence. This procedure (Fig.2.6; see subsection 2.4.4.), with pace and magnitude of changes tailored to individual animal, was upgraded during following sessions till the animal showed good performance at 1s of sound presentation (silence periods varied between animals). After 2 days in a row of good performance at this stage (stable success rate and good reaction time, below 1s) the mode was changed from upgrading to random where each licking window lasted 1s and ITI (intertrial interval) typically had either 2 - 4 s or 1-3s. Stable success rate and fast reaction times, below 1son this level allowed for introduction of Noise Detection Task.

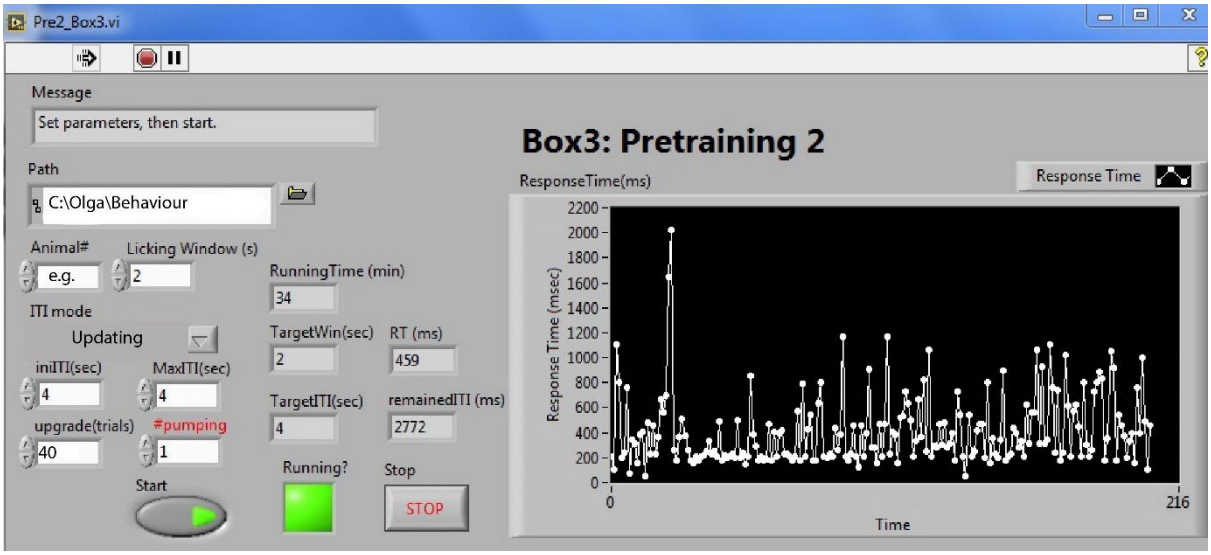


Fig.2.6. Screen from example session of Pretraining2.

Example screen from a Pretraining2 session; upgrading mode

Number of licks and RTs were tracked. ITI was upgraded after each 40 successful trials

2.4.3. Noise Detection Task

This was the final step of the experiment during which the valuable data was collected for further analysis. Below, differences in parameters used for NDT development together with Temporal Expectations experiments and NDT in chemogenetic experiments are described in Sections 2.4.3.1. and 2.4.3.2. respectively.

2.4.3.1. Development of Noise Detection Task and Temporal Expectations experiments

In this paradigm the sound intensity was fixed throughout session at 70 or 60Db (78dB in first 9 animals, see subsection 2.4.4.). On each trial the reward could be collected after the time of foreperiod (time of silence before sound presentation). The beginning of the foreperiod and a trial itself, was marked by switching light on or off (see point 4 in 2.4.4.). This feature was necessary to indicate to the animal the start of the session to allow it for estimation of the time of reward. Usage of light as a cue was well integrated with applied techniques, and additionally would be compatible with optogenetics, should the results were to be compared using this technique in the future research. In the initial sessions only one type of foreperiod was presented, whereas further sessions had between 2 and 4 foreperiods that were presented at various ratios (Fig.2.7.). ITI had between 1-3s or 2-4s. The reaction time for each FP was evaluated. This task had as a goal investigation of changes in reaction times due to manipulation of foreperiod duration or of ratio of presentation of different foreperiods. In a batch of 6 animals where foreperiod was not used (only ITIs of different duration) sound intensities between 40dB (50dB)-70dB were played on different trials.

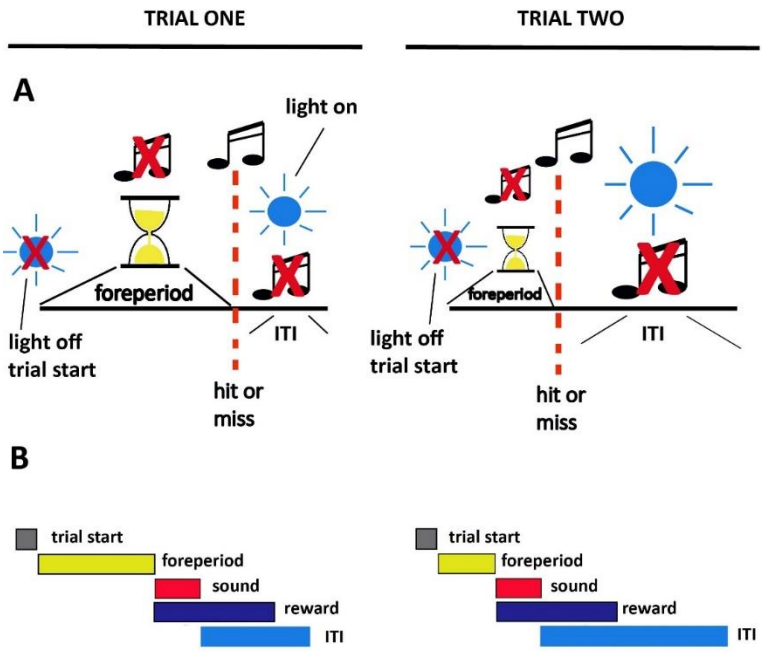


Fig.2.7. Timeline of 2 consecutive trials in a NDT session

A: Scheme of 2 consecutive trials with different durations of foreperiods and ITIs is presented.
B: Timeline of 2 consecutive trials with FPs and ITIs of different durations is presented. Stages of a trial: trial start (light off) –grey; foreperiod duration (yellow); sound presentation (red); reward (navy blue); ITI (blue). Duration of sound presentation and reward delivery were constant across trials. Duration of FPs and ITIs was changes between trials

2.4.3.2. Noise Detection Task in chemogenetic experiments

In early sessions only tones at 70dB were presented and separated with ITIs of duration same as during random mode sessions of Pretraining 2. With time, sessions' complexity increased and sounds were presented in consecutive trials at different intensities that spanned between 70-40dB (Fig.2.8.). Hit rates and reaction times for each sound intensity were evaluated. Part of the animals in the last session was also subjected to 'catch trial', a trial where no sound was played which was a way to measure animal's level of impulsivity.

Once the animal was performing for at least 3 sound intensities, alternating sessions with ip injections of either clozapine-N-oxide (CNO), activating DREADDs, or saline (SLN), as a vehicle, were performed. All CNO and SLN sessions included a catch trial.

During those sessions the paradigm was identical to NDT trials with the exception of injection of either CNO or SLN that took place 10-15 min before the start of a session. CNO was administrated on every second day.

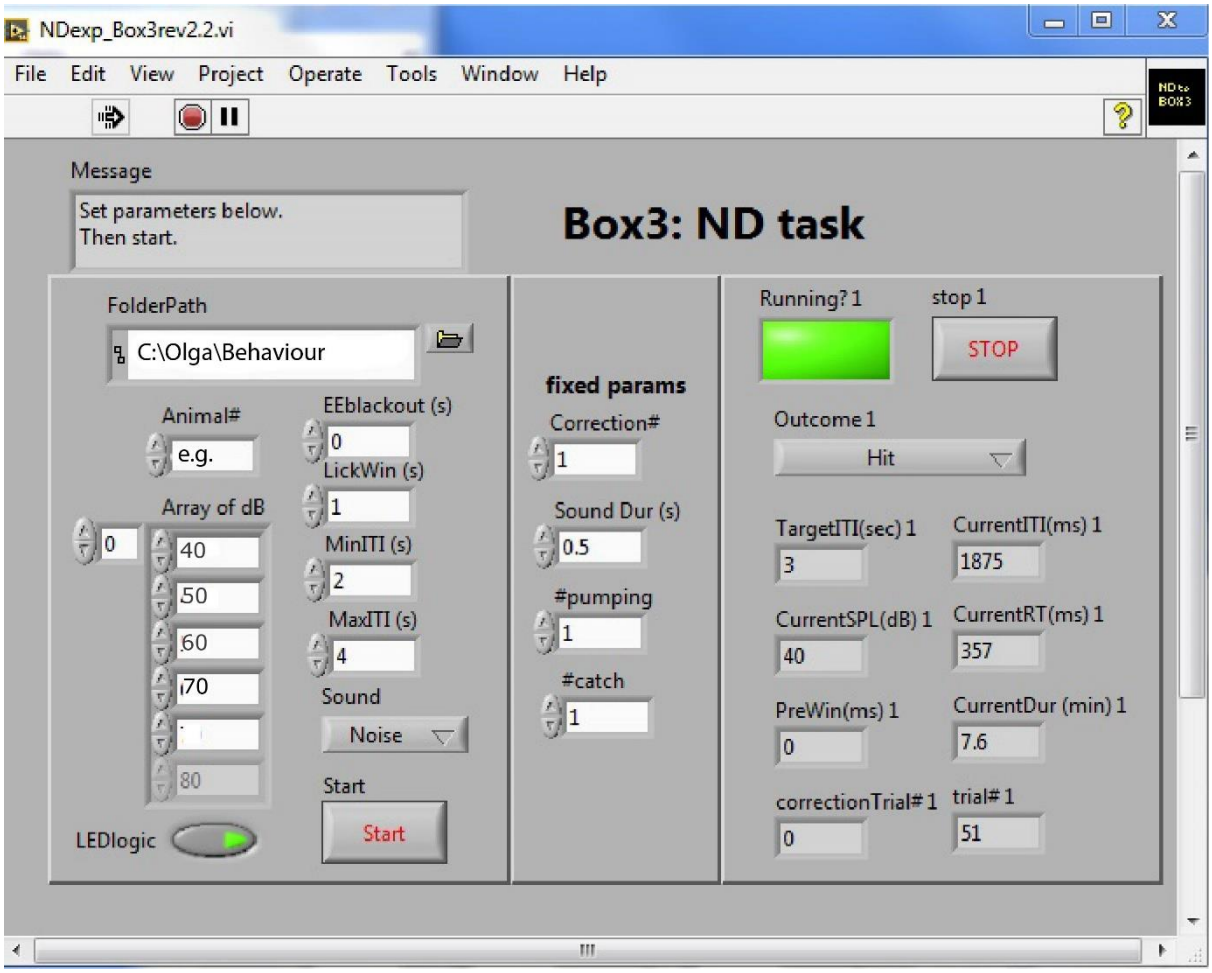


Fig.2.8. Screen from example session of NDT

Example session. Sound intensities applied: 40-70dB. Duration of the sound 0.5s; ITI 2-4s

Hit rate and reaction time for each ssound intensity were measured

2.4.4. Optimisation process

Before establishing final values of parameters, while training first several animals, various versions of different parameters were tested. Below, those variations are enumerated.

1) Time of training: although the majority of animals was trained once a day 5 days a week during all stages, 2 animals were trained twice a day for7 days during Pretraining. This schedule was not continued as the animals seemed more stressed and tired (took longer to initiate performance, poor results). Also, due to obtaining access to water after morning session, their motivation in the evenings was low.

2) Reward type: initially also 10% milk in water and strawberry milk were tried. 10% milk in 0.1% saccharine was chosen as all animals accepted this reward from first session.

3) Pace of upgrading licking window vs ITI ratio during Pretrainig2. Although initially only one second changes were applied (8s l.w.-1sITI,7s-2s etc.) this schedule was causing decrease in motivation, especially for animals/sessions with lower general amount of licks. Therefore, if the animal presented robust performance in 8s-1s scheme, in the next session it was upgraded to 6s-1s or 5s-1s due to the fact that in final task, NDT, the animal did not need to wait for more than 4s.

4) Light-sound schedule. For the animals trained in NDT for temporal expectations, 2 schedules were tried out during Pretraining2 and NDT sessions. For first 17 animals light appeared at the beginning of trial (after ITI), whereas for the rest of the animals switching off of the light after ITI signalised start of new trial. Dependency between scheme type and performance was not observed.

5) Sound intensities during NDT sessions for temporal expectations. Initially sound at 78dB was applied throughout sessions (first 8 animals). Later, the intensity got reduced to 70dB followed by further reduction (on some sessions) to 60dB. The reduction of sound intensity was implemented to prevent potential correlation between sound intensity and reaction time. As for the higher intensities reaction time

is generally faster, this could make it more difficult to see differences in RT for different FPs at high sound intensity.

6) Water deprivation. Different schedules of water delivery after training were tested. Time for which water was available spanned from 45min to 2h a day in different animals. In several animals also hydrogel (clearh2o) was tested. Most animals were water deprived from Sun to Fri although 7 days a week water deprivation was also tested.

2.5. In vivo electrophysiology under anaesthesia

2.5.1. Acute electrophysiological recordings from auditory cortex

Previously to the procedures initiation the animal was introduced into a state of anaesthesia using intraperitoneal 20% urethane (1,5g/kg) injections.

2.5.1.1. Craniotomy

Animal was placed in the stereotaxic frame (Narishige) on a heating pad (WPI). The protective gels were removed and skull surface was cleaned. The 2mm² recording area marked during previous surgery was re-measured when necessary and drilling (NSK-Nakanishi, Volvere Max) followed to expose the brain surface. During the procedure moist of brain surface was kept using 0.9% saline solution.

After completion of this stage animal was moved to the recording chamber (Industrial Acoustic Company).

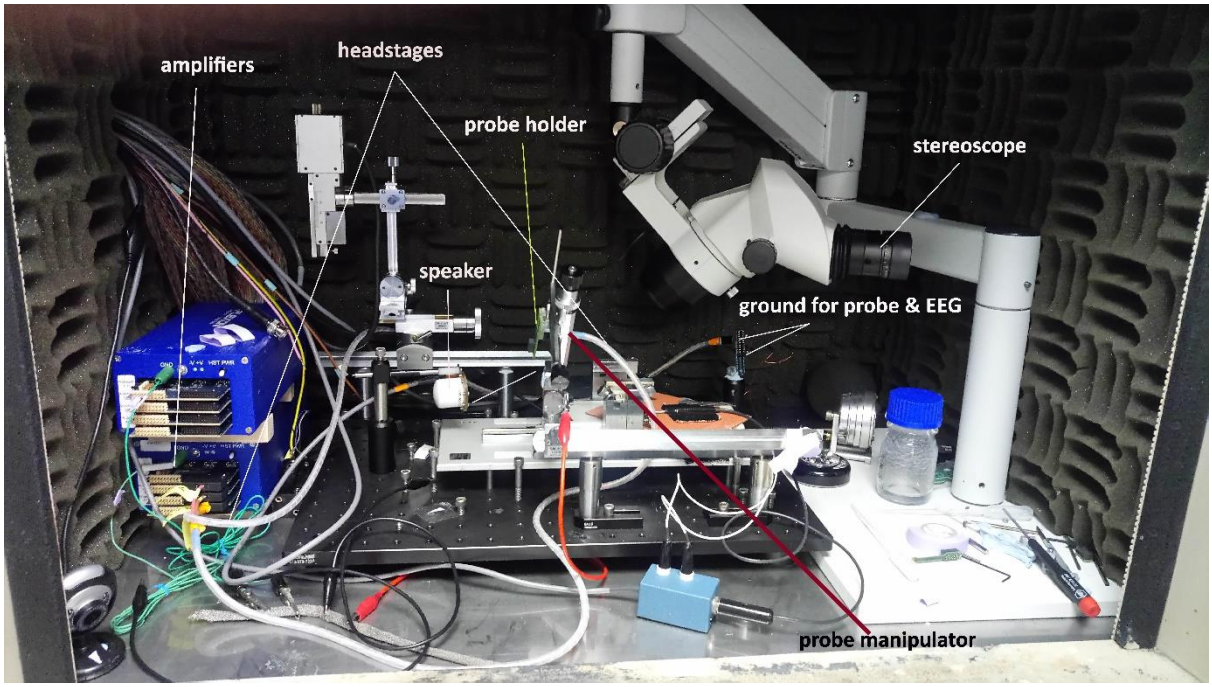
2.5.1.2. Silicon probe recordings from A1

Animal was placed on a heating pad (DC Temperature Cotrol System,WPI) and head-fixed in stereotaxic frame (Narishige) inside recording chamber (MAC-3, Industrial Acoustic Company, NY,Fig.2.9.A). Body temperature was kept stable using DC temperature controller (WPI). Brain surface was repeatedly being moisted using 0.9% saline. The tip of silicon probe with iridium electrode array sites, (NeuroNexus linear 32-channel single shank or 2shanks probe with 25-100µm spacing, Fig.2.9.B, C) marked with DiI dye (Life Technologies; for further histological confirmation of the position of probe), was placed in the exposed recording area. The electrode array was

connected to the headstage. Recording was supplemented with EEG channel, from a skull screw placed rostrally.

At first, white noise was applied (LabView) through the speaker located 15cm from the animal for detection of evoked auditory responses (to confirm correct position of the probe). Vasculature pattern on the brain surface was a helpful indicator during auditory cortex search period. Once the probe was inserted in the correct position, the electrode was lowered to the final depth (750-1100µm depending on the animal and probe) and the area was covered with 1% agar solution and left for 30-60min for signal stabilization. In the next step the actual recording took place.

A



B

Fig.2.9. Silicon probe and equipment for electrophysiological recordings scheme

A: equipment configuration

B: silicon probe scheme (Neuronexus, modified)

2.5.1.3. Chemogenetics experiment

Following 5-10 min of spontaneous activity recording a sequence of 50 trials of broadband white noise (500ms sound duration with 500ms off and 1s ITI) with intensities between 0-80 dB (10 dB step) was presented. Acoustic stimuli were generated digitally (sampling rate 97.7 kHz, TDT, Tucker-Davis Technologies) and delivered in free-field through a calibrated electrostatic loud-speaker located 15 cm in front of the animal. Subsequently CNO (1 mg/kg) injection was applied and couple hundreds of above described trials were presented. Recording was finished with another 5-10 min of spontaneous activity recording after which animal was perfused.

2.6. Histology

In the first step brain is harvested immediately after perfusion and fixation (Section 2.6.1.1.), followed by histology where brain sections are stained with antibodies visualising pattern of expression of used DREADD virus as well as PV^+ cells expression Section 2.6.1.2.). Finally, sections are imaged to confirm viral expression (Section 2.6.1.3.).

2.6.1. Histology

2.6.1.1. Perfusion

After completing the experiment, the animal was injected with either an overdose of urethane or with 0.2ml of euthatal & lidocaineand (Henry Schein) and moved onto perfusion bench. There, it underwent transcardial perfusion with 0.9% saline followed by fixation using 4% paraformaldehyde (PFA) (Acros Organics) in PBS. Successively, the brain carefully removed with surgical tongs, was harvested and stored overnight in 4% paraformaldehyde, PFA at 4ºC. The next day the brain was transferred into 30% sucrose (Sigma-Aldrich for tissue dehydration. Sample was further processed no earlier than 36-48h after that step.

2.6.1.2. Immunohistochemistry

Sections of 100µm thickness containing auditory cortex were obtain using microtome (Leica SM2010R). Double staining was performed to visualise overlay of the area infected with DREADD virus (pAAV5-hSyn-DIO-hM3D (Gq)-mCherry) as well as presence of PV^+ cells. First, sections were incubated in blocking solution (10% normal

goat serum and 0.3% Triton X in PBS). Then, for PV^+ cells staining primary monoclonal anti-parvalbumin (Sigma-Aldrich P3088; 1:4000) and secondary anti-mouse IgG Alexa Fluor488 (Abcam; 1:500) antibodies were used. For DREADD staining primary polyclonal Ds-Red (Takara 632496; 1:1000) and secondary anti-rabbit IgG Alexa Fluor 594 (Abcam; 1:500) antibodies were used. Between staining with primary and secondary antibodies sections were stored overnight in 4°C and washed. Then, sections were transferred on gelatin-coated microscope slides, coverslipped and sealed with DePeX mounting medium (VWR).

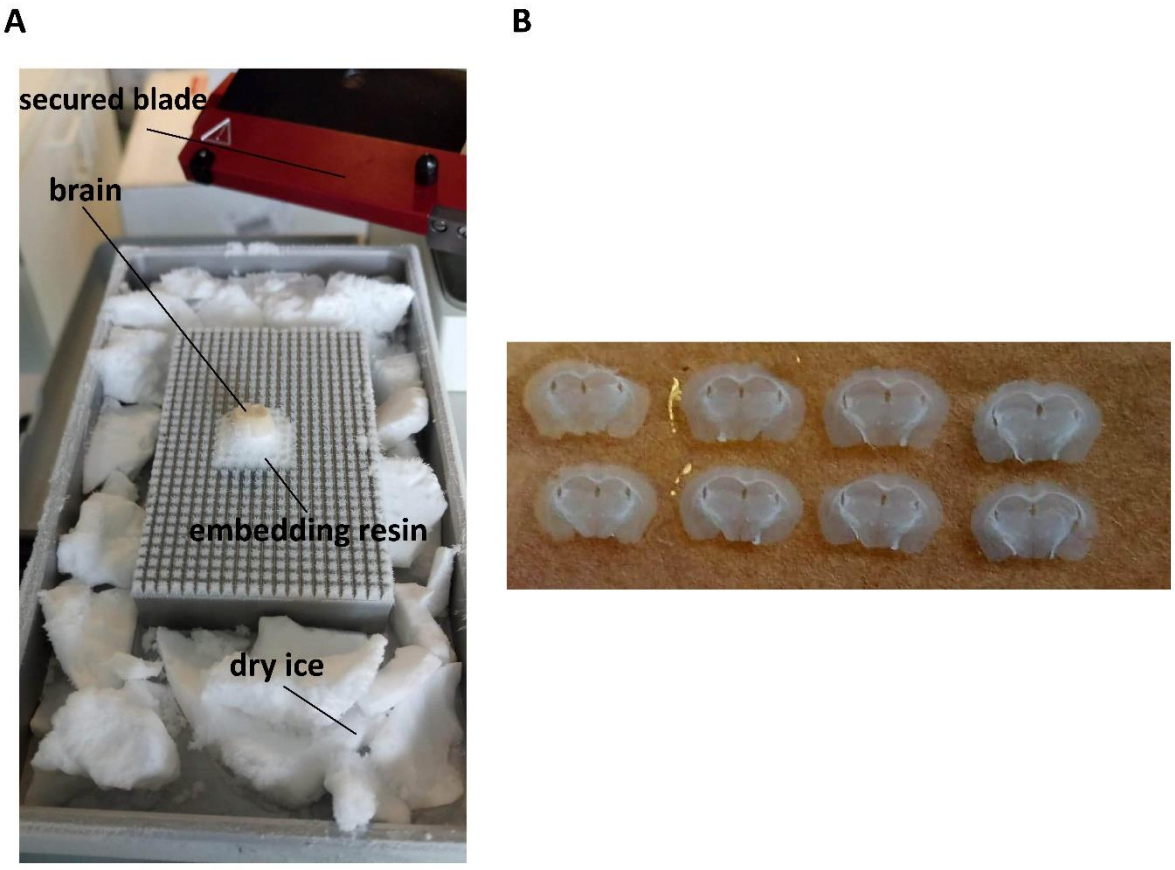


Fig 2.10. Preparation of sections using microtome

A: Preparation of sections on microtome
B: Sections after cutting

2.6.1.3. Imaging

Ready slides were observed under epi-fluorescent upright microscope (Nikon, Eclipse E600) and images obtained using MetaMorph or WinFluor imaging softwares processed using Image-J.

2.6.2. Histological identification of silicon probe track

To identify the probe track, DiI signals were also assessed. Its position was compared with mCherry signal visualising position of viral vector and with position of PV-Cre positive cells (stained in green).

2.7. Data analysis

2.7.1. Behavioural data analysis

2.7.1.1. Pretraining

Animals' performance in both stages of Pretraining was evaluated using scripts written in Matlab (Mathworks). Results for a single animal and whole cohort were presented separately for both Temporal expectations experiments and Chemogenetic experiments. In Pretrainin1 lick rate (number of licks per minute) and lick number per session was calculated for a single animal. Lick rate and number of animals per session was calculated for whole cohort. To finish this stage, animals had to perform either over 100 licks in 2 consecutive sessions or perform over 300 licks during single session. Statistical significance of changes in lick rate in consecutive sessions for whole cohort was evaluated using one-way ANOVA and Tukey's post hoc test. In Pretraining2, hit rate and mean RT (also median RT in temporal expectations experiments) were evaluated for single animal for each session. For all cohort hit rate and mean RT were evaluated using reapeated measures ANOVA and Bonferroni post-hoc test. Number of animals per session was also evaluated.

2.7.1.2. NDT for Temporal expectations experiments

Here, the difference in RTs for different foreperiods (in variants of 2FPs and 4FPs per session) were analysed. Sessions taken into account needed to meet inclusion criteria: at least 100 trials per session, at least 60 % of hit rate per session and at least 2 FPs per session presented. Data selected for analysis was 'cut' after 10 inactive trials in a row. Foreperiods that were analysed were between 200ms and 2000ms and were presented at different ratios. Further, statistical significance was tested using either

t-test (for variants with 2 FPs per session) or ANOVA (for variants with 4 FPs). The influence of ITIs on RTs was tested as well. The statistical analysis was performed using ANOVA and Tukey's test.

2.7.1.3. NDT for Chemogenetic experiments

Here, data from sessions under each of three conditions (CTL, CNO, SLN) was evaluated. First, data from a single animal was shown. Next data for all cohort was analysed. The inclusion criteria for CTL sessions were at least 100 trials, at least 60% hit rate and at least 3 sound intensities applied. For CNO and SLN sessions the inclusion criterion was at least 3 sound intensities applied. Probability to go vs sound intensities and RTs vs sound intensities were analysed between 3 different conditions. For the statistical analysis for each sound intensity one-way ANOVA and Tukye's post hoc test were applied. All graphs were prepared using Matlab and Illustrator (Adobe).

2.7.1.4. Histological verification

As the final step in Chemogenetic experiments histological data was analysed. Previously prepared slides were observed under epifluorescent upright microscope (Nikon, E-600). The position of taken section was established according to Paxinos and Franklin's atlas 'The mouse brain in stereotaxic coordinates, 4th edition',2012. The position of mCherry signal and PV^{+}cells signal was marked and their overlay was checked (magnification x4). The position of the probe from electrophysiological recording was also verified.

2.7.2. Electophysiological data analysis

The quality of recording was initially evaluated using Neuroscope. The process of spike sorting was divided into automatic spike detection and clustering followed by manual clustering. Those steps were performed in Klusters and further analysis was performed using Matlab. In the manual clustering stage clusters with good quality were selected and grouped as Multiunit activity cluster. This stage allows to minimise the amount of spike sorting errors which occur when spikes originating from different neurons are grouped together, due to synchronous spiking, or when not all spikes generated by a neuron are clustered together. For that spike wave-form, principal

component analysis, autocorrelogram as well as position on the recorded channel are taken into account.

The analysis performed using Matlab allowed to visualise differences in spike rates (50ms time window) during evoked responses before and after CNO administration. Spikes rate were calculated for all used intensities. Additionally, analysis of all 4 steps of the experiment was performed (spontaneous activity, evoked PreCNO injection, evoked PostCNO injection, spontaneous). Further, analysis of the same 4 stages was performed for no-sound epochs. Statistical confirmatory analysis was performed using one-way ANOVA and Tukey's post hoc test

Chapter 3. Results - Optimisation of behavioural procedures

3.1. Objectives

The aim of the experiments presented below was two-fold. Firstly, to establish a behavioural procedure enabling study of the relationship between the presentation of auditory cues with different parameters and a behavioural outcome measured as the hit rate and reaction time of an animal performing the task. Secondly, this paradigm was tested and modified in experiments measuring temporal expectations, in search of optimal parameters. Importantly, this tool was created in a way that would allow behavioural modulation by chemogenetics (Chapter 4) and, in the future, could be paired with electrophysiological recordings in awake animals to elucidate its neural underpinnings. Meanwhile, Chapter 5 presents data from electrophysiological recordings in anaesthetised animals, during sound presentation before and after modulation of neural activity using chemogenetics, in a study evaluating influence of this technique on spontaneous and sound-evoked activity in the auditory cortex.

3.2. Overview

In this chapter, experiments aiming to establish an auditory-based behavioural paradigm, further tested in temporal expectations experiments, are presented. Below, general information about the animals used during this process is shown.

At the beginning of each section, inclusion criteria for data sets are presented. Section 3.3. shows results for the Pretraining sessions, containing both Pretraining1 and Pretraining2. Subsection 3.3.1. presents exemplary results from a single animal, whereas subsection 3.3.2. presents pooled data from a group of animals meeting the initial criteria in Pretraining1 as well as examples of a slow-learner and a fast-learner in Pretraining2

Section 3.4. shows data for the final behavioural task, Noise Detection Task (NDT). Here, the effect of various foreperiods (FPs), crucial for the temporal expectations, and their combinations as well as the effect of different intertrial intervals (ITIs) on reaction time is shown. Subsections 3.4.2. and 3.4.3. analyse the differences in reaction time depending on the duration of foreperiods, their number and ratio of

presentation. The main hypothesis assumed that RTs would decrease with the increase of FPs' duration. Additional hypothesis presumed that ratio of presentation of FPs would affect their respective RTs, with less frequent FPs provoking faster RT. The dependency between FPs and RTs was studied for both 2 or 4 FPs. Subsection 3.4.4. presents data showing the relationship between ITI duration and reaction time. Section 3.5. summarises the results presented in this chapter whereas section 3.6. discusses limitations of the used method.

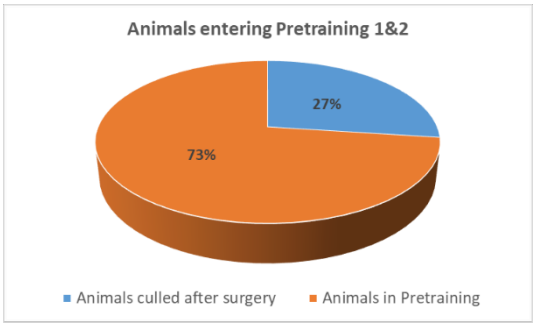
Below table (Table.3.1.), summarizes general information about the animals used in experiments described in Chapter 3.

Number of animals	35	Min-Max Post-surgery weight (g)	22.1-39.3
Mouse line	Wild type (C56BL/6)	Pretraining Min-Max (days)	6-51
Gender	Male	NDT Min-Max (days)	4-58
Min-Max age (weeks)	8-10	Number of FP/ITI animals	20 / 6
Min-Max Pre-surgery weight(g)	21.2-37.1	Surgery- sacrifice (days)	27-78

Table.3.1. All animals' information

Figure 3.1. presents percent of animals entering subsequent stages of behavioural task. In panel A, the initial 100% is equal to the number of animals (n = 48) that underwent surgery for NDT. Pretraining1 & 2 are not discriminated as the same number of animals began training in both stages. In panel B, 100% represents the number of animals (n = 35) that started Pretraining2. The major cause of loss of animals were problems with head-cap. Some animals did not enter the final stage of the task, NDT, due to poor performance in Pretraining2.

A



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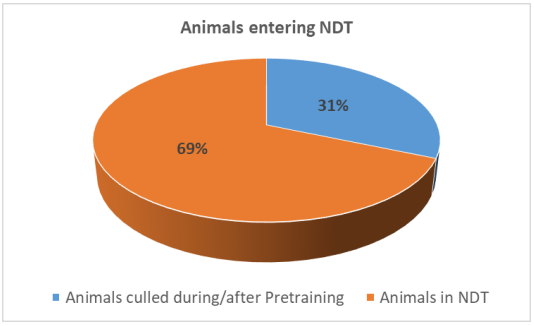


Fig.3.1. Percent of animals entering subsequent stages of training

A: Animals entering Pretraining. 100% (n=48) animals. **Blue**- animals culled after surgery, 27% (n = 13), **orange**- animals in Pretraining, 73% (n = 35)
B: Animals entering Noise Detection Task (NDT). 100% (n = 35) animals. **Blue**- animals culled during/after Pretraining, 31% (n = 11), **orange**- animals in NDT, 69% (n = 24)

3.3. Pretraining

Subsection 3.3.1. shows Pretraining1 and Pretraining2 for a single animal. Subsection 3.3.2. presents results of Pretraining1 for the whole cohort and examples of a slow-learner and a fast-learner from Pretraining2.

The objective of Pretraining was to shape robust behaviour in response to sound cues as well as a fast reaction time (below 1s), necessary for completing NDT. Pretraining was divided into two stages. The first stage was dedicated to familiarizing the animal with the conditions of the training, equipment and, most importantly, so that the animal could make a connection between its performance and obtaining a reward. In the second stage, the animal's response robustness and its reaction time were shaped.

3.3.1. Single animal example

Data shown in Pretraining1 and Pretraining2 sessions presents results from the same animal.

3.3.1.1. Pretraining1

As this stage had as the principal goal teaching the animal dependency between its action and obtaining the reward, timing of action was not important. The task was completed when, in 2 consecutive sessions, the animal made at least 100 licks during each one (inclusion criterion). The animal easily learned the task, as proven by the increase in lick rate in the 2nd session (Fig.3.2. A) as well as by lick number obtained in both sessions (Fig.3.2 B). As the animal met the inclusion criteria, it could continue shaping in Pretraining2.

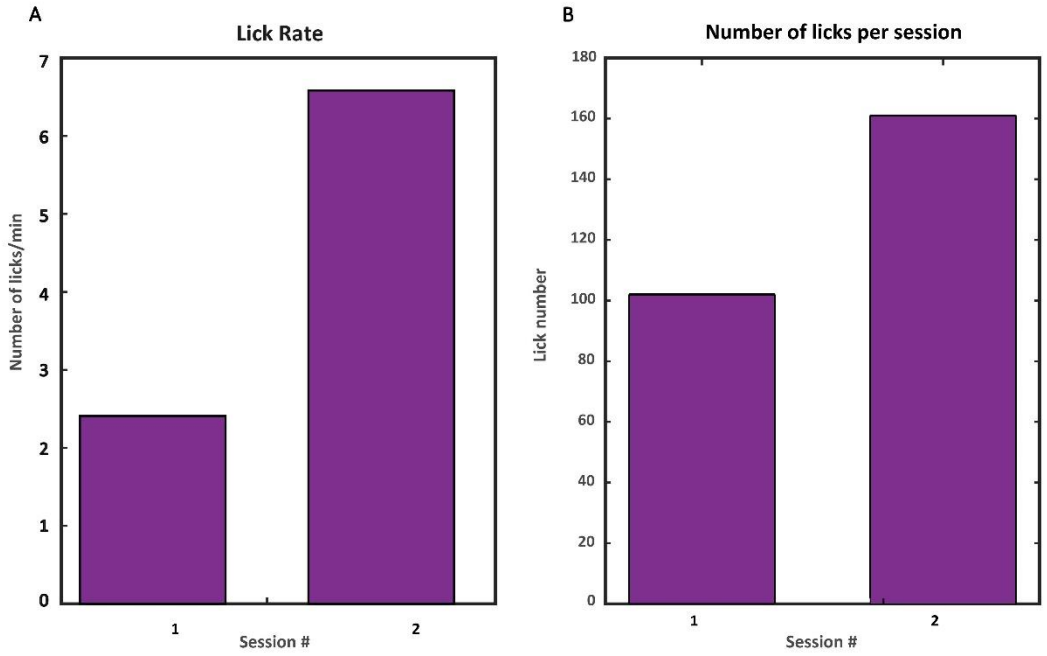


Fig.3.2. Single animal results. Pretraining1

A: Lick rate (number of licks/min) during Pretraining1 sessions

B: Number of licks during Pretraining1 sessions

3.3.1.2. Pretraining2

In Pretraining2, the animal's behaviour was shaped in subsequent sessions where the time to respond (licking window, LW) was getting shorter while the time between trials (ITI) was getting longer. Starting with an 8s licking window and 1s ITI, the parameters were changed repeatedly after 40 successful trials to shorter licking window and longer ITI. The changes in parameters happened both within session and

in between sessions and were continued until the animal was able to perform within a 1s licking window. To check durability of performance at this point, a *random mode* was introduced where the animal was allowed a 1s licking window, while the ITI changed randomly on every trial and was set to 1-3s. The ability of the animal to perform at this stage completed Pretraining2. The objective of this stage, as well as criteria for eligibility for next stage-NDT, was to teach the animal to respond quickly (below 1s) and to wait for the next sound cue over prolonged ITIs of different durations.

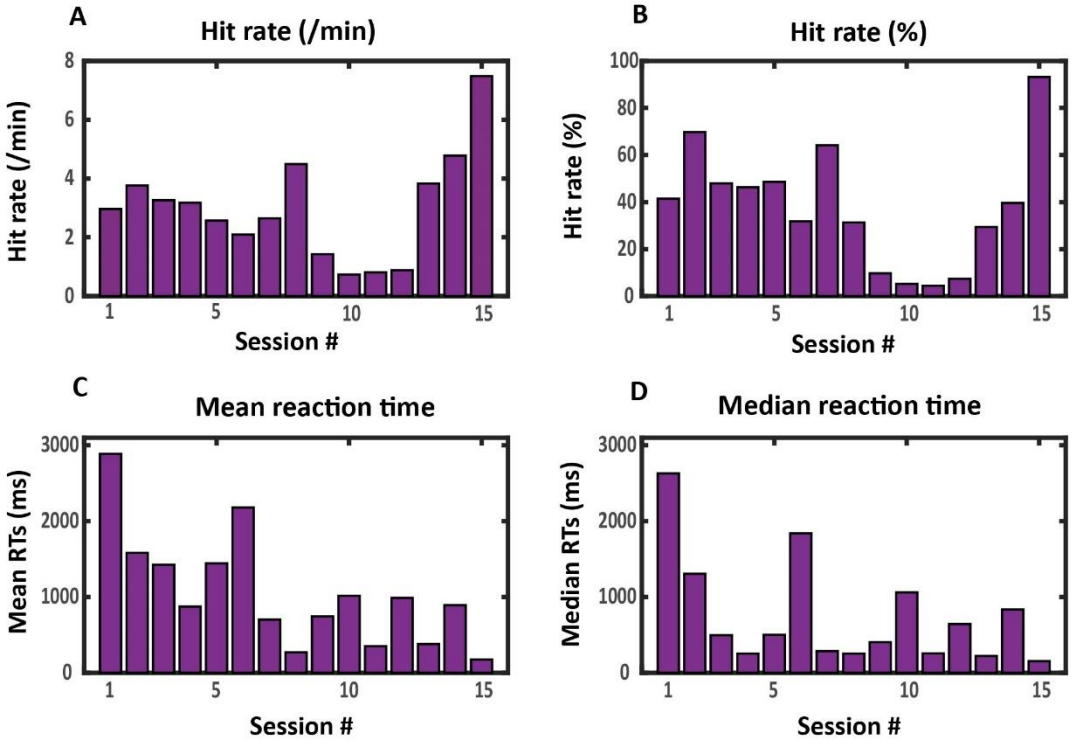


Fig. 3.3. Single animal results. Pretraining2

A: Hit rate (licks) per minute on subsequent sessions **B:** Hit rate (percent) on subsequent sessions

C: Mean reaction time (ms) on subsequent sessions **D:** Median reaction time (ms) on subsequent sessions.

Above, Fig.3.3. presents a summary of performance for the animal during 15 sessions of Pretraining2. The hit rate for each session is shown as the number of valid licks per minute (Fig.3.3. A) and as a percentage of successful trials (Fig.3.3. B). As can be seen, there was variability in performance with a drop in the middle sessions followed by recovery in the last 3 sessions. This pattern, which was also typical for other animals, is further explained in Fig.3.4. Panels C and D of Fig.3.3. show mean and median reaction times respectively. Mean reaction time decreased steadily in subsequent sessions and stayed constantly below 1s from session 7 (SD=747ms). The ability of the animal to restore good hit rate at the end of the training (Fig.3.3. A, B) together with constant performance below 1s in the second half of the training showed that the animal mastered the skills necessary to enter the NDT stage.

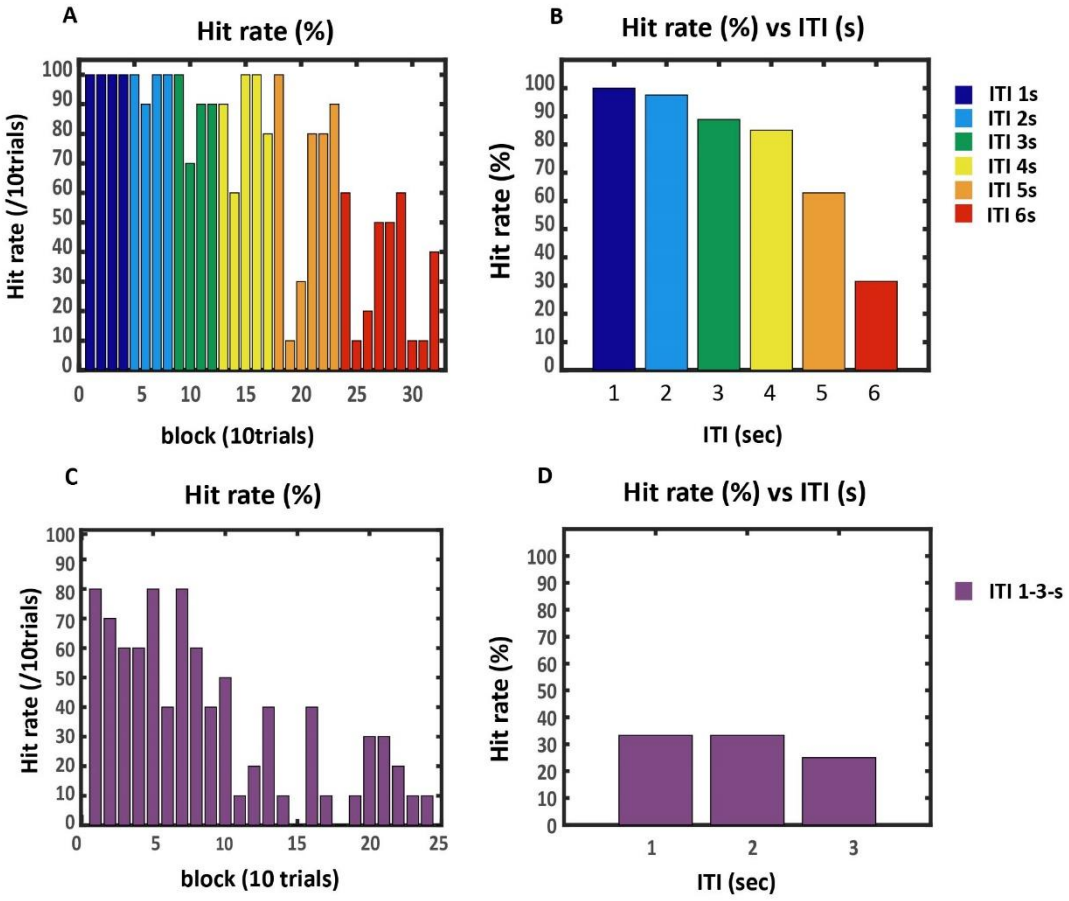


Fig.3.4. Two sessions form Pretraing2

A: *Updating mode* session. Success rate along session
B: *Updating mode* session. Correlation between success rate and ITI
ITIs were increasing by 1s after every 40 successful trials. LW started at 8s and decreased by 1s simultaneously with ITI increase.
C: *Random mode* session. Success rate along session
D: *Random mode* session. Correlation between success rate and ITI
Randomly applied ITIs had span between 1 and 3s. LW in all trials equalled to 1s.

Above figure (Fig3.4.), presents more details from two selected sessions from Fig.3.3. Panels A and B present the 2nd session of training with *updating mode*. Here, the animal was starting with an 8s licking window and 1s ITI. After 40 successful trials, the licking window was shortened and ITI increased by 1s. As the level of difficulty was low and the animal was familiar with the methods from the previous day, the

initial hit rate (Fig.3.4. B) was 100% and slowly decreased with the increase of difficulty and duration of the session, as by collecting reward in each successful trial the animal was decreasing its motivation. The results are worse in panels C and D (Fig.3.4.) which present the first session with *random mode.* Here, the animal had only 1s to respond in every trial, while the randomly applied ITI was between 1-3s. The greater difficulty of this session (comparing to previously presented one) might have influenced the level of performance of the animal and contributed to the changing dynamics of performance shown in Fig.3.3.

3.3.2. Pooled data from all animals. Slow and fast-learner examples

Below, pooled data from all 35 animals is presented for Pretraining1, while examples of a slow-learning animal and of a fast-learning animal are presented for Pretraining2.

3.3.2.1. Pretraining1

Below, a summary of Pretraining1 for 35 animals is presented. As shown in panel A (Fig.3.5.), the number of licks per minute was growing, indicating progress in training. There was a significant increase in lick rate from session 1 to sessions 2 and 3, although the increase from session 2 to 3 was shown to be insignificant (one-way ANOVA: p value=4.70395×10^{-5}, F = 8.76; Tukey's post hoc test: p value s1/2=0.0010, p value s1/3 = 0.0003). Lower lick rate in session 4 occurred because only 2 animals (Fig.3.5. B), with worse results, performed this additional session. Panel B (Fig.3.5.) depicts the number of animals vs the amount of sessions they needed to perform in, to complete this stage of training. The majority of animals needed only 2-3 sessions to meet the criterion of 2 consecutive sessions with at least 100 licks. Four animals, after performing several hundred trials in a short time, began Pretraining2 after just one session of Pretraining1.

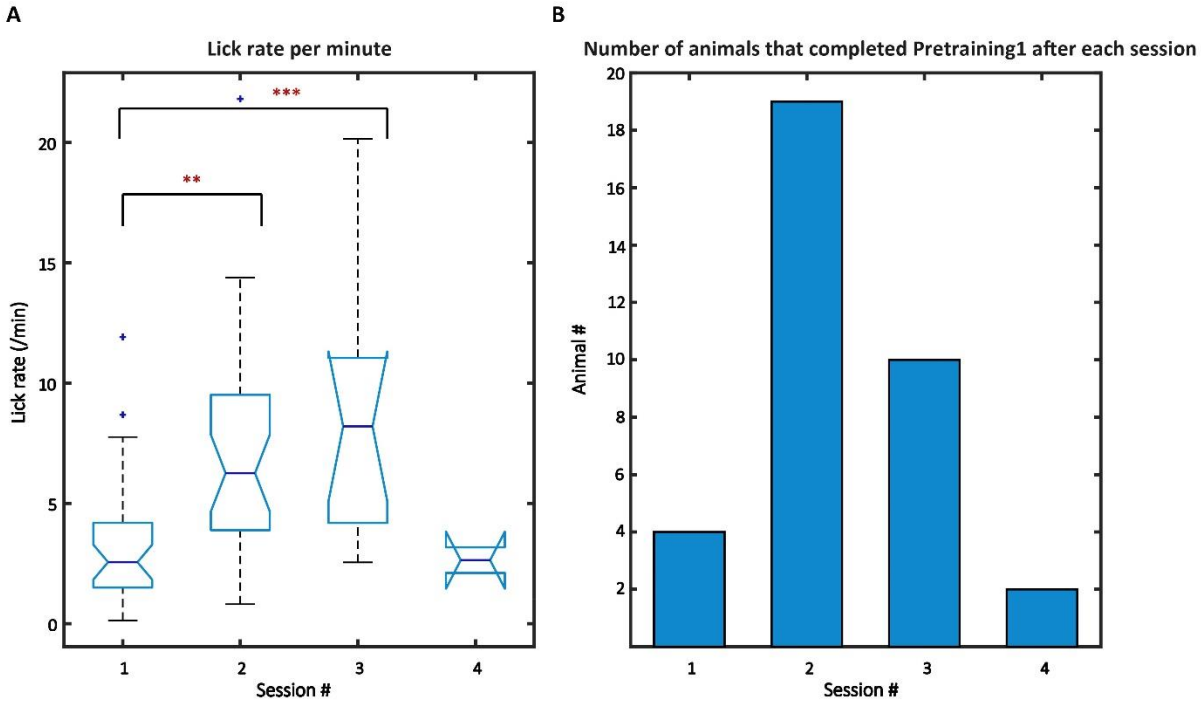


Fig.3.5. Pretraining1. Pooled data from 35 animals

A: Lick rate per minute during Pretraining1 sessions.

B: Number of animals that completed Pretreining1 in each session.

3.3.2.2. Pretraining2

Figure (Fig.3.6.) presents an example of results of Pretraining2 for a slow-learner (Fig.3.6. A-C) and a fast-learner (Fig.3.6.D-F).. The inclusion criterion were sessions with at least 100 trials. Panels A, D and B, E present dynamics of hit rate per minute and hit rate in percent, respectively, in consecutive sessions of training for each animal. Panels C and F (Fig.3.6.) show changes in mean reaction time in successive sessions for a slow and fast-learner respectively.

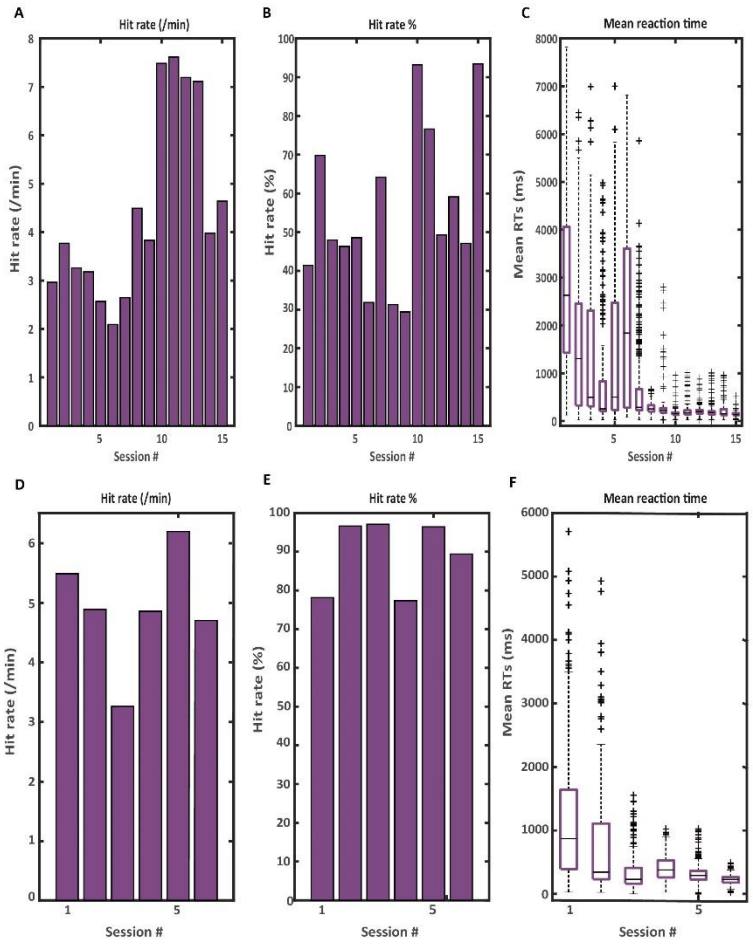


Fig.3.6. Pretraining2. Examples of a slow-learner and a fast-learner

A-C: Slow-learner (n=1) A: Hit rate per minute in subsequent sessions **B:** Hit rate per session **C:** Mean reaction time per session (ms) **D-F: Fast-learner (n=1) D:** Hit rate per minute in subsequent sessions **E:** Hit rate per session **F:** Mean reaction time per session (ms)

The percent hit rate for the slow-learner (Fig.3.6. B), similarly to the single animal result shown in Fig.3.3. B, showed daily fluctuations which might partially originate from changes of parameters used in subsequent sessions as explained in Fig.3.4. For the fast-learner, those changes were smaller and more uniform (Fig.3.6.E). Additionally, fast-learner needed only 6 sessions (Fig.3.6.E) compared to 15 of the slow-learner (Fig.3.6.B) to complete Pretraining2. The difference in results between animals was also clearly visible in mean reaction times (Fig.3.6.C,F). Fast-learner presented quicker responses from session1 and improved performance more rapidly. As the main objective of this stage of training was to shape fast responses,the animals that performed below 1s and had an increase in hit rate in the final sessions of

Pretraining were moved to NDT. The animals whose performance was not shaped during this stage terminated the experiment.

3.4. Temporal Expectations

This section shows the process of optimisation of the Noise Detection Task (NDT) which was tested in experiments exploring the influence of temporal expectations on animal's behaviour. The motivation behind this test was the assumption that longer foreperiods (time at the beginning of trial preceding the auditory cue which signals possibility of obtaining the reward) should shorten the reaction time. Below, results for a varied number of foreperiods of different durations and presentation ratios are presented.

3.4.1. General information

In the following sections, analysis of dependency between foreperiods' (FPs) duration and reaction time is presented. Inclusion criteria for the sessions were: at least 2 types of FPs; at least 100 trials per session; at least 60% hit rate per session. Table 3.2. gives an overview of foreperiods analysed in following sections. Number of sessions and animals taken into account during analysis is also presented.

Number of animals	Total number of sessions	Number of FPs	Ratio S:L sessions	Min FP duration (ms)	Max FP duration (ms)
6	10	2	50:50	200	1500
2	10	2	20:80	200	1500
2	6	2	80:20	200	1500
3	8	4	50:50	200	2000

Table.3.2. Foreperiods used in analysed data

3.4.2. Influence of foreperiods on reaction time. Two foreperiods

Fig.3.7. presents dependency between foreperiods and reaction time in 3 combinations of ratio: 50:50, 80:20 and 20:80. In each case, combined values of shorter FPs (see Table.3.2.) are compared against combined values of longer foreperiods. . In panel A (Fig.3.7.A), the main hypothesis that a longer foreperiod results in faster reaction times was tested.No statistically significant difference in RTs between FPs was seen for 50:50 ratio (Fig.3.7.A) as established using t-test. Therefore, ratios with shorter FP occurring more frequently (Fig.3.7.B) and longer FP occurring more frequently (Fig.3.7.C) were subsequently tested. In both cases significant difference in RTs was confirmed using t-test (p-value= 0.0079 and p-value = 0.0006 respectively). Altogether, results presented in Fig.3.7. show that it was the frequency of a FP rather than its duration what impacted the RT.

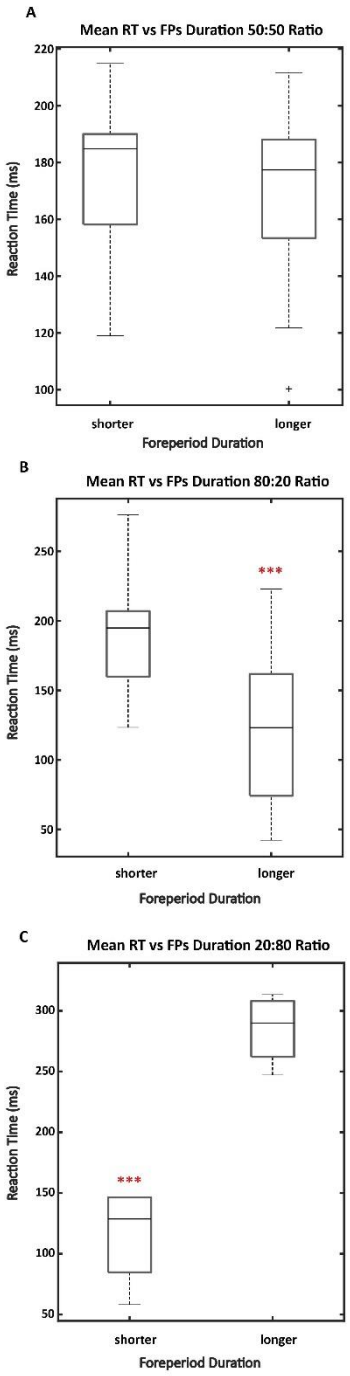


Fig.3.7. Reaction Time vs Foreperiod duration. Two FPs, different ratios

A: Ratio 50:50 Shorter FPs, combined values of: 200ms and 500ms Longer FPs, combined values of 1000ms and 1500ms; animal n=6, session n=10; p-value = 0.6813
B: Ratio 80:20 FPs values as in point A;animal n=2 sessions, n=6; p-value = 0.0079
C: Ratio 20:80 FPs values as in point A; animal n=2,sessions n=5; p-value = 0.0006

3.4.3. Influence of foreperiods on reaction time. Four foreperiods

Further, it was tested if the number of FPs, occurring at even distribution, in a session influences RT. Below, Fig.3.8. shows the dependency between foreperiods and reaction time.. Sessions with different foreperiods, classified as short, medium-short, medium-long and long were combined. Applied one-way ANOVA did not detect statistical significance (p-value = 0.8597).

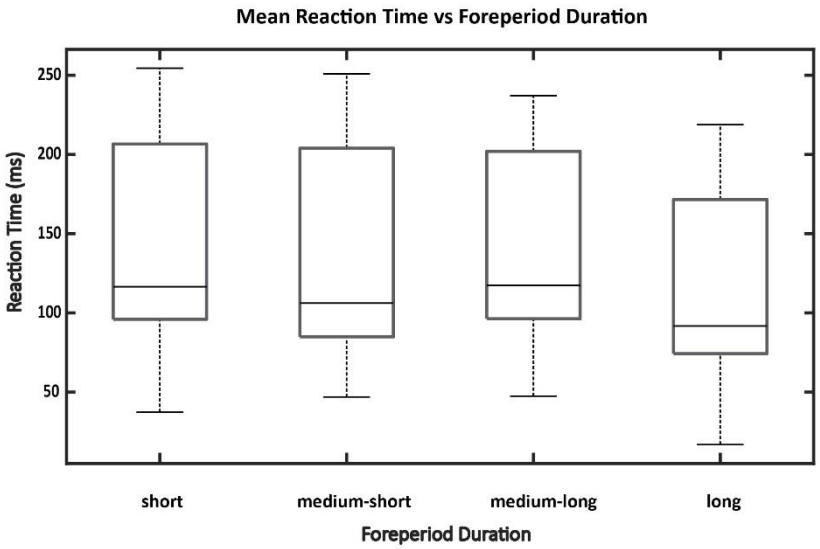


Fig.3.8. Reaction Time vs Foreperiod duration. Comparison of 4 FPs.

Comparison of RT between 4 types of FPs (See Table.3.2.); Short FPs (200,500ms),medium-short FPs (500,1000ms), medium-long FPs (1500ms), long FPs (1900,2000ms);animal n= 3, sessions n=8; p -value = 0.8597 F=0.25

3.4.4. Influence of ITI duration on reaction time

In the last step of analysis, ITIs were taken into account. Fig.3.9 shows the relation between intertrial interval (ITI 1-3s and 2-4s sessions combined) and reaction time. One-way ANOVA was used as the test for significance of results. Detected statistical significance (p-value = 0.003, F = 8.51) was further analysed using a post hoc test to establish between which ITIs significant difference in RTs occurred. Tukey's test showed differences between pairs short-medium (p value=0.003) and medium-long (p value=0.0049).

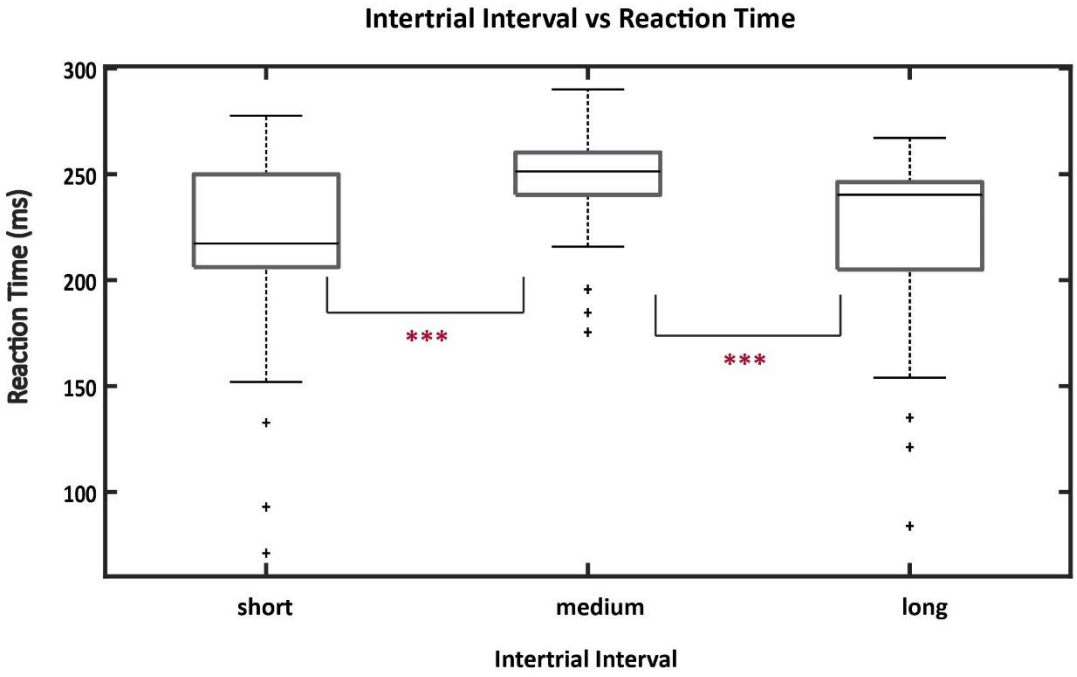


Fig.3.9. Influence of ITI duration on mean reaction time

RT vs ITI. Short:1&2s, medium:2&3s, long:3&4s combined;animal n=5, sessions n=46; p-value = 0.003, F=8.51

3.5. Summary

In Chapter 3, the stages of development of a behavioural paradigm, the Noise Detection Task, were presented and used for the analysis of temporal expectations. Data for both selected animals and the cohort was presented in Pretraining subsections, whereas NDT was used to examine the relation between different FPs/ITIs and RTs. Following the Pretraining1, which the animals were able to complete within no more than 4 sessions, results from Pretraining2 were shown where animals could be divided into slow and fast-learners. Although fast-learners were able to master the task within just a few sessions, obtaining high hit rate and fast RTs, slow-learners needed numerous sessions of training while obtaining modest final results, especially in hit rate. This discrepancy most probably followed into NDT. The analysis of temporal expectations was made using different ratios of presentation of FPs of different duration. Additionally, the effect of a higher number

of presented FPs was tested. Statistical analysis (t-test) revealed a statistically significant difference in RTsbetween FPs presented at different ratios in 2 opposing schemes. As this effect was not present for equal distribution ratio (neither for 2 nor for 4 FPs), it appears that FP presentation ratio and not FP duration played a key role in modulating animal's RT. Animals performed faster after less frequent FP, independently of its duration.. Additionally, the dependency between ITI and RT wastested. One-way ANOVA analysis showed a significant difference in reaction times. Further post hoc analysis, Tukey's test, showed that the statistically significant difference in reaction times applies to pairs short-medium and medium-long ITIs. The significance of temporal expectations results is further discussed in Chapter 6. In Chapter 4, a modified version of NDT was combined with chemogenetics to study the effects of manipulation of auditory cortex activity on behavioural outcomes.

3.6 Caveats and limitations

In Pretraining1, the majority of animals completed the task without a problem within 2-3 sessions. Worse performance of those that needed additional sessions originates most probably from stress related to head-fixed conditions and water deprivation schemes.

In Pretraining2, despite the decrease of reaction times in subsequent sessions indicating progress in training as well as increase of hit rate in initial sessions, the performance in later sessions was characterised by day to day variability and moderate hit rate as seen in the example of slow-learner. These shortcomings in performance may be related to various factors, such as levels of motivation and impulsivity, stress related to water deprivation, a slow pace of adjustment to more difficult conditions (longer ITIs and shorter licking windows) or general low reaction time.

In NDT the main factors that could influence animals' performance were: levels of motivation and impulsivity, stress related to the head-fixed condition and changes in the paradigm's parameters when behavioural output under different conditions was tested.

The motivation of animal is vital for performing a task as it influences the rate of animal's response (Salamone and Correa, 2012). In the Go/ No-Go paradigm an animal is capable of performing numerous trials that can reach even several hundred over a session. However, natural biases such as motivational level can influence the behaviour of an animal (Komiyama et al., 2010). This means, that over a course of a single session animal can experience various motivational states which can modulate its responses. As, typically, outcomes of trials are averaged for each session the dynamic of motivation is not included in analysis what can blur the results (Busse et al., 2011). In a study by Berditchevskaia et al. in a Go/No-Go discrimination task it was shown that the behaviour at the beginning of a session is caused mostly by over-motivation, whereas at the end of the session the balance shifts towards goal-directed behaviour. Therefore, they suggested that although deprivation is crucial in the learning phase, it's influence can be negative once the task was learned. They concluded that the middle part of a session is characterised by optimal behaviour, with high hit rate related to both engagement level and good discrimination. They confirmed these results by evaluating lick rate which, at the end of the session was lower but applied in a more strategic way (Berditchevskaia et al., 2016).

Food and water restriction have been used in numerous studies as a factor increasing motivation levels (Toth and Gardiner, 2000; Tucci et al., 2006; Bekkevold et al., 2013; Guo et al., 2014). Rodents cope better with water restriction then food restriction, which can lead to health problems already in the initial phase of training (Treichler and Hall, 1962). Water restriction is vital to maintain a significant level of motivation in animals throughout the training period (Bodyak and Slotnick, 1999).

The distance between lickometer and mouse is an important parameter, as when positioned too close can elicit compulsive licking, while when too far away lowers the level of animal's motivation (Guo et al., 2014). In the experiments described in this thesis the position of lickometer was adjusted to each animal separately as well as during subsequent sessions, where especially at the beginning of the training animals displayed variability in preferred distance from lickometer. For the future refinement of the method, increasing the distance from lickometer in more advanced sessions

could be considered to decrease compulsive licking. However, the caveat of such action is a decrease in animal's performance.

Various studies use very strict water restriction schemes delivering 1ml of water per day and maintain water deprivation 7 days a week, which often leads to loss of 20% of initial body weight (Guo et al., 2014). Guo et al. claim that at least 15% body weight decrease is necessary for robust behaviour and that animal's health should be investigated in depth after reaching 70% pre-deprivation body weight. However, such strict methods can't be applied everywhere. In the experiments performed for this thesis 20% body weight loss was a limit indicating the need for termination of experiment or change in water restriction scheme. Here, in the water restriction scheme in different batches animals were allowed between 45min and 2h post training access to water. Also, water restriction using hydrogel was tested. Animals were mostly restricted from Sun to Fri although 7days a week restriction has also been tested. Free access to water from Fri to Sun morning did not impair animals' performance on Mon sessions. Apart from weight loss, strict water restriction may also increase the level of compulsive licking. For the future refinement of the procedures, hydrogel option or automated systems for precise water delivery could be investigated more in-depth. Limiting the time of water availability is important, however depending on stress levels, especially after initial sessions, not all animal try to rehydrate straightaway. Another possible change is implementing water restriction a week before the initiation of the training. However, as mice need at least 5-days recovery period and training period can last up to 2 months, additional week increases the possibility of infection or instability of head-post which is crucial not only for behavioural studies but also for electrophysiological recordings that were performed in part of the animals after completing behavioural training.

As for the type of reward, sweet water has been proved to yield better results than pure water (Guo et al., 2014). The other parameter that could be changed is the amount of delivered reward. This parameter was set individually for each animal depending on behaviour in initial sessions of Pretraining. Nonetheless, the caveat of

increased reward amount may lead to a decrease in the number of sessions performed by an animal.

Chapter 4. Results – Effects of chemogenetic manipulation in the auditory cortex on task performance.

4.1. Objectives

Experiments described in this chapter were dedicated to investigate the necessity of the auditory cortex for sound detection and the influence of impairment of this structure on behavioural task outcomes under the NDT paradigm, described previously both in Chapter 2 and Chapter 3. To achieve auditory cortex impairment, chemogenetics was applied. In this technique, viral vector containing, in these experiments, activating DREADDs is injected into the structure. Several weeks later DREADDs are activated by ip injection of its ligand-CNO. In presented experiments inhibitory interneurons, parvalbumin-positive cells, were targeted, therefore DREADDs increased their activity which subsequently should have led to a decrease/inhibition of the activity of principal cells. From a behavioural point of view, the incorporation of chemogenetics was assumed to decrease the hit rate and additionally increase RTs.

The neural underpinnings of those results were further investigated in Chapter 5 where electrophysiological recordings in anaesthetised animals were carried out during sound presentation both before and after modulation of the auditory cortex activity using chemogenetics.

4.2. Overview

In this chapter's overview, general data on animals used in the following experiments is presented. At the beginning of each section, inclusion criteria for data sets are presented. Section 4.3. shows results for the Pretraining sessions, containing both Pretraining1 and Pretraining2. Subsection 4.3.1. presents exemplary results from a singular animal, whereas subsection 4.3.2. presents pooled data from a group of animals meeting the initial criteria for Pretraining1 as well as an example of a slow-learner and a fast-learner in Pretraining2.

Section 4.4. shows data for the final behavioural task, Noise Detection Task (NDT), under 3 conditions: control (CTL) – undisturbed behaviour; modulation with chemogenetics- under influence of pAAV-hSyn-DIO-hM3D(Gq)-mCherry expressed in PV^+ cells of auditory cortex and activated via CNO administration - (CNO) and after intraperitoneal vehicle (0.9% saline) administration - (SLN). Subsection 4.4.1. presents data from a selected animal whereas subsection 4.4.2. shows pooled results from a group of animals. In Section 4.5. histological data from experiments is provided to illustrate area infected by the virus. Section 4.6. summarises results presented in this chapter and finally section 4.7. discusses the limitations of the applied method.

The below tables present general information about the animals used in experiments described in this chapter. Table 4.1. presents basic information about a single animal (#83) used as an example whose results from Pre1, Pre2 and NDT are discussed in the following subsections. Table 4.2. summaries information about all animals used for experiments described in Chapter 4.

ID	#83	**Chemogenetics**	Yes
Mouse line	PV-Cre	**Pretraining (days)**	8
Gender	Male	**NDT (days)**	23
Age (surgery)	10 weeks	**Electrophysiology**	Yes
Weight Pre/Post Surgery	27.0g / 27.8g	**Surgery to sacrifice (days)**	57

Table.4.1. Animal's information – selected example

Number of animals	7	**Min-Max Post-surgery weight (g)**	24.5-30.8
Mouse line	PV-Cre	**DREADDs type**	hM3D(Gq)-mCherry
Gender	male	**Pretraining Min-Max (days)**	8-11

Min-Max age (weeks)	8-10 weeks	**NDT Min-Max (days)**	10-21
Min-Max Pre-surgery weight(g)	23.1-29.8	**Surgery- sacrifice Min-Max (days)**	53-65

Table.4.2. All animals' information

Figure 4.1. illustrates the percentage of animals in different stages of the experiment. Out of 15 animals that underwent the surgery, 7 completed the full task performing in both Pretraining stages and the final NDT stage of the task under 3conditions stated previously. The decrease in the number of animals in subsequent stages of the experiment was due to detachment of the head-cap or post-surgery infection of the animal.

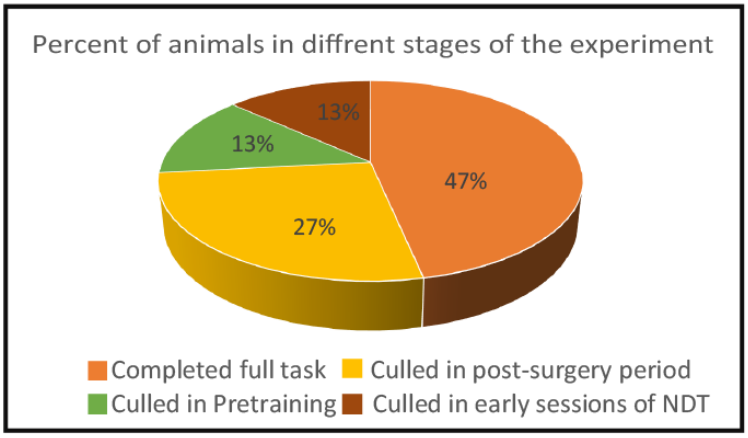


Fig.4.1. Percent of animals in different stages of the experiment

Figure shows how many of the initial 15 animals that underwent surgery entered subsequent stages of experiment. **Orange** (47%, 7 animals) completed full task; **Yellow** (27%, 4 animals) culled in post-surgery period; **Green** (13%, 2 animals) culled during Pretraining, **Red** (13%, 2 animals) culled in early sessions of NDT.

4.3. Pretraining

Subsections 4.3.1. and 4.3.2. show Pretraining1 and Pretraining2 sessions, , for the animal used as an example all cohort as well as for an example of a slow and a fast-learner.

The aim of Pretraining is to shape an animal's behaviour so that its responses are robust and fast. This stage is very important for the experiment as behaviours

acquired at this point will be used at NDT stage, where the ultimate results are collected.

4.3.1. Single animal example

Data shown in Pretraining1 and Pretraining2 sessions presents results from the same animal.

4.3.1.1. Pretraining1

The aim of this part of training was to teach the animal the connection between its reaction – making a lick - and obtaining the reward. At this stage, timing of action was not important. The task was completed if the animal made at least 100 licks in 2 consecutive sessions (Fig.4.2.).

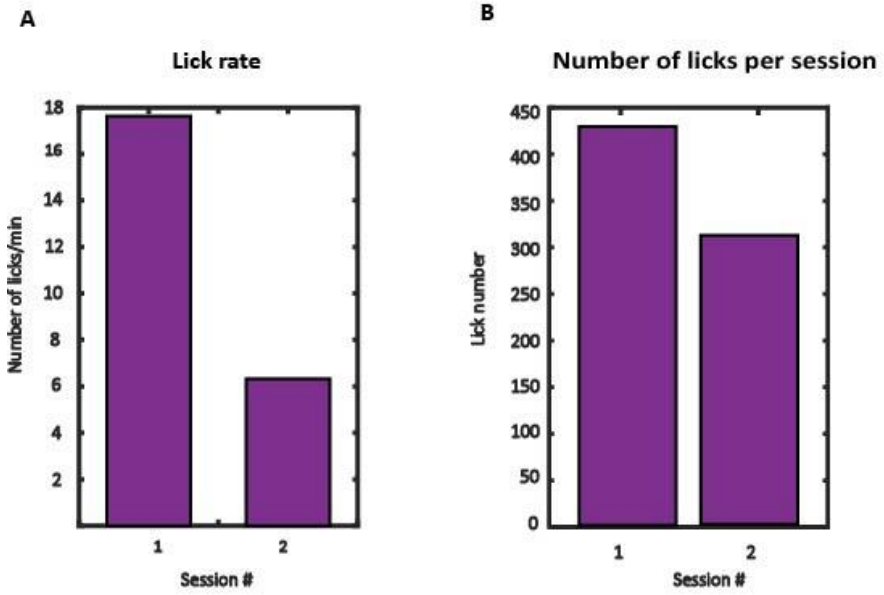


Fig.4.2. Pretraining1. An example from single animal.

A: Lick rate (number of licks per minute) during Pretraining1 sessions
B: Number of licks during Pretreining1 sessions.

This animal, despite having longer intervals between licks in Session 2, presented robust behaviour in both sessions, making hundreds of responses in each of them.

This result indicated that the animal learned the connection between making a lick and obtaining reward, therefore it was ready for Pretraining2.

4.3.1.2. Pretraining2

The aim of this stage was to further shape behaviour of the animal. In addition to monitoring hit rate (success rate of responses), reaction time was another crucial parameter. *Licking window*- time to obtain reward - was shortened within each session as well as in almost every subsequent session until it reached the targeted 1s window in final sessions of Pretraining2 (Fig. 4.3). The objective of this training was that the animal would be capable of completing sessions with over 50% hit rate while the *licking window* was set to 1s.

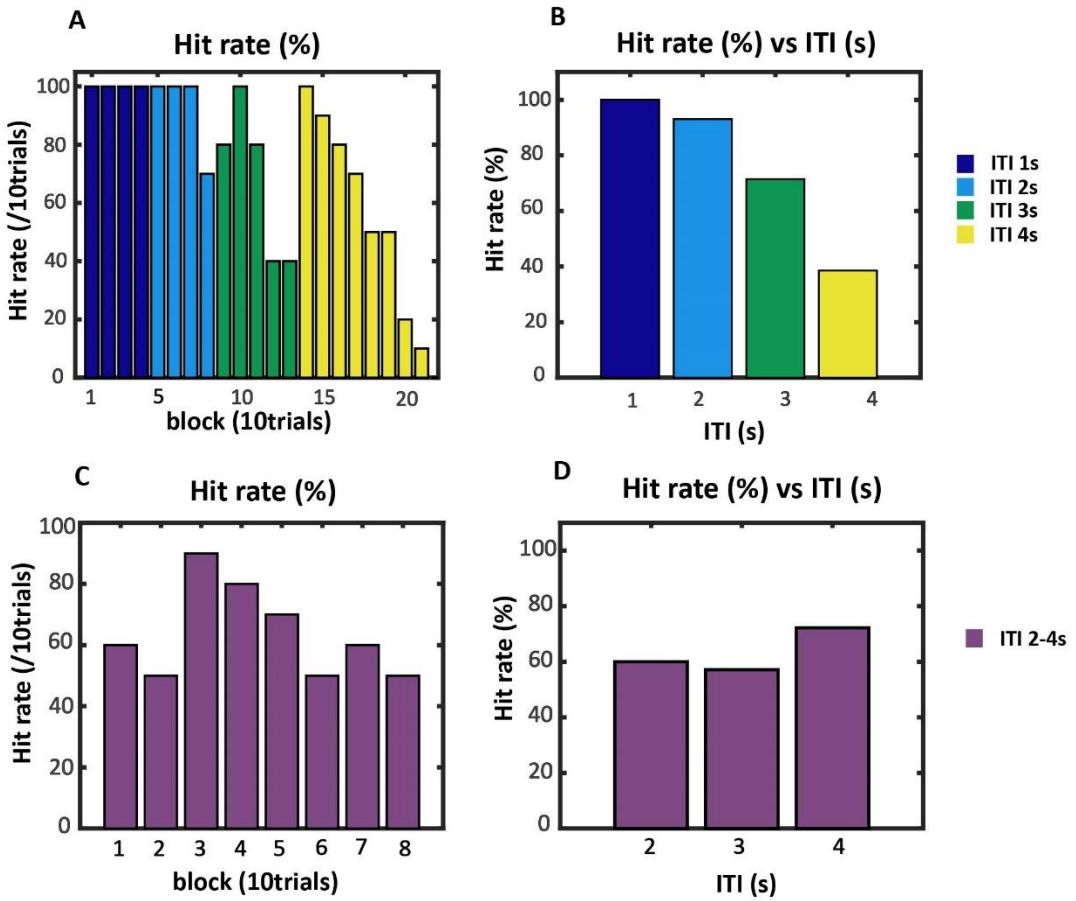


Fig.4.3. Two sessions form Pretraining2

A: *Updating mode* session. Success rate along session

B: *Updating mode* session. Correlation between success rate and ITI

ITIs were increasing by 1s after every 40 successful trials. LW started at 7s and decreased by 1s simultaneously with ITI increase.

C: *Random mode* session. Success rate along session

D: *Random mode* session. Correlation between success rate and ITI

Randomly applied ITIs had span between 2 and 4s. LW in all trials equalled to 1s.

In Fig. 4.3. two sessions, from beginning (updating mode) and end (random mode) of Pretraining2 stage, are presented. Panel A shows the hit rate (%) for a session where the animal started with an 7s licking window and 1s ITI and ended with a 5s licking window and 4s ITI. Panel B shows the relation between hit rate and ITI duration. Panel C and D show respective results for the final session of Pretraining2 where the licking window was constantly set to 1s while ITI was set to 2-4s.

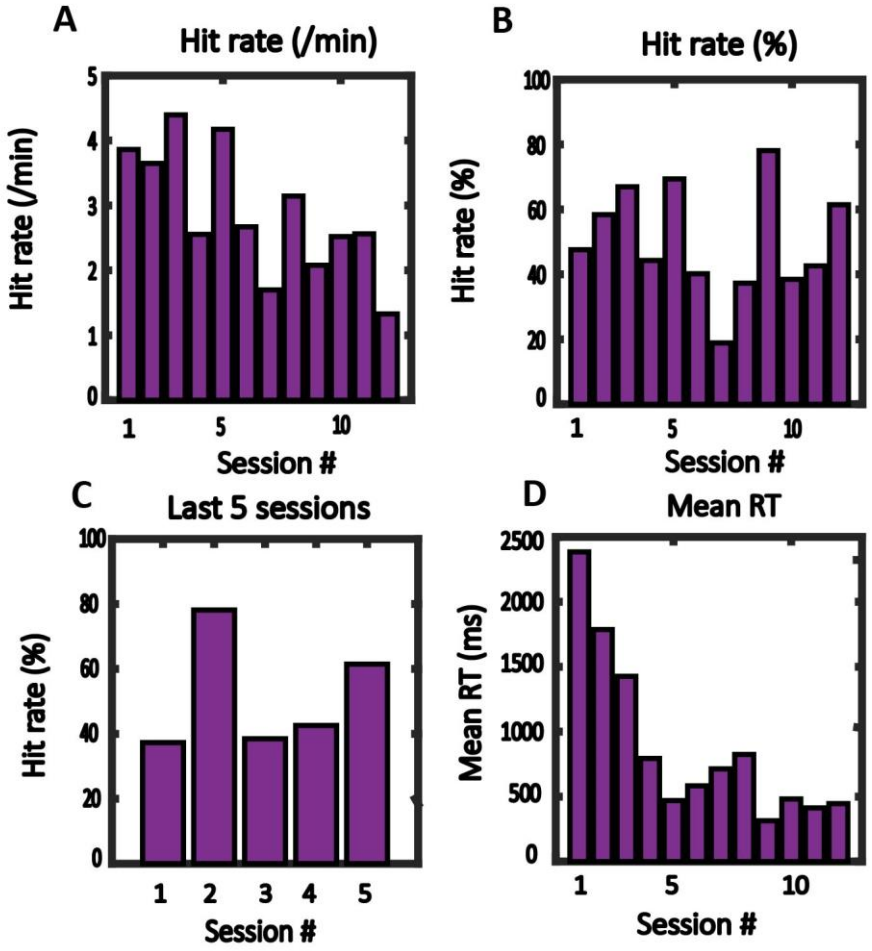


Fig.4.4. Pretraining2. An example from a single animal.

A: Lick rate per minute on subsequent sessions **B:** Hit rate on subsequent sessions **C:** Hit rate on last 5 subsequent sessions **D:** Mean reaction time on subsequent sessions (ms)

The results from Pretraining2 showed the robustness of the animal's behaviour, evident in the number of licks and improvement in reaction time of the action (Fig.4.4.). Although the hit rate showed variability in subsequent sessions (Fig.4.4.B), the last 5 sessions showed a steady hit rate (Fig.4.4.C). The variability along Pretraining2 might partially originate from the change in parameters in subsequent sessions (e.g., longer ITIs, shorter licking window), as shown in Fig.4.3., which

required more sessions to adjust to the more difficult task. The reaction time of the animal decreased steadily and after several sessions, the mean reaction time (Fig.4.4. D) was shorter than 1s.

Overall, the results from Pretraining confirmed that the animal learned the association between licking behaviour and liquid reward and proved the behaviour was robust and fast enough to enter the last stage of experiment, NDT.

4.3.2. Pooled data from all animals. Slow and fast-learner examples

Below, results from all 7 animals are presented similarly to Subsection 4.3.1. For Pretraining2 an example of a slow-learner and a fast-learner is given.

4.3.2.1. Pretraining1

To complete Pretraining1, each of the animals had to complete two subsequent sessions with at least 100 licks.

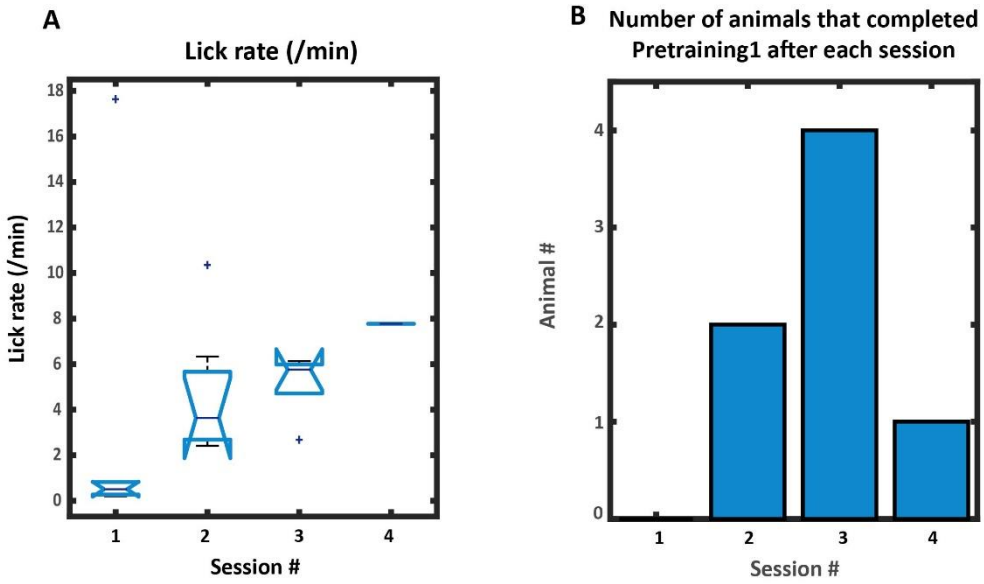


Fig.4.5. Pretraining1. Pooled data from 7 animals.

A: Lick rate per minute during Pretraining1 sessions
B: Number of animals that completed Pretraining1 in subsequent sessions.

The majority of the animals needed 3 sessions to complete the task (Fig.4.5. B). Nevertheless, the robustness of behaviour was increasing in subsequent sessions (Fig.4.5. A), including for the only animal that required 4 sessions of Pretraining1.

4.3.2.2. Pretraining2

Here, as previously described in Subsection 4.3.1.2., the ultimate goal was to teach the animal to make licks during a 1s window.

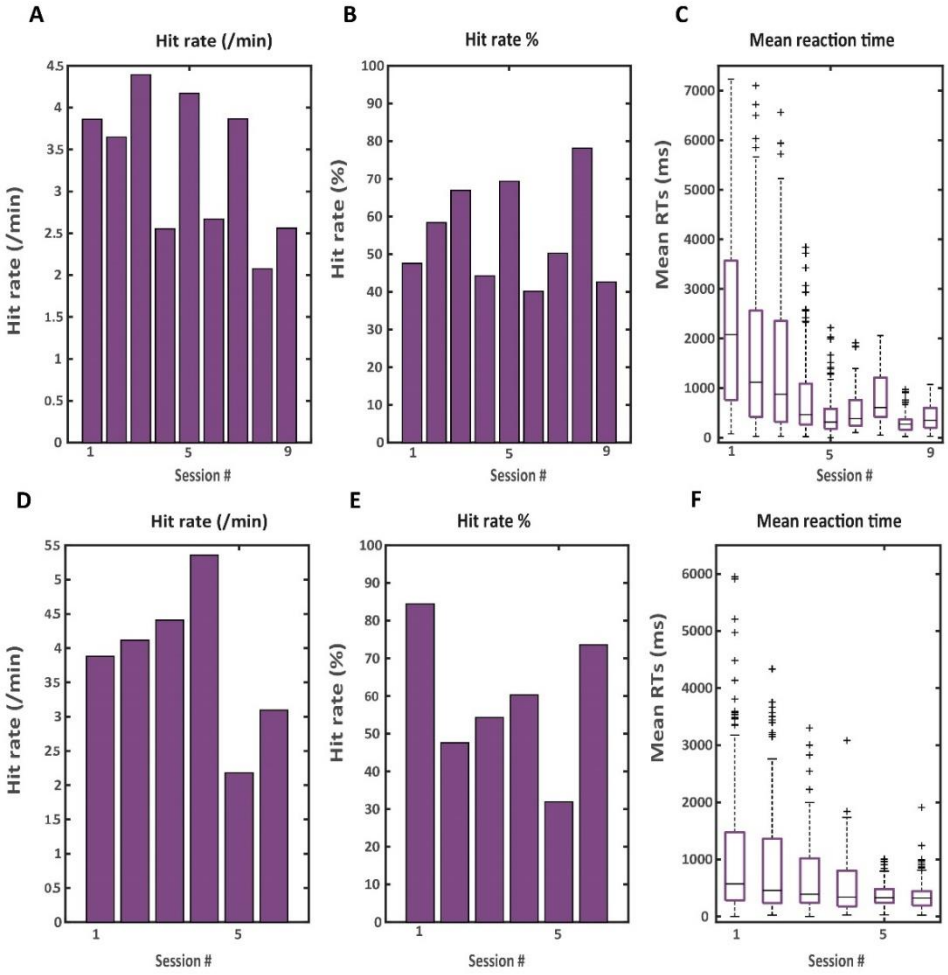


Fig.4.6. Pretraining2. Examples of a slow-learner and a fast-learner

A-C: Slow-learner (animal n=1)A: Hit rate per minute in subsequent sessions **B:** Hit rate per session (%) **C:** Mean reaction time (ms) **D_F: Fast-learner (animal n=1) D:** Hit rate per minute in subsequent sessions **E:** Hit rate per session (%) **F:** Mean reaction time per session (ms)

The hit rate was showing variability over sessions and was decreasing during more difficult sessions (Fig.4.6.B,E) which,, as explained on the example of Fig.4.3, was related to the changes in parameters where each subsequent session had longer ITIs and shorter licking windows. Those changes were even more pronounced for slow-learner (Fig.4.6.B).. Reaction times were steadily decreasing over subsequent sessions with better results for the fast-learner (Fig.4.6.F) than slow-learner (Fig.4.6.C). Decreasing RTs reaching sub-second values and ability to perform with over 60% hit rate allowed to move animals to NDT.

4.4. Noise Detection Task (NDT) in baseline and after modulating the activity of auditory cortex with chemogenetics

The aim of this experiment was to examine the necessity of the auditory cortex for sound detection. Therefore, NDT (described previously in Chapters2 and 3) was implemented under 3 conditions: Control (CTL) undisturbed paradigm, after impairment of auditory cortex activity using chemogenetics (CNO) and after intraperitoneal injections of vehicle, 0.9% saline (SLN). Below, the changes in behavioural outcomes under those three conditions are described.

4.4.1. Single animal example

In this subsection results for a single animal are presented. The inclusion criteria for CTL sessions were at least 100 trials per session, 60% hit rate per session and presentation of stimuli with at least 3 different sound intensities.

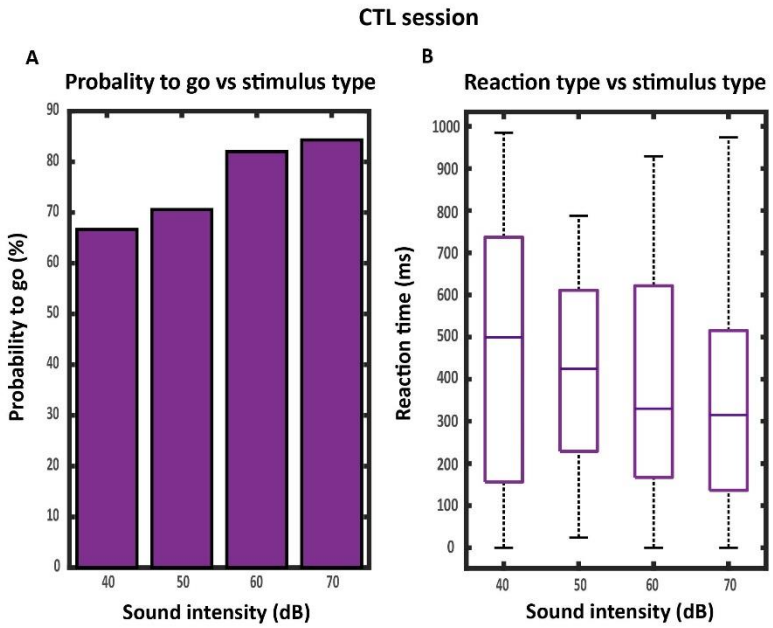


Fig.4.7. Example of a CTL session.

A: Probability to go (in percent) against sound instensity
B: Mean reaction time (ms) against sound intensity

In this control session of NDT, hit rate was increasing with soud intensity meaning the animal was more likely to respond to louder sounds (Fig.4.7.A). The reaction time does not show a clear pattern, as confirmed by one-way ANOVA (p-value = 0.3991, F=0.99) (Fig.4.7.B).

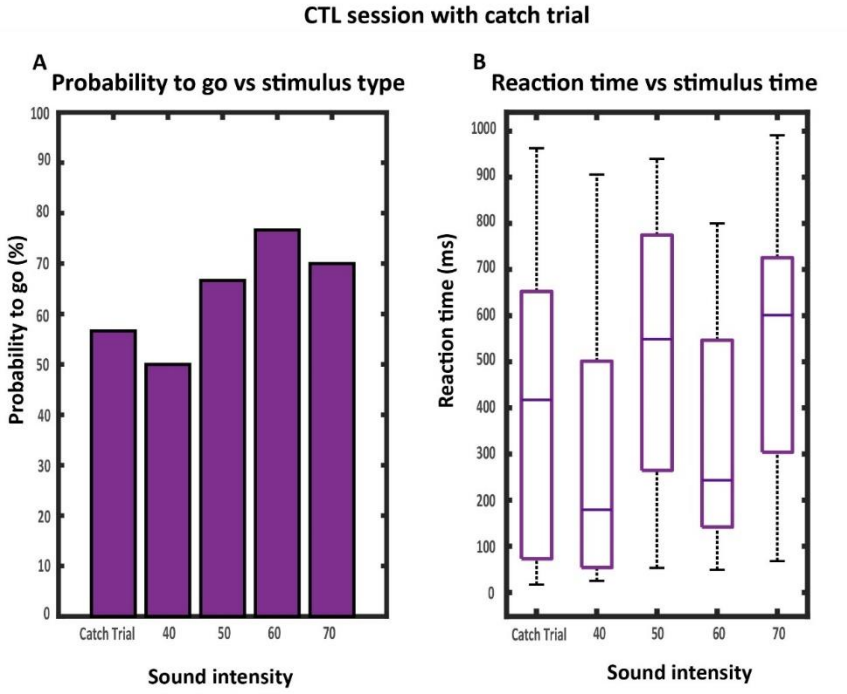


Fig. 4.8. Example of the CTL session with catch trial.

A) Probability to go (in percent) against sound instensity
B) Mean reaction time (ms) against sound intensity

The additional parameter presented in this session (Fig.4.8.) comparing to the previous CTL session, is the implementation of catch trials. During the catch trial, no sound is presented and therefore it helps to estimate the level of the animal's impulsivity when its hit rate is compared against hit rates following stimulus presentation at different sound intensities. The catch trial was implemented during the last session of CTL condition when the animal was already familiar with the task. In this case the animal was making responses on over 50% of catch trials (Fig.4.8. A). Although it's a high level, that may be connected to either animal's impulsivity or a strategy to obtain reward, it's still lower than responses provoked by sounds of high intensity. This session lacks a clear pattern for the timing of responses (Fig.4.8. B) and presents a broad spectrum, including the catch trials (ANOVA pvalue = 0.0955, F= 2.04).

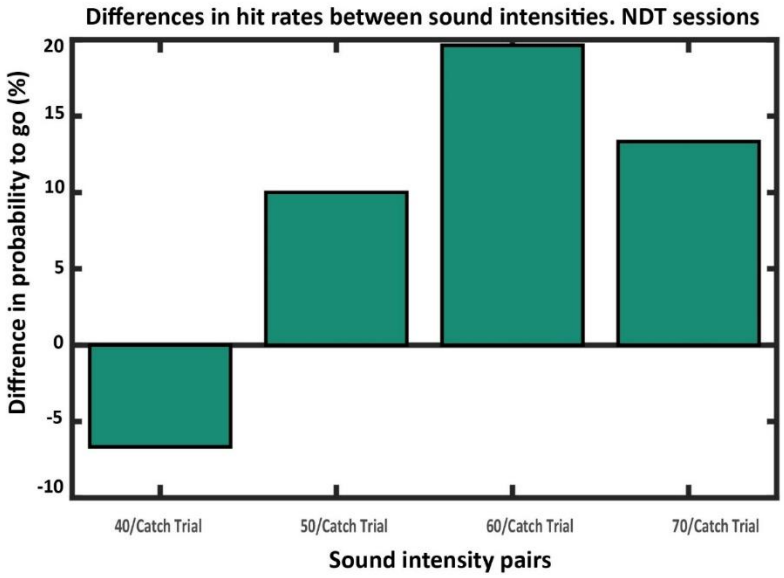


Fig. 4.9. Differences in hit rate for all sound intensities against catch trial.

Each column represents difference (in percent) between hit rate in trials with particular sound intensity and hit rate for catch trials.

Figure 4.9. shows that although hit rates for 50-70dB were higher than for the catch trial, it did not apply for 40dB. In this case, it's possible that due to the difficulty of the trials with the lowest sound intensity, the animal had low efficacy on those trials. A higher hit rate on catch trials may represent the strategy of the animal – trying to obtain the reward even in the absence of the sound which at the same time may represent its impulsivity.

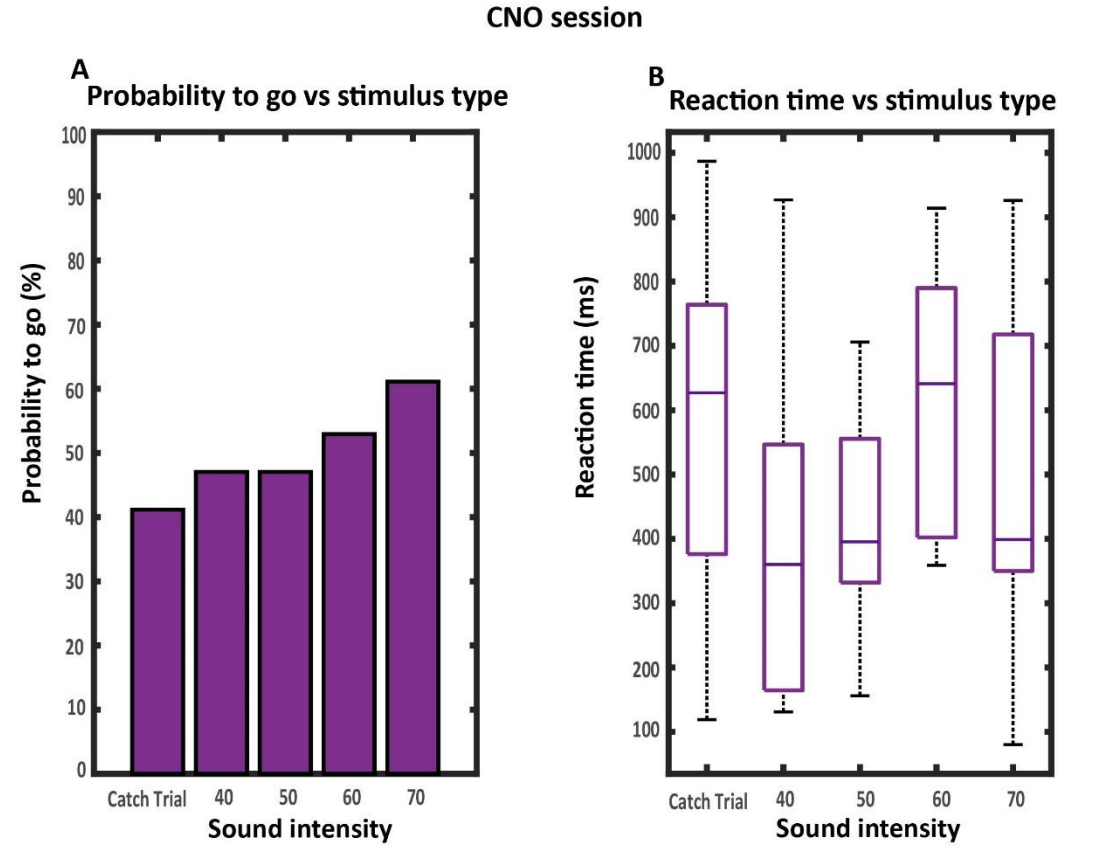


Fig.4.10. Example of a CNO session.

A: Probability to go (in percent) against sound instensity
B: Reaction time (ms) against sound intensity

Here, the results for a CNO session are presented (Fig.4.10.). Catch trials were presented in all CNO sessions. Panel A (Fig.4.10.) presents hit rate where, as previously, louder sounds elicited response more frequently. However, the hit rate is lower than for both CTL sessions presented above. Probability to go for catch trial is high again (Fig.4.10. A) but lower than for any sound intensity presented. Reaction time does not show a clear pattern as confirmed by ANOVA (p value = 0.4016, F= 1.04).

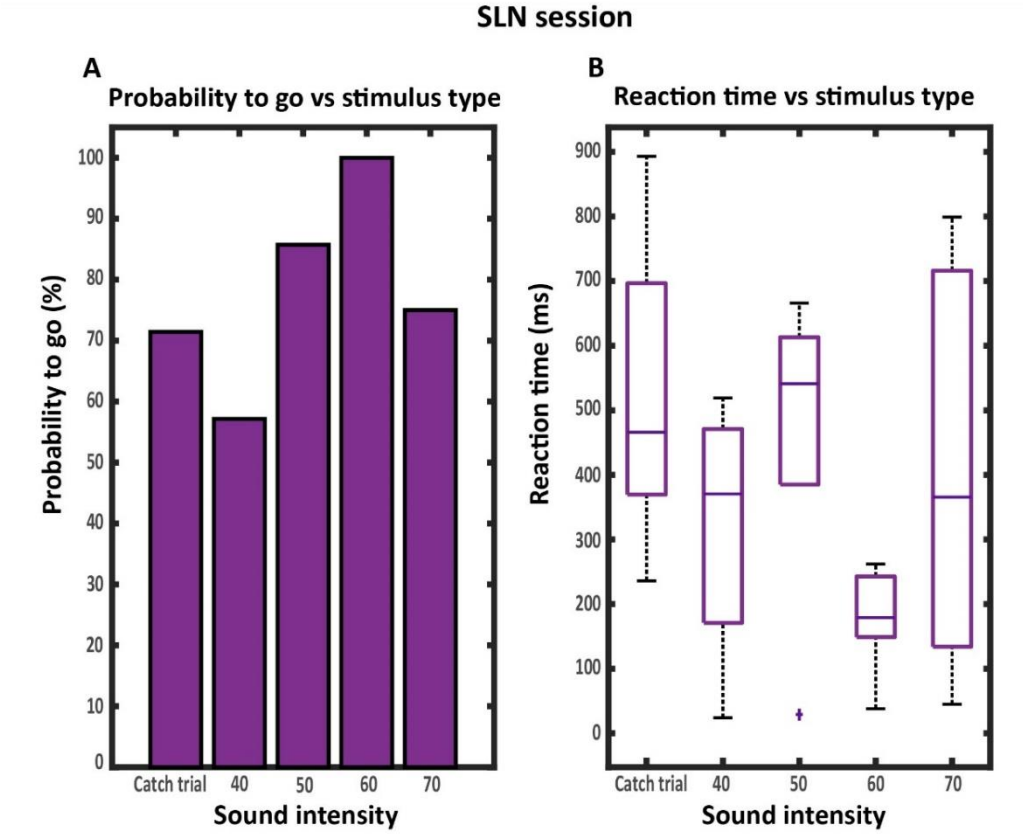


Fig.4.11. Example of a SLN session.

A: Probability to go (in percent) against sound instensity
B: Reaction time (ms) against sound intensity

In SLN sessions, intraperitoneal injections of saline were applied as a control for injections applied in CNO sessions. Probability to go in SLN session (Fig.4.11. A) was higher for all sound intensities comparing to the CNO session. Although the probability to go for catch trials was lower than for 70, 60 and 50dB it was higher than in CTL and CNO groups. The reaction time (Fig.4.11. B) pattern for different sound intensities again did not show statistically significant differences as confirmed by ANOVA (p value = 0.0993, F=2.21).

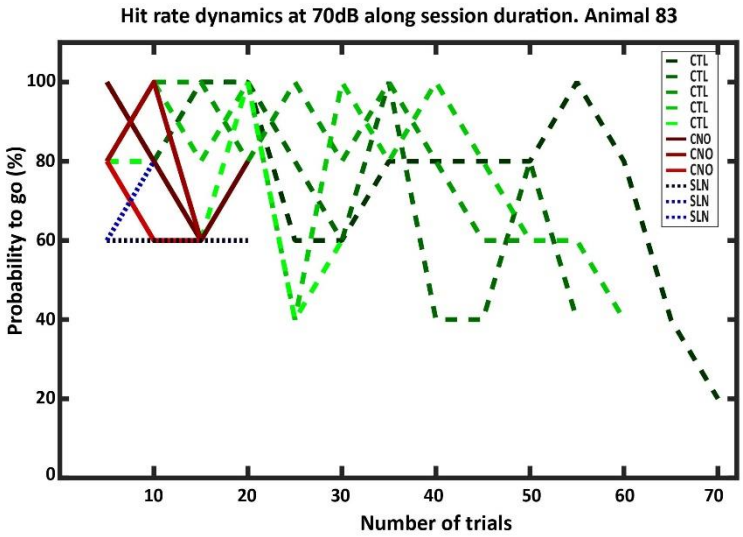


Fig.4.12. Hit rate dynamics along session at 70dB trials

Probability to go during 70dB trials along session. animal n=1 Shades of **green**: CTL sessions; shades of **red**: CNO sessions; shades of **blue**: SLN sessions

In figure 4.12., trials at 70dB were extracted from each session and hit rate for them was presented. Number of trials shows that animals performed for the longest time on CTL sessions while on CNO and SLN sessions responses were less robust (each session was stopped after 10 subsequent inactive trials). Despite the fluctuations in hit rate present in every session, a momentary decrease in behaviour does not impede a rebound in activity later in the session (as shown in individual lines, each presenting different session).

Overall, the hit rate (probability to go) for the presented CTL and SLN sessions was higher than for CNO sessions. Reaction time from singular sessions did not provide a clear pattern in any of the groups. Bearing in mind the variability in behaviour not only within a session but mostly in between sessions, in the next subsection (4.4.2) data from pooled animals fulfilling criteria is presented.

4.4.2. Pooled data from all animals

Below, Figure 4.13. presents how many sessions under each condition (CTL, CNO and SLN) had been performed by each of the animals taken into account during data analysis.

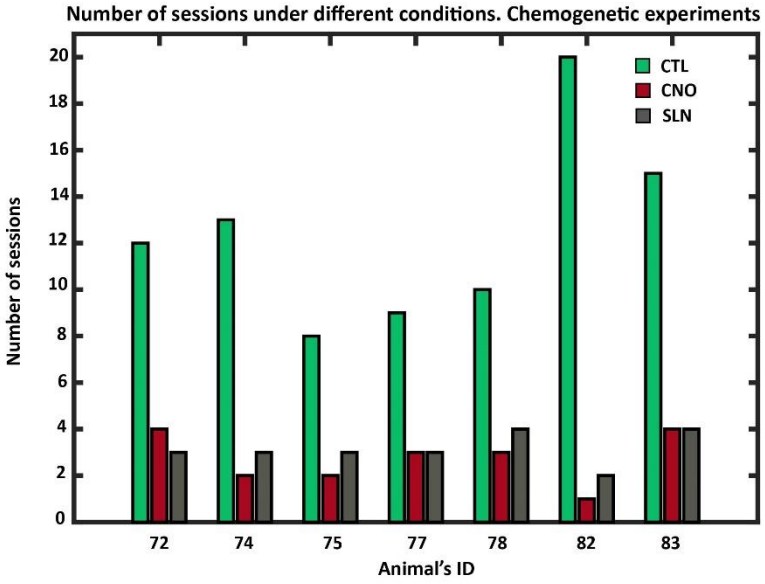


Fig.4.13. Number of sessions under CTL, CNO and SLN conditions. 7 animals

Number of sessions in each condition for each of the 7 animals. **Green** –CTL sessions; **red**- CNO sessions; **grey**- SLN sessions

Below, Fig.4.14 and Fig.4.15 show, respectively, pooled hit rates (probability to go) and reaction times for all sound intensities under 3 conditions (CTL, CNO and SLN).

In Fig.4.14, results across 3 conditions were grouped for each sound intensity. One-way ANOVA was further implemented to test for statistical significance between 3 conditions for each sound intensity. Due to dispersion of results, only the group for 70dB showed a significant difference in probability to go (pvalue = 0.0107, F=7.4). Tukey's test for post-hoc comparisons revealed that the difference affects CTL and CNO groups (pvalue = 0.0104).

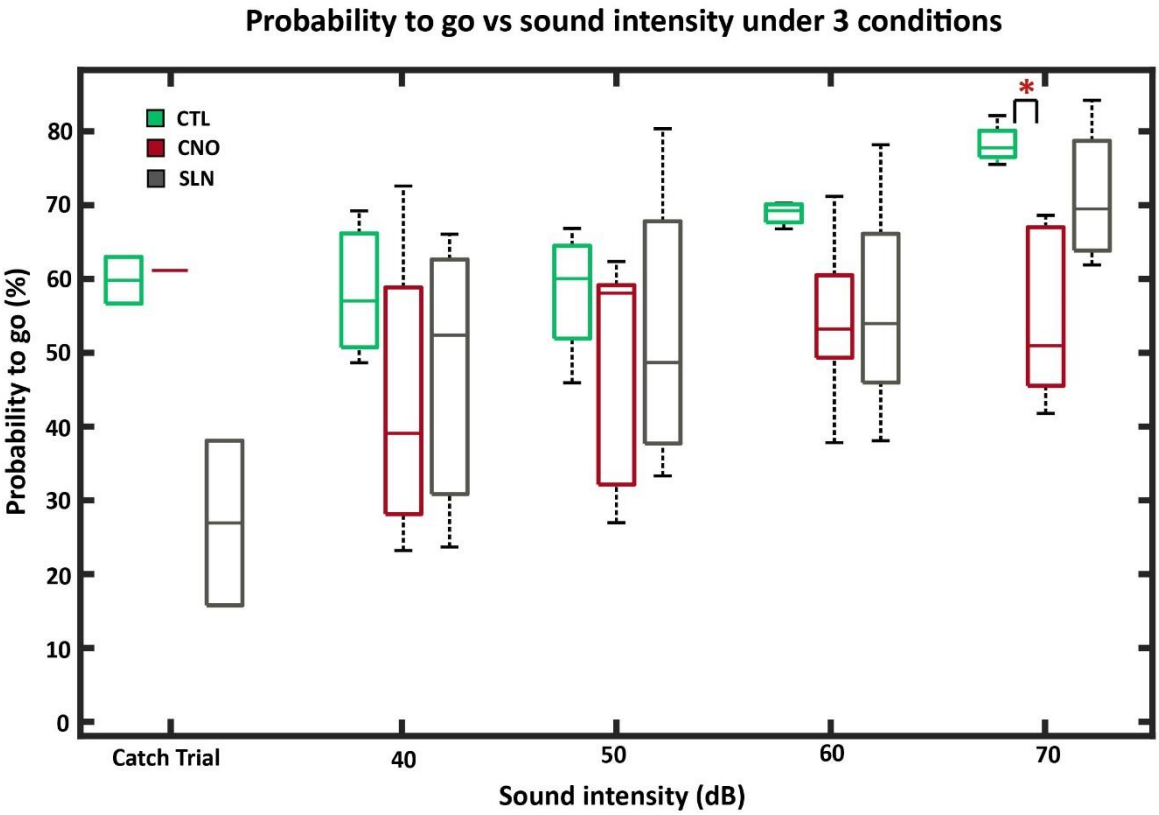


Fig.4.14 Probability to go (%) vs sound intensity (dB) under 3 conditions, pooled data.

Green- Control (CTL), **red** – modulation with chemogenetics (CNO), **grey**-vehicle injections (SLN); ANOVA: 70dB (p value = 0.0107, F=7.4)

In Fig.4.15, reaction times across 3 conditions were grouped for each sound intensity. One-way ANOVA was further implemented for each sound intensity to test for statistical significance. Due to dispersion of results, only the group for 60dB showed significant difference in reaction times (p value = 0.0323, F = 4.93). Tukey's test showed that the difference affects CNO and SLN groups (p-value = 0.0393).

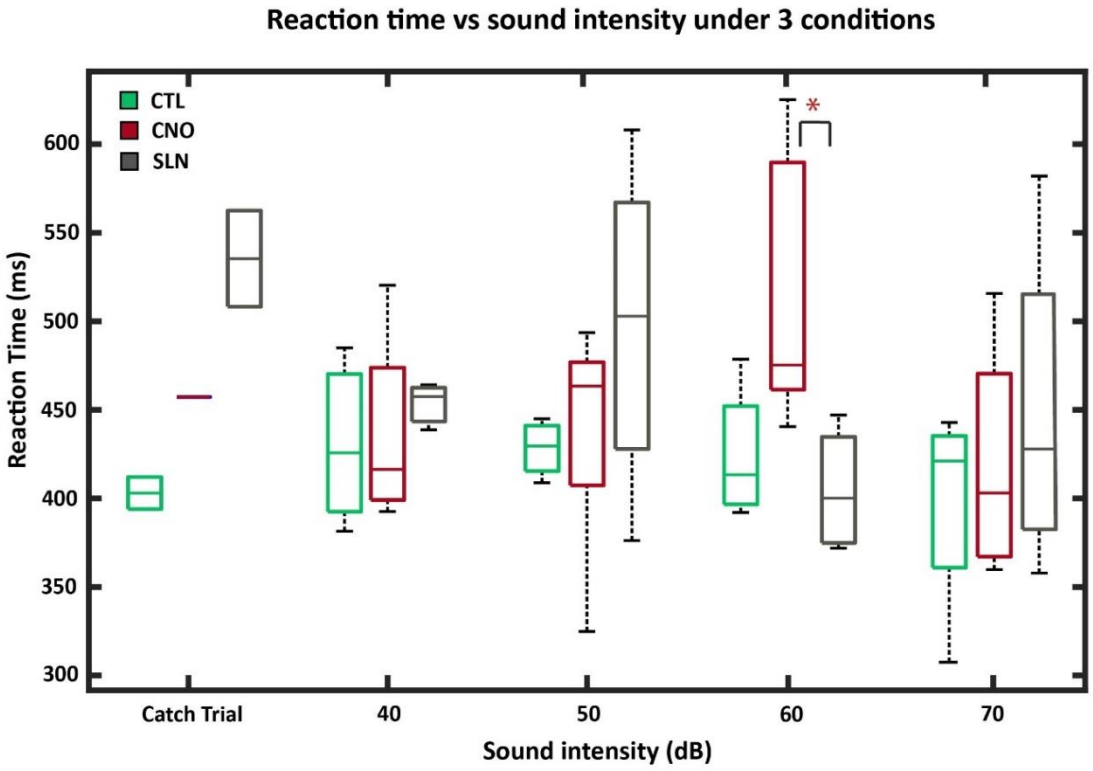


Fig.4.15 Reaction time (ms) vs sound intensity (dB) under 3 conditions, pooled data.

Green- Control (CTL), **red** – modulation with chemogenetics (CNO), **grey**-vehicle injections (SLN); ANOVA 60 dB (p value = 0.0323, F=4.93).

Due to high probability to go in catch trials in part of the sessions, d' (ratio of FA/ overall Hit rate) was calculated for each of 3 types of sessions. FA was most prominent in CTL sessions reaching 11% of overall response rate, followed by 8% in CNO sessions and 7% of overall responses in SLN sessions.

4.5. Histological data

Below, histological data after double staining for each of 7 animals is presented. The aim of this procedure was 3-fold. To check if 1) there was expression of DREADDs in auditory cortex (marked in red by mCherry), 2) PV^+ cells were detected (marked in green) and 3) expression of virus and PV^+ cells overlaid (marked in yellow). The coordinates presented below are based on Paxinos and Franklin's 'The mouse brain in stereotaxic coordinates, 4th edition',2012. The volume of each of 4 injections per animal (apart from mentioned exceptions) equalled to 150nL at a depth of 500µm.

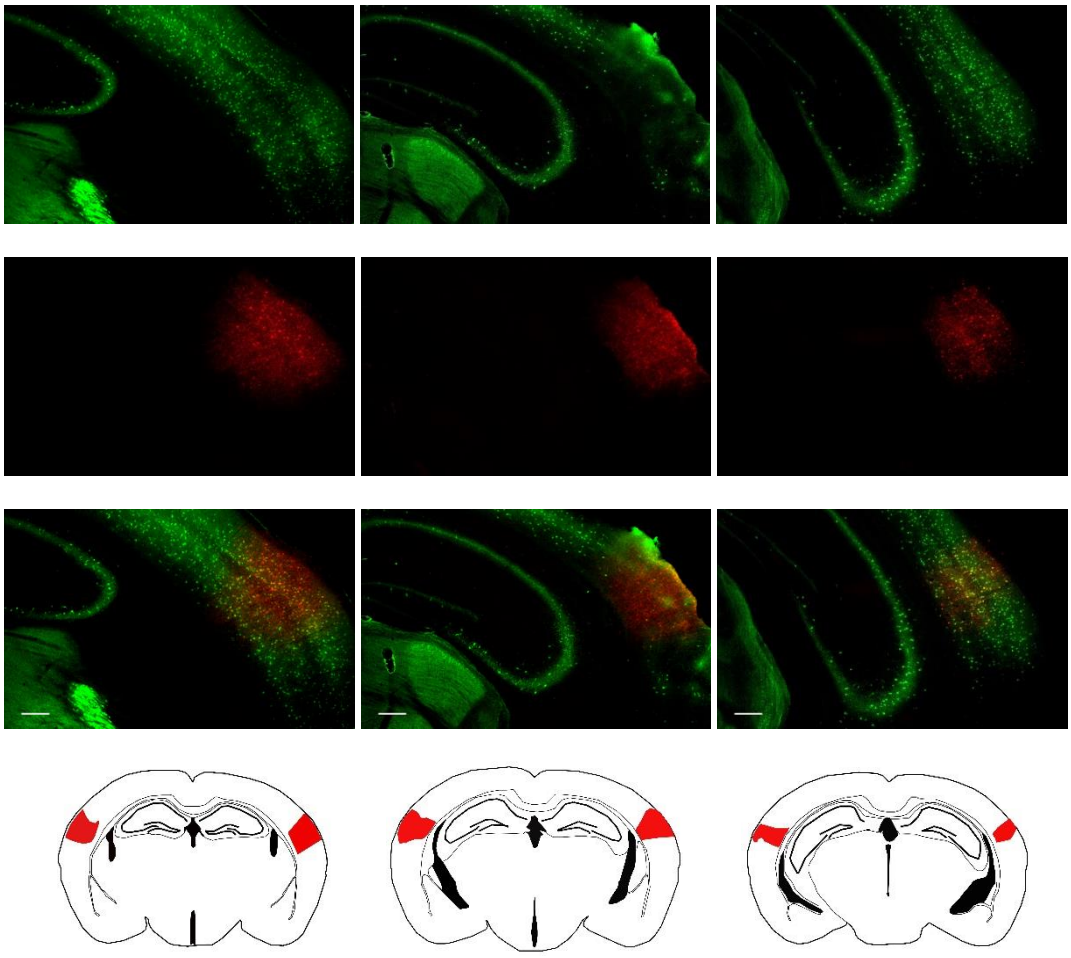


Fig.4.16. Histological analysis. Animal #83

A) Green- PV^+ cells. **B) Red-** mCherry expression. **C)** PV^+ cells and virus overlay D) schemes of virus expression. Bregma: -1.79mm,-2.15mm,-2.45mm . Scale bars 100µm.

Fig.4.16. shows histological data for animal 83, which was presented as an example of a single animal across this chapter. PV cells were visible and abundant (Fig.4.16. A). Expression of virus containing hSyn-DIO-hM3D(Gq)-mCherry was successful (Fig.4.16B). The virus was restricted to the area of the auditory cortex. Areas of expression of PV cells and virus containing the chemogenetics agent were successfully overlaid (Fig.4.16.C). Although the expression of DREADDs is present in auditory cortex (Fig.4.16. D), including part of A1 (from Bregma -1.79mm rostral to -2.79mm caudal) it does not cover the full extension of auditory structures in caudal direction (Bregma -3.63mm). Incomplete coverage of auditory cortex structures by virus containing DREADDs most probably contributed to mild impairment in functioning of the structure.

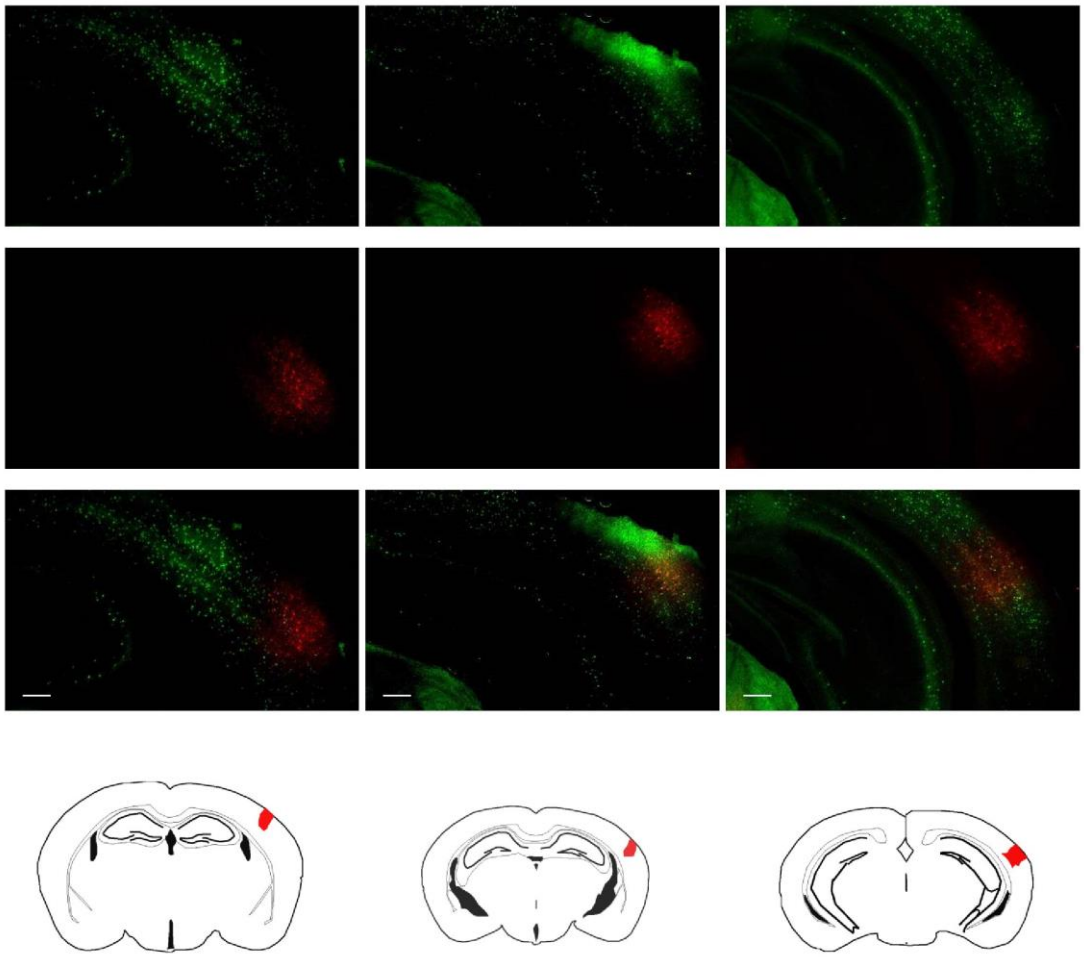


Fig.4.17. Histological analysis. Animal #82. Single injection

A) Green- PV^+ cells. **B) Red-** mCherry expression. **C)** PV^+ cells and virus overlay D) schemes of virus expression. Bregma: -1.67mm, -2.27mm, -2.79mm. Scale bars 100µm.

Due to technical problems, this animal was injected only in one site. As a result of the limited delivery of virus, for which the exact volume couldn't be established, this animal did not contribute to condition CNO in results described in subsection 4.4.2. However, as the animal completed all procedures and all sessions of training, data under CTL and SLN conditions was included in the pooled results.

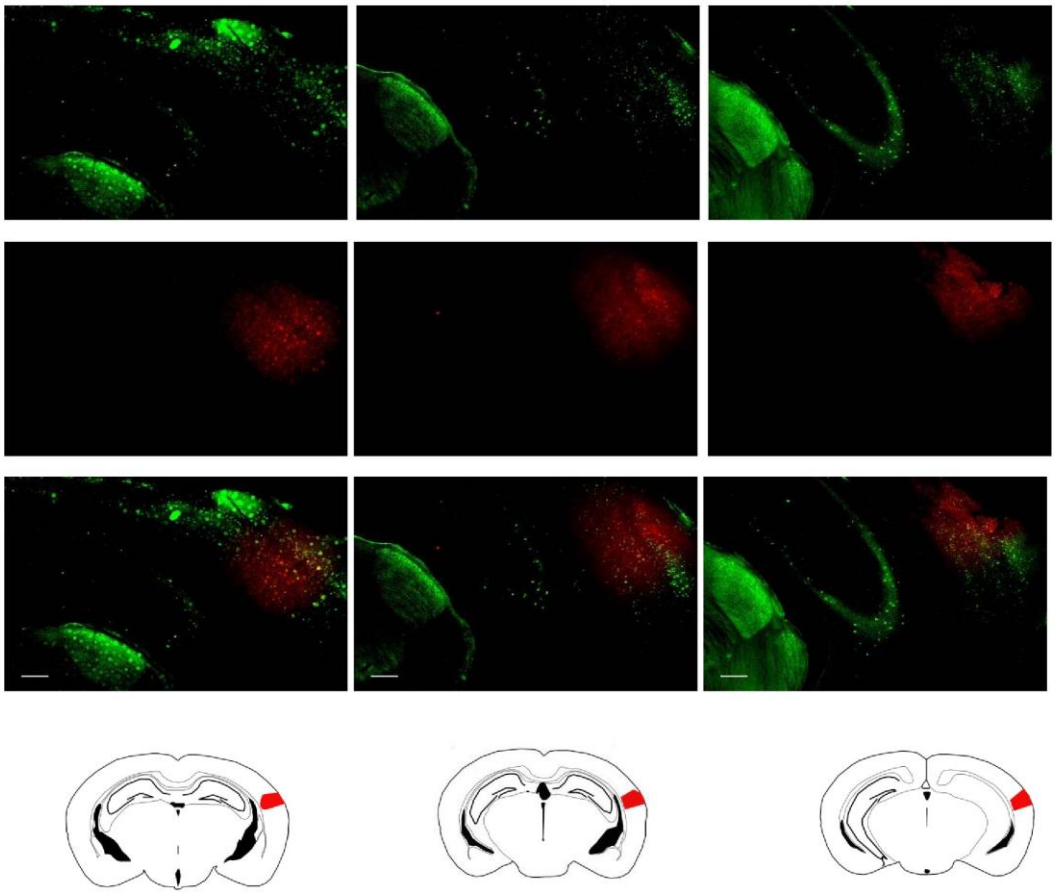


Fig.4.18. Histological analysis. Animal #78. Unilateral injetions

A) Green- PV^+ cells. **B) Red-** mCherry expression. **C)** PV^+ cells and virus overlay D) schemes of virus expression. Bregma: -2.27mm,-2.45mm,-2.69mm. Scale bars 100µm.

Due to technical problems, this animal was injected in 2 sites on one hemisphere. All data was included in the results. Panel A (Fig4.18. A) shows moderate presence of PV^+ cells. In panel B (Fig.4.18. B) expression of the virus carrying DIO-hM3D(Gq)-mCherry was presented. The expression of mCherry was localised in the auditory cortex structures including A1 (Fig.4.18. D), however it did not fully cover the structure in caudal direction (expression limit: Bregma -.2.79mm, structure limit Bregma: -3.63mm). The unilateral injections, together with partial coverage of auditory cortex, added to very mild influence of DREADDs on activity of auditory cortex as seen in subsection 4.4.2.

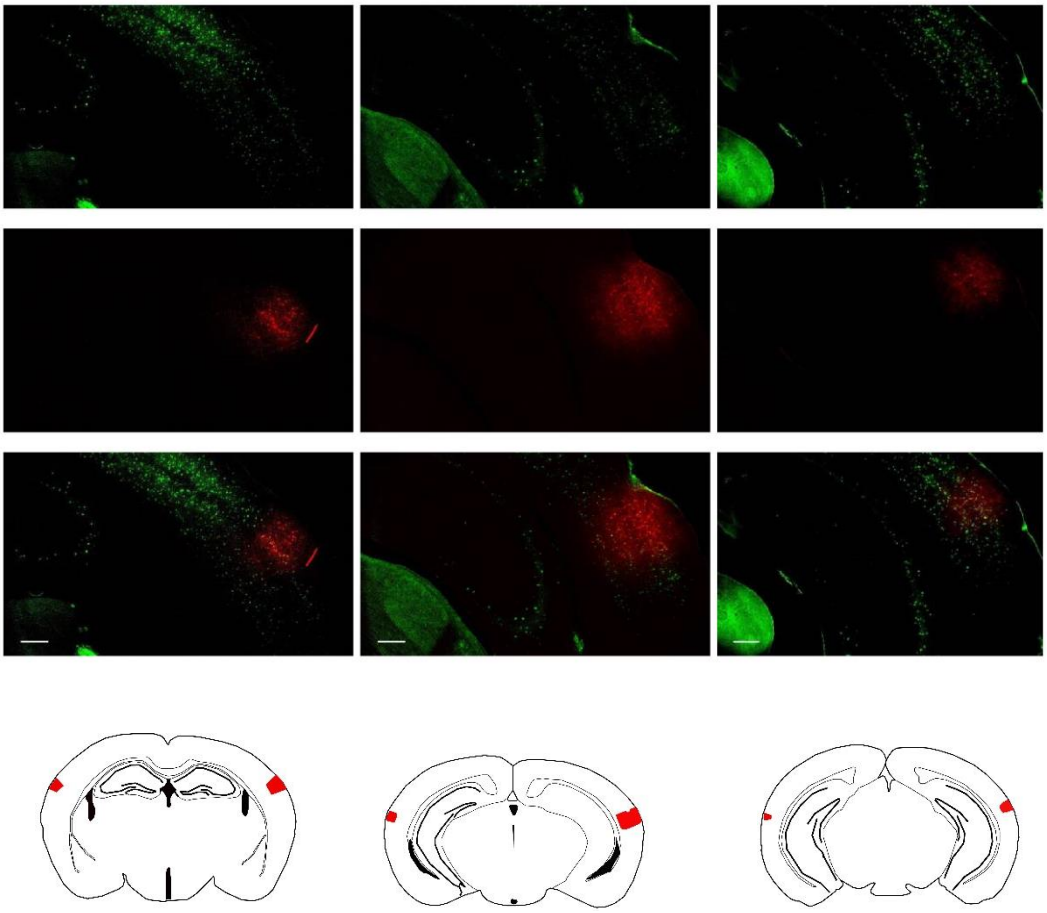


Fig.4.19. Histological analysis. Animal #77

A) Green- PV^+ cells. **B) Red-** mCherry expression. **C)** PV^+ cells and virus overlay D) schemes of virus expression. Bregma: -1.79mm,-2.69mm,-3.15mm. Scale bars 100µm.

Fig.4.19., panel A shows PV^+ cells. In panel B mCherry expression is depict. Although it's placed in auditory cortex quite restricted area of expression caused that significant part of the structure was left intact (Fig.4.19, D). The overlaid results were shown in panel C.

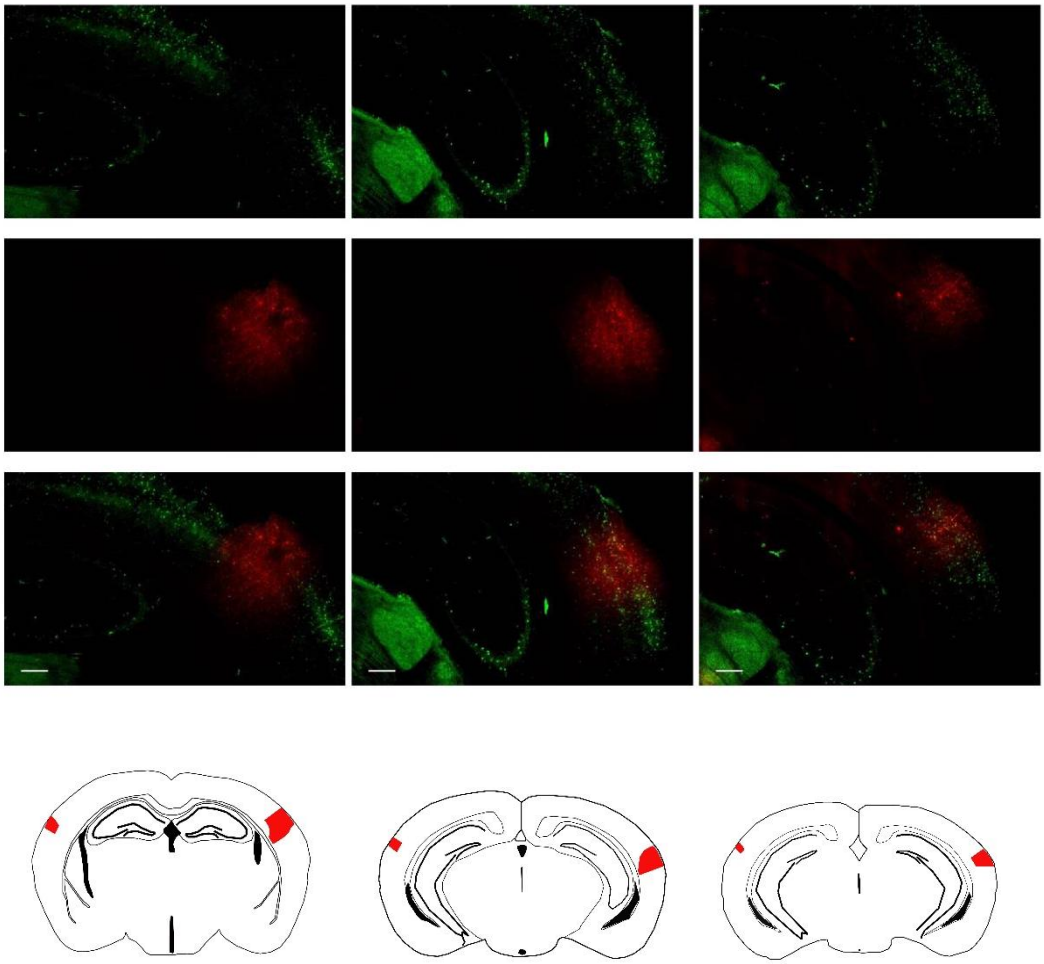


Fig.4.20. Histological analysis. Animal #74

A) Green- PV^+ cells. **B) Red-** mCherry expression. **C)** PV^+ cells and virus overlay D) schemes of virus expression. Bregma: -1.91mm, -2.69mm,-2.79mm. Scale bars 100µm.

Panel A (Fig.4.20.A) shows PV^+ cells. Panel B (Fig.4.20. B) presents mCherry expression at different coordinates. Expression on the left side is visibly weaker than on the right side (Fig.4.20.D). The expression targeted auditory cortex together with part of A1, however the coverage of the area was not extended enough in the caudal position (mCherry limit Bregma: -2.91mm, AuD Ctx limit Bregma: - 3.63mm). Unequal coverage of targeted areas across hemispheres and lack of detected mCherry in the caudal parts of Aud Ctx possibly contributed to mild influence of DREADDs on the investigated structure.

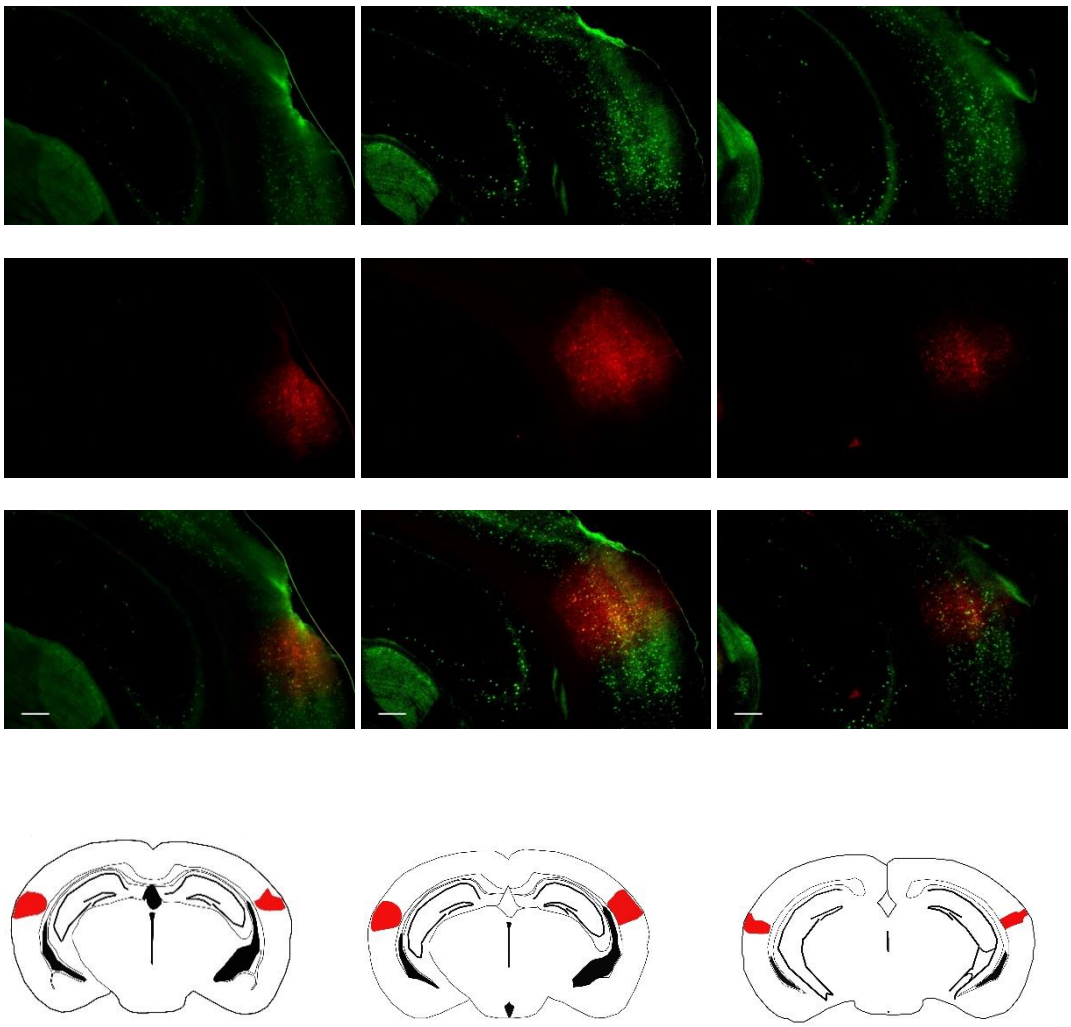


Fig.4.21. Histological analysis. Animal #72

A) Green- PV⁺ cells. **B) Red-** mCherry expression. **C)** PV⁺ cells and virus overlay D) schemes of virus expression. Bregma: -2.45mm,-2.53mm,-2.79. Scale bars 100µm.

Fig.4.21., panel A shows abundant expression of PV⁺ cells. Panel B depicts mCherry expression. The full observed expression of virus is showed in panel D. The expression coincides with Aud Ctx including area A1, however caudally it does not exceed position Bregma: -3.07mm meaning not whole area of auditory cortex could be influenced by activation of pAAV-hSyn-DIO-hM3D(Gq)-mCherry. As mentioned in all previous histological analysis, this fact contributed to very mild results of DREADDs activation presented in subsection 4.4.2.

4.6. Summary

In Chapter 4, Noise Detection Task under 3 conditions was described. Firstly, in subsections 4.3.1 and 4.3.2., Pretraining stages for a single example animal, all investigated group and a slow and a fast-learner were presented. The reaction time of animals was decreasing in subsequent sessions, proving that they learned the association between their performance and obtaining the reward. The hit rate (probability to go) was showing variability between sessions, yielding average results. Possible reasons for this were mentioned in subsection 4.7. and will be further discussed in Chapter 6.

In section 4.4., results for NDT under 3 conditions (CTL, CNO, SLN) were presented. Subsection 4.4.1. showed examples of sessions from a single animal. The reaction time did not show a clear pattern due to dispersion of data points. The probability to go was lower in CNO session but due to variability of data the statistical analysis was performed only on all cohort, for which the results were presented in subsection 4.4.2. There, one-way ANOVA proved significant differences in probability to go between CTL and CNO groups at 70dB and in reaction times between CNO and SLN groups at 60dB. The possible reasons for data dispersion and differences in results between CTL and SLN groups will be further discussed in Chapter 6. Lastly, in subsection 4.5. histological data for all animals is presented. The presence of PV^+ cells and areas infected with virus appear to coincide, although the abundance of PV^+ cells vary across animals. The area with visible mCherry expression corresponds to the auditory cortex however, does not cover it fully leaving the most caudal part unaffected. Partial impairment of the auditory cortex contributed to mild differences visible between tested groups as showed in subsection 4.4.2. Caveats related to those results, together with limitations of DREADDs technique will be further described in Chapter 6.

Altogether, lack of statistically significant changes in RTs and probability to go for the majority of sound intensities after activation of DREADDs, the variability of results between CTL and SLN groups as well as only partial coverage of auditory cortex by DREADDs prevents the conclusion of chemogenetics-dependent changes in

presented behaviour. Apart from already mentioned caveats, this situation could be to some degree related to A1-independent performance of the task. This possibility will be further analysed in Chapter 6.

4.7. Caveats and limitations

Similar to Chapter 3, the average performance in probability to go in Pretraining2 may be related to diverse factors such as: head-fixed conditions, water deprivation scheme, animal's impulsivity and levels of stress. The parameters used in the task and their influence will be further discussed in next chapter.

In the final task, NDT, apart from moderate probability to go, high dispersion of reaction times was observed, what precluded any final conclusion. Additionally, unexpected differences between CTL and SLN groups were observed. The possible reasons for this, including both those related to Pretraining as well as use of chemogenetics itself, will be further discussed in Chapter 6. Finally, although both PV^+ cells presence and mCherry expression illustrating area infected by virus coincide in the auditory cortex, the structure was not completely affected by chemogenetic agent. This issue will be further described in Discussion.

Chapter 5. Results- Electrophysiological recordings from auditory cortex in anaesthetised mice. Effects of chemogenetics manipulation on spontaneous and evoked activity

5.1. Objectives

This experiment served as a study evaluating the effects of manipulation of neural activity in auditory cortex, both spontaneous and sound evoked. The chemogenetics agent used for this purpose has a potential to further relate behavioural outcomes in awake animals (as described in Chapter 4) with underlying neural mechanisms of decision-making and elucidate the role of primary auditory cortex in the process. Future experiments would combine behavioural test with electrophysiological recordings before and after neural activity modulation using chemogenetics. Meanwhile, the current experiment consisted of registration of neural activity from auditory cortex of mice anaesthetized with 20% urethane. During an acute session, divided into 4 parts (Fig. 5.1.), spike rates in auditory cortex were measured. The comparison of spike rates between conditions served to investigate the effect of implemented method on neural activity levels and therefore to provide insight into the necessity of auditory cortex in sound detection that is an indispensable stage for auditory decision-making.

5.2. Overview

Presented data comes from animals fulfilling inclusion criteria: 1) observed auditory responses before initiation of the core experiment (white noise presentation, visual evaluation of evoked responses) and 2) observed changes in neural activity after CNO injection activating DREADDs, visualised in early stage analysis.

In subsequent sections, 5.3 and 5.4, results for an animal not subjected to behavioural training and an animal previously trained in Go/No-Go task are shown. Below table (Table 5.1. B), presents details about animals used in experiments.

The rest of the animals, for which data is not presented (Table 5.1.A), were discarded for several reasons: 1) death before the end of the experiment 2) lack of auditory

responses before the experiment start (assessed using white noise) 3) lack of CNO effect and 4) technical issues.

A

Number of Animals	Previous behavioural training	Type of anaesthesia	Type of DREADDs	Recording probe
3	yes	20% urethane	pAAV5-hSyn-DIO-hM3D (Gq)-mCherry	Neuronexus linear 32 channels
4	no	20% urethane	pAAV5-hSyn-DIO-hM3D (Gq)-mCherry	Neuronexus linear 32 channels

B

Behavioural test	Mouse line	Surgery to recording (days)	Virus amount	CNO amount	Probe type	Auditory responses	CNO effect
No	PV^+	20	4x150nL	1mg/kg	Neuronexus 32 channels 25µm spacing	yes	yes
Yes	PV^+	49	4x150nL	1mg/kg	Neuronexus 32 channels 25µm spacing	yes	yes

Table.5.1. General information about animals in chemogenetics experiments
A: Animals that underwent electrophysiological recording procedures
B: Animals presented in this chapter

In the figure 5.1. a detailed scheme of timeline of recording was shown. Recording was divided in 4 stages: 5 min of spontaneous activity, evoked activity before CNO administration, evoked activity after CNO activation and 5 min of spontaneous

activity. Time of CNO administration is also marked. To obtain evoked responses, trains of sounds from 0dB to 80dB (step 10dB, random order) were presented. Order of sounds was randomly distributed on each trial.

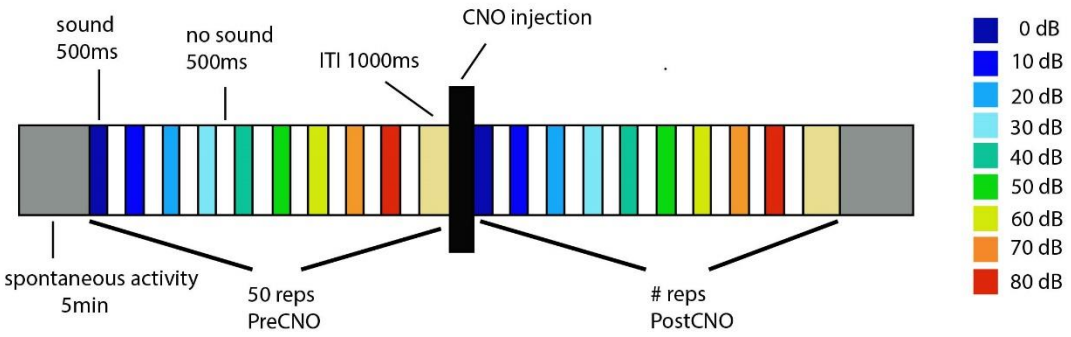


Fig.5.1. Scheme of recording paradigm

Scheme shows the timeline of 4 stages of recording. **1)** 5min of spontaneous activity **2)** Evoked responses before application of CNO. 50 repetitions of trains of sounds from 0dB to 80dB (step of 10dB, random order) **3)** Evoked responses after CNO. Several hundreds of repetitions (depending on recording) of trains of sounds from 0dB to 80dB (step of 10dB, random order) **4)** 5min of spontaneous activity

5.3. Data from an animal without previous behavioural training

Data from this animal was obtained at the end of the optimisation process for DREADDs experiments. Therefore, the coordinates and amount of applied pAAV5-hSyn-DIO-hM3D (Gq)-mCherry, as well as amount of used CNO, is the same as for the animal presented in section 5.4 and as for animals presented in Chapter 4.

In Fig.5.2. spike counts across experiment timeline are presented. Panel A shows spike count across 1min bins across 4 stages of the experiment while panel B shows spike counts across 10 trials bins for each sound intensity applied during PreCNO and PostCNO stages. The moment of CNO administration is presented in black in both panels.

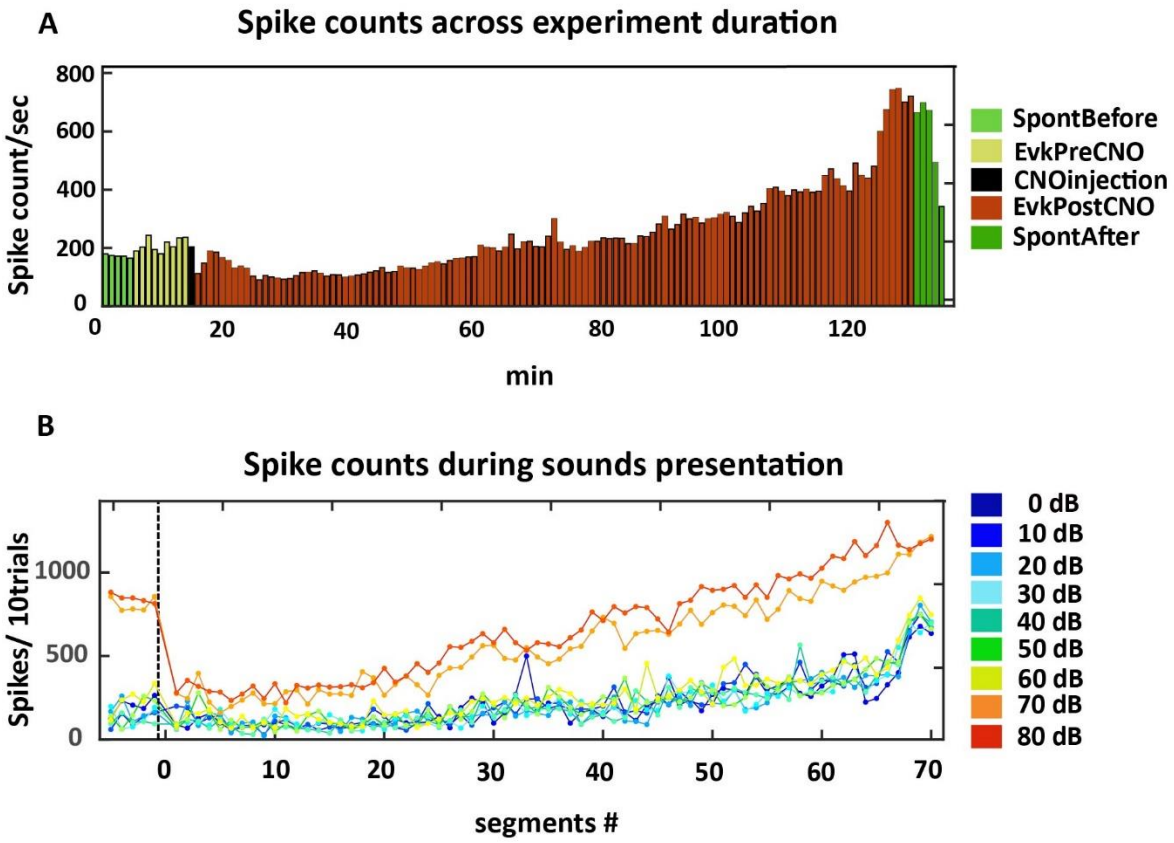


Fig.5.2. Spike counts across experiment's timeline

A: Spike count/sec across 1min segments. **Light green** -5min spontaneous activity, **yellow** – evoked responses before CNO administration, **black**-CNO administration, **brown**- evoked responses after CNO administration, **green**- 5min spontaneous activity. CNOinjection (black) delimites PreCNO period from PostCNO period

B: Spike counts across 10 trials segments and across sound intensities

Vertical black like indicates CNOinjection and delimites PreCNO period from PostCNO period

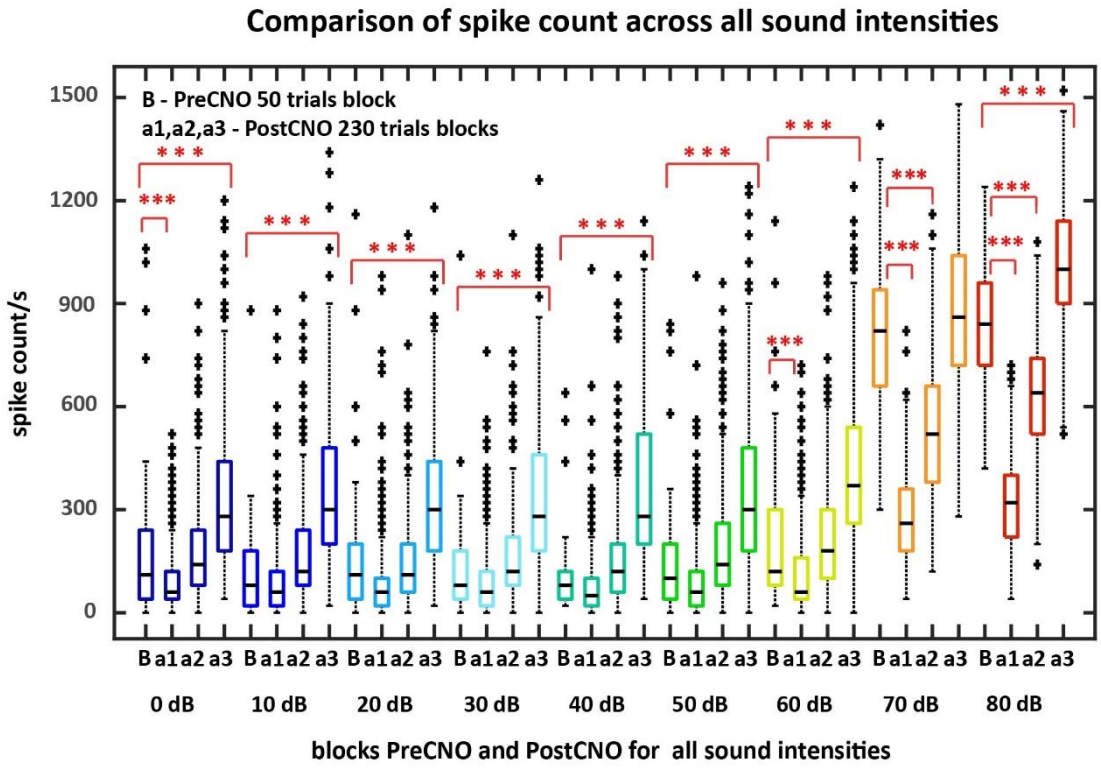
5.3.1. Comparison of neural activity during evoked responses Pre vs Post CNO injection

To assess the effect of CNO injection on auditory cortical activity, neural activity following presentation of sounds of varying intensity, from 0dB to 80dB, was compared between 2 conditions 1) before CNO injection – undisturbed evoked responses and 2) after CNO injection, and therefore activation of DREADDs in PV^{+} cells of auditory cortex, that was aiming to reduce the activity of the structure. One-way ANOVA was implemented for each sound intensity to test for statistical

significance. Once detected, Tukey's *post hoc* test was used for more in depth analysis.

Figure 5.3. (Fig. 5.3. A) presents spike counts/second per block for each sound intensity during PreCNO injection period (block of 50 trials) and PostCNO injection period, which due to long time of recording, was divided into 3 equal blocks (230trials each, last 10 trials of evoked responses were excluded). Panel B (Fig.5.3. B) presents p values between blocks.

A



B

	B - a1	B - a2	B - a3	a1 - a2	a1 – a3	a2 – a3
0 dB	**0.0008**	0.9921	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
10 dB	0.9062	0.1067	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
20 dB	0.1078	0.9830	**<0.0005**	**0.0085**	**<0.0005**	**<0.0005**
30 dB	0.8051	0.3350	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
40 dB	0.7000	0.1165	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
50 dB	0.1704	0.5468	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
60 dB	**0.0016**	0.9934	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
70 dB	**<0.0005**	**<0.0005**	0.0762	**<0.0005**	**<0.0005**	**<0.0005**
80 dB	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**

Fig.5.3. Comparison of spike counts pre and post CNO injections across all sound intensities

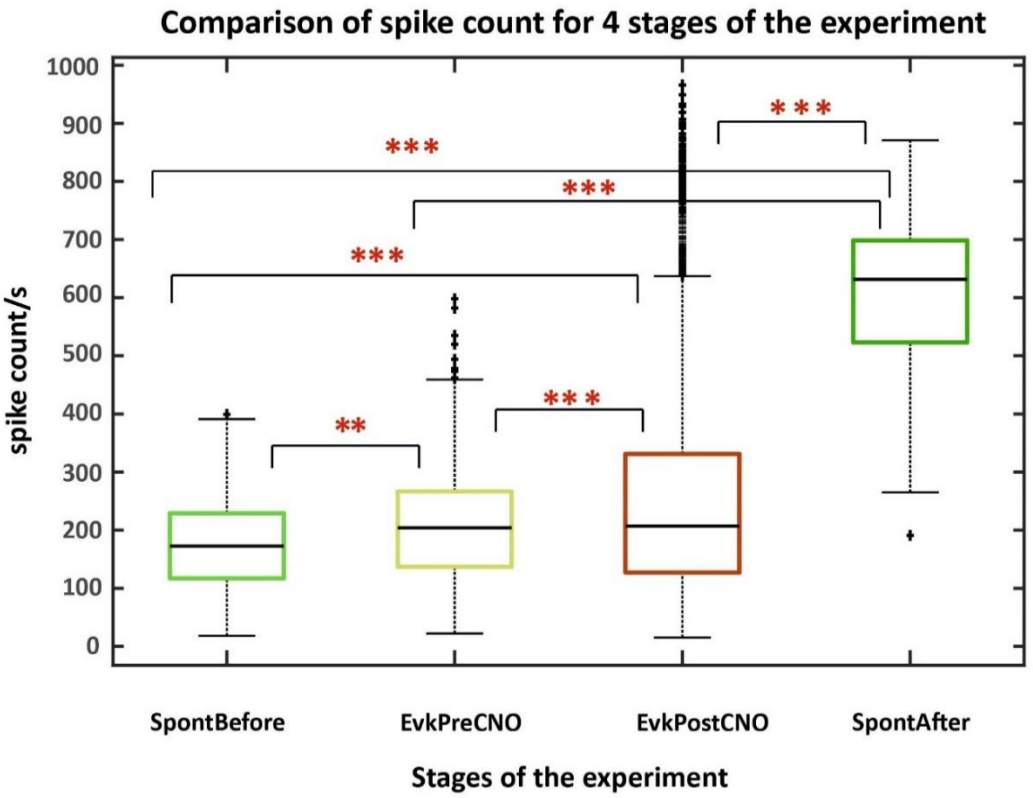
A: B – PreCNO block (50 trials) a- PostCNO blocks (total of 690 trials divided into 3 blocks of 230trials) a1- first 230 trials, a2- second 230 trials, a3- last 230 trials **B:** p-values for each sound intensity, post hoc test; in bold <0.05;

As depicted in panel B (Fig. 5.3. B), statistically significant differences between PreCNO and a1 were detected for 0dB and 60-80dB. In B-a2 pair difference was detected only for 70dB and 80 dB, whereas between B and a3 all but result for 70 dB were significant. Moreover, results for all PostCNO pairs showed significant differences.

5.3.2. Comparison of spike counts between 4 stages of the experiment

In the next step, differences between all four stages of the experiment (5min of initial spontaneous activity, PreCNO -evoked responses before CNO injection, PostCNO - all evoked responses after CNO injection, and 5 min of spontaneous activity at the end of recording) were compared (Fig.5.4. A). The aim of this analysis was to study the changes in neural activity dynamics during all experiment to verify if changes elicited by CNO lasted beyond PostCNO period. One-way ANOVA was used for initial statistical analysis (p value = $1.82112x10^{-317}$, F=535.46), followed by Tukey's test (Fig. 5.4., B).

A



B

SpB-EvkPre	SpB-EvkPost	SpB-SpA	EvkPre-EvkPost	EvkPre-SpA	EvkPost-SpA
0.0061	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**

Fig.5.4. Comparison of spike counts across 4 blocks of data representing 4 stages of experiment.

A: Comparison of spike counts between 4 stages of experiment (5min of initial spontaneous activity, PreCNO, PostCNO, and 5 min of spontaneous activity at the end of recording) **B:** p-values (Tukey's test) between each pair of experiment's stages

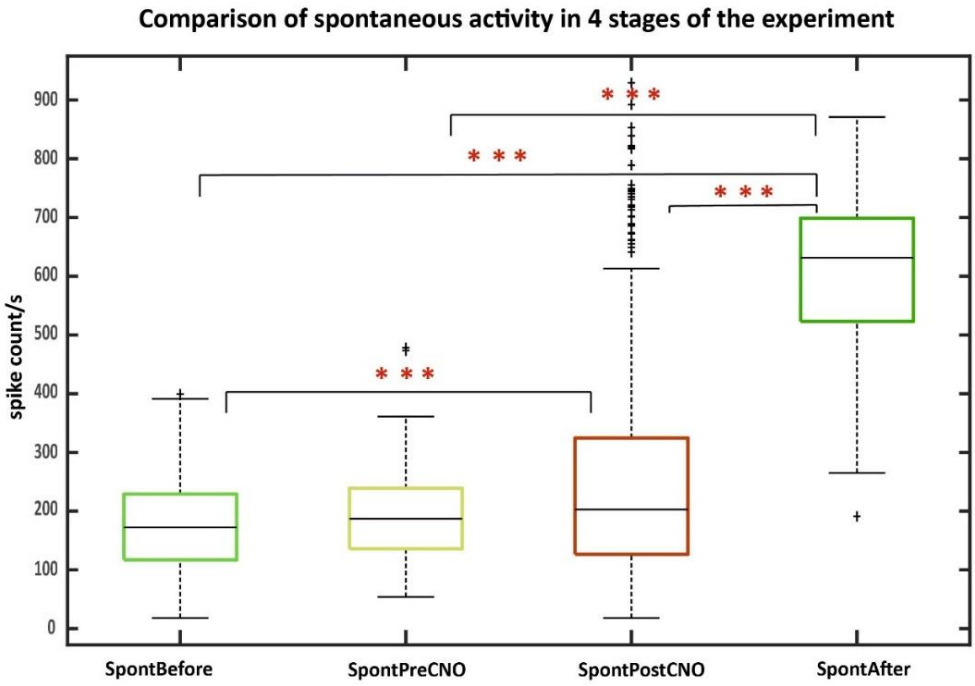
As shown in panel B (Fig.5.4., B), statistical significance in spike counts was detected for all pairs. As Fig. 5.2. showed more detailed differences between evoked

responses, below, in section 5.3.3. activity during 'no-sound' periods is looked at more in depth.

5.3.3. Comparison of neural activity between 4 stages of experiment during 'no-sound' periods

In the last step changes in neural activity during no sound presentation (defined as types of 'spontaneous' activity) were compared between experiment's stages. This analysis looked at potential changes occurring in baseline activity in periods of repeated sound presentation or/and as a result of CNO administration. Below figure presents spike rates during 4 stages of the experiment, while no sound was presented. SpontBefore: 5minutes of spontaneous activity at the beginning of the recording; SpontPreCNO: 50 repetitions of 1000ms period when no sound was presented (ITI) before DREADDs activation; SpontPostCNO: 700 repetitions of 1000ms ITIs after DREADDs activation; SpontAfter: last 5 minutes of recording without sound presentation (Fig.5.5., A). One-way ANOVA detected statistically significant difference (p value= 1.37029×10^{-245}, F=593.08).

A



B

SpB-SpPre	SpB-SpPost	SpB-SpA	SpPre-SpPost	SpPre-SpA	SpPost-SpA
0.5629	**< 0.0005**	**< 0.0005**	0.0906	**< 0.0005**	**< 0.0005**

Fig.5.5. Comparison of spike counts for no-sound periods during 4 stages of the experiment

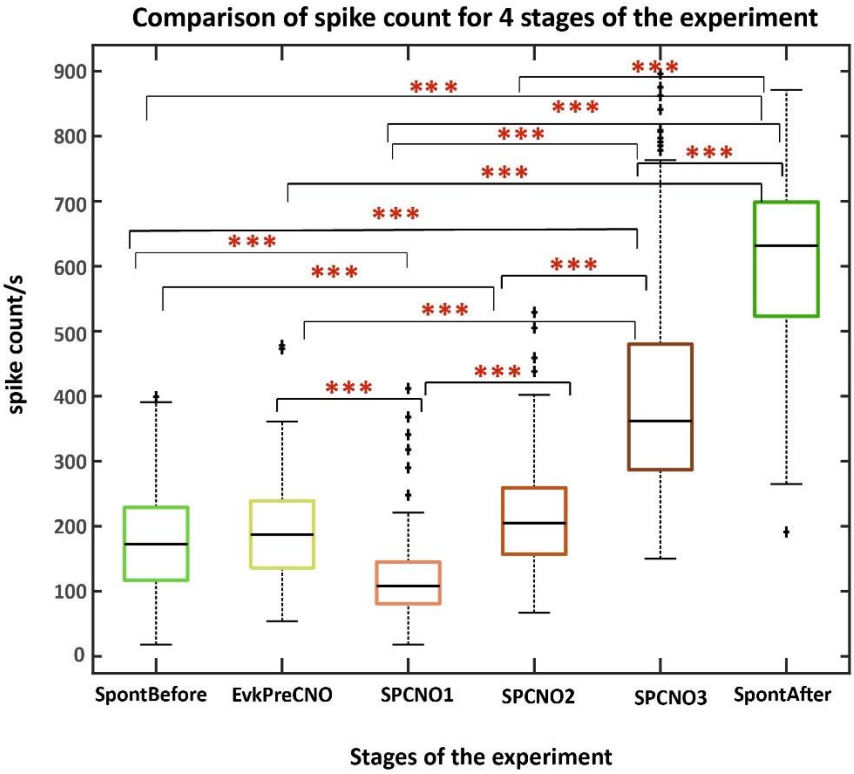
A: Spike counts per second for each of stages of the experiment. SpontBefore: 5minutes of spontaneous activity at the beginning of recording; SpontPreCNO: 50 repetitions of 1000ms period when no sound was presented(ITI) before DREADDs activation; SpontPostCNO: 700 repetitions of 1000ms it is after DREADDs activation, SpontAfter: last 5 minutes of recording without sound presentation
B: Post hoc analysis; p values between experiment stages pairs.

In panel B (Fig.5.5., B), p values obtained by applying Tukey's post hoc test, after implementation of one-way ANOVA, are presented. Differences in spike counts were

significant between 4 pairs with exception of pairs: 5 initial min of spontaneous activity vs activity in PreCNO during no sound periods (ITIs) and no-sound periods PreCNO vs PostCNO.

However, as seen both in Fig.5.2 A and Fig.5.3. A, due to long duration of PostCNO period, averaging activity for this whole period obscures changes in dynamics. Therefore, in Fig. 5.6. analysis was performed with SpontPostCNO devided into 3 blocks: SPCNO1, SPCNO2 and SPCNO3, similar to scheme presented in Fig.5.3. A.

A



B

SpB-SpPre	SpB-SPCNO1	SpB-SPCNO2	SpB-SPCNO3	SpB-SpA	SpPre-SpA
0.5220	**<0.0005**	**0.0002**	**<0.0005**	**<0.0005**	**<0.0005**
SpPre-SPCNO1	SpPre-SPCNO2	SpPre-SPCNO3	SPCNO1-SpA	SPCNO2-SpA	SPCNO3-SpA
<0.0005	0.9742	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
SPCNO1-SPCNO2	SPCNO1-SPCNO3	SPCNO2-SPCNO3			
<0.0005	**<0.0005**	**<0.0005**			

Fig.5.6. Comparison of spike counts for no-sound periods during 4 stages of the experiment

A: Spike counts per second for each of stages of the experiment. SpontBefore: 5minutes of spontaneous activity at the beginning of recording; SpontPreCNO: 50 repetitions of 1000ms period when no sound was presented (ITI) before DREADDs activation; SPCNO1, SPCNO2 andSPCNO3: 690 repetitions of 1000ms ITIs after DREADDs activation, SpontAfter: last 5 minutes of recording without sound presentation

B: Post hoc analysis. p values between experiment stages pairs.

One-way ANOVA confirmed statistical significance between compared stages of the experiment (p value = 0, F = 794.91). Tukey's post hoc test revealed differences between all but SpontBefore-EvkPreCNO and EvkPreCNO-SPCNO2 groups. This data suggests that baseline activity did not change with application of sound itself but only after CNO injection. Those changes were evident both during stages with and without sound application (EvkPostCNO, SpontAfter).

5.3.4. Histological data

Below, histological data is presented. The aim of this procedure was 4-fold. To check 1) position of the recording probe as well as if 2) there was expression of DREADDs in auditory cortex (marked in red by mCherry), 3) PV^+ cells were detected (marked in green), and 4) expression of virus and PV^+ cells overlaid (marked in yellow). The coordinates presented below are based on Paxinos and Franklin's 'The mouse brain in stereotaxic coordinates, 4th edition',2012. Volume of each of 4 injections equalled to 150nL at depth of 500µm.

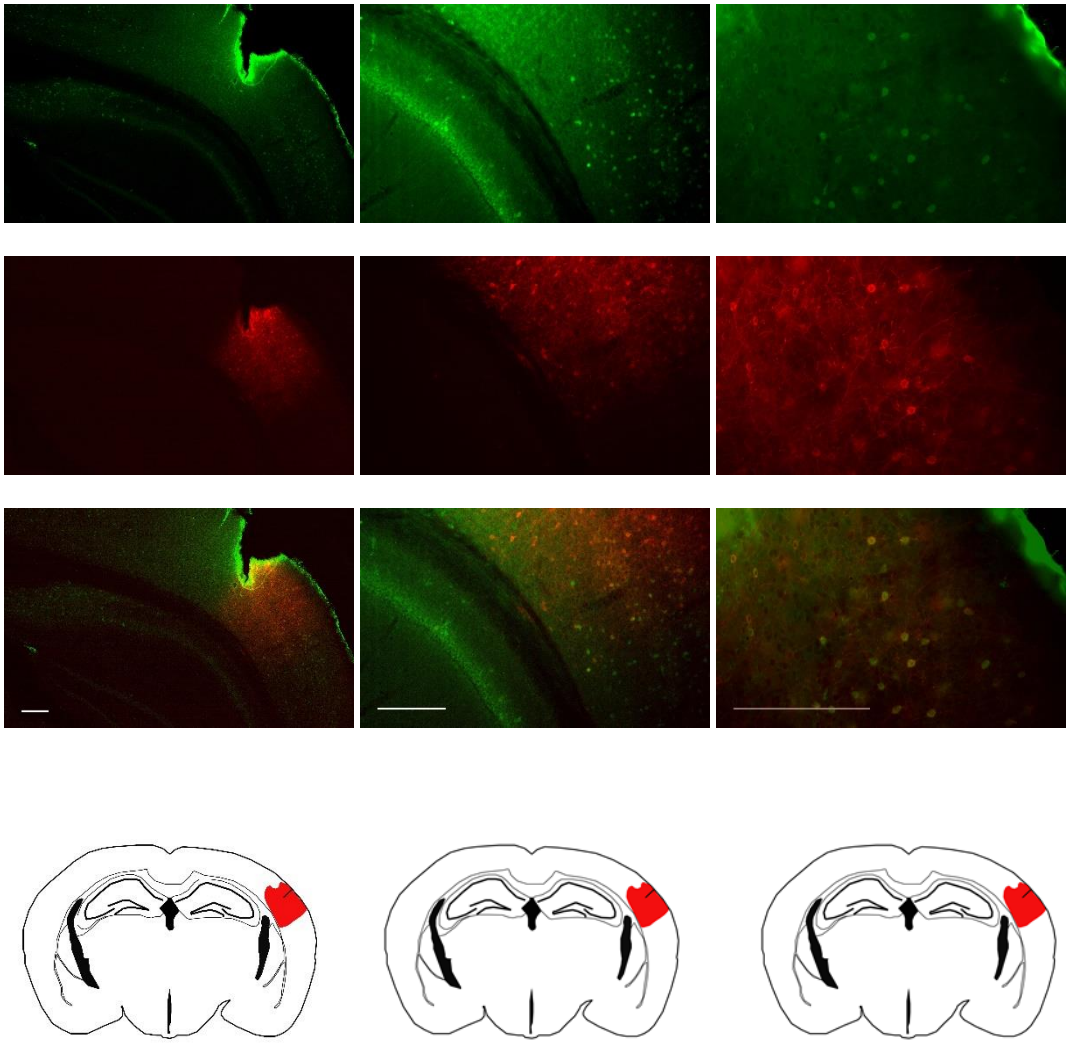


Fig. 5.7. Histological analysis.

A) Green- PV^+ cells. **B) Red-** mCherry expression. **C)** PV^+ cells and virus overlay left to right magnification:x4, x10, x20 **D)** schemes of virus expression, probe location marked in the scheme. Bregma: -2.03 mm. Scale bars 100µm.

Panel A shows abundant PV^+ cells. Panel B shows expression of virus in anterior part of auditory cortex. In panel C the overlay of PV cells and virus is clearly visible. As seen in the brain scheme, the probe was located in Bregma: -2.03mm.

5.4. Electrophysiological recording after last session of behavioural training

Below data shows results from electrophysiological recording of an animal which previously completed behavioural training.

Below (Fig.5.8.), spike counts across experiment's timeline are presented. Panel A shows spike counts across 1min bins across 4 stages of the experiment while panel B shows spike counts across 10 trials bins for each sound intensity applied during PreCNO and PostCNO stages. The moment of CNO administration is presented in black in both panels.

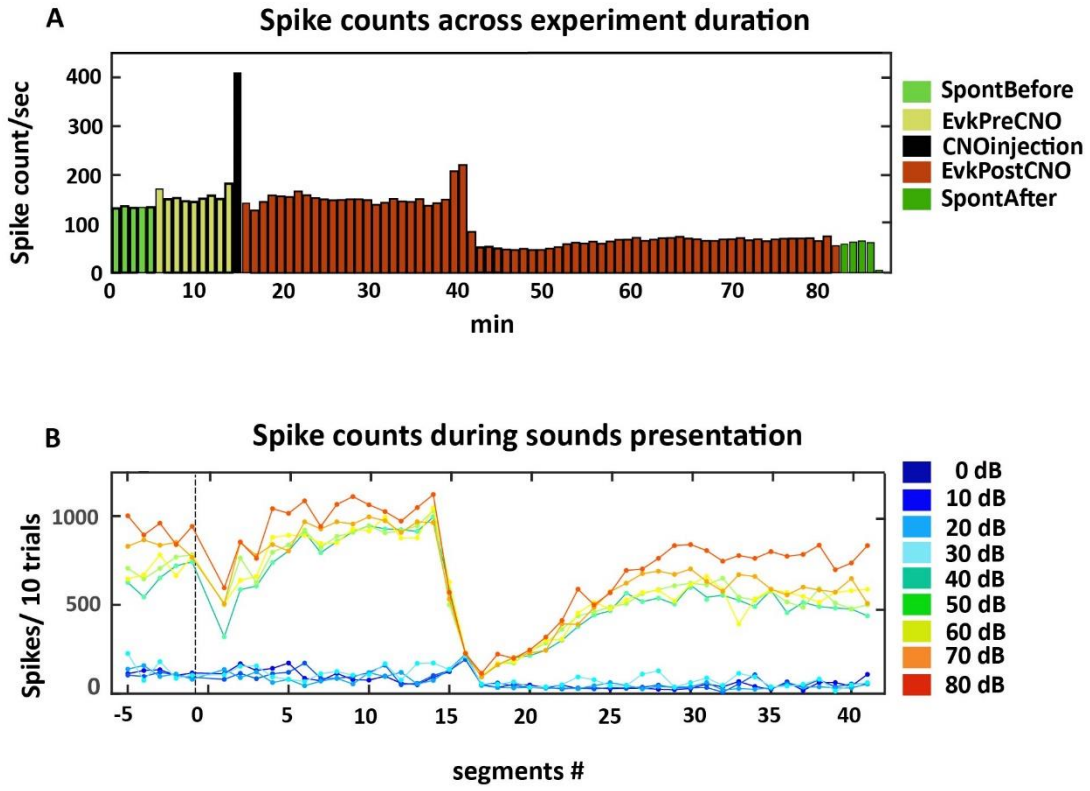


Fig.5.8. Spike count across experiment's timeline

A: Spike count/sec across 1min segments. **Light green** - 5min spontaneous activity, **yellow** – evoked responses before CNO administration, **black**-CNO administration, **brown**- evoked responses after CNO administration, **green**- 5min spontaneous activity. CNOinjection (black) delimites PreCNO period from PostCNO period

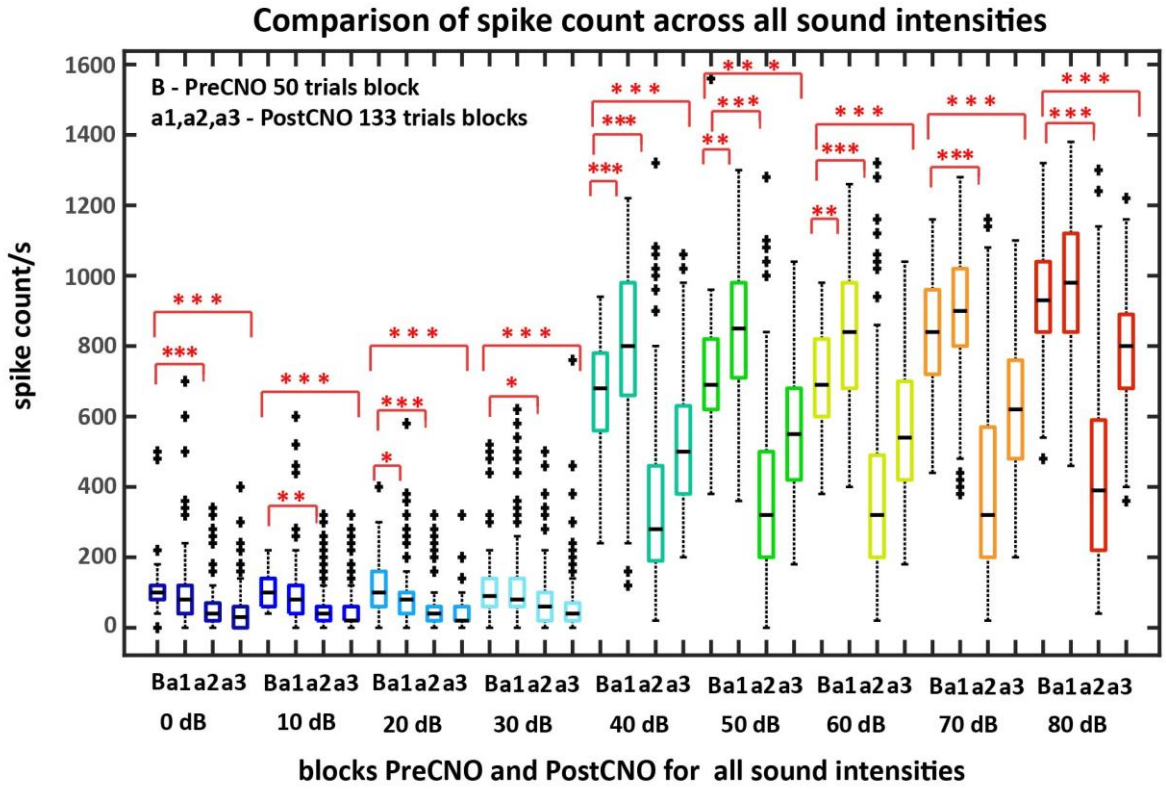
B: Spike counts across 10trials segments and across sound intensities, vertical black like indicates CNOinjection and delimites PreCNO period from PostCNO period

5.4.1. Comparison of neural activity during evoked responses Pre vs Post CNO injection

Similar to subsection 5.3., neural activity following presentation of sounds of varying intensity, from 0dB to 80dB, was compared between 2 conditions 1) before CNO injection – undisturbed evoked responses and 2) after CNO injection, to determine the necessity of auditory cortex for sound detection. One-way ANOVA was implemented for each sound intensity to test for statistical significance. Once detected, Tukey's *post hoc* test was used for more in depth analysis.

Figure 5.9. (Fig. 5.9. A) presents spike counts/s per block for each sound intensity during PreCNO injection period (block of 50 trials) and PostCNO injection period, which due to long time of recording, was divided into 3 equal blocks (133trials each, last 11 trials of evoked responses were excluded). Panel B (Fig.5.8. B) presents p values between blocks.

A



B

	B - a1	B - a2	B - a3	a1 - a2	a1 – a3	a2 – a3
0 dB	0.6551	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	0.5907
10 dB	0.9991	**0.0026**	**<0.0005**	**<0.0005**	**<0.0005**	0.6994
20 dB	**0.0316**	**<0.0005**	**<0.0005**	**0.0021**	**<0.0005**	**0.0273**
30 dB	0.8557	**0.0105**	**<0.0005**	**0.0092**	**<0.0005**	0.5585
40 dB	**0.0007**	**<0.0005**	**0.0006**	**<0.0005**	**<0.0005**	**<0.0005**
50 dB	**0.0048**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
60 dB	**0.0019**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
70 dB	0.3805	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
80 dB	0.5195	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**

Fig. 5.9. Comparison of spike counts pre and post CNO injections across all sound intensities

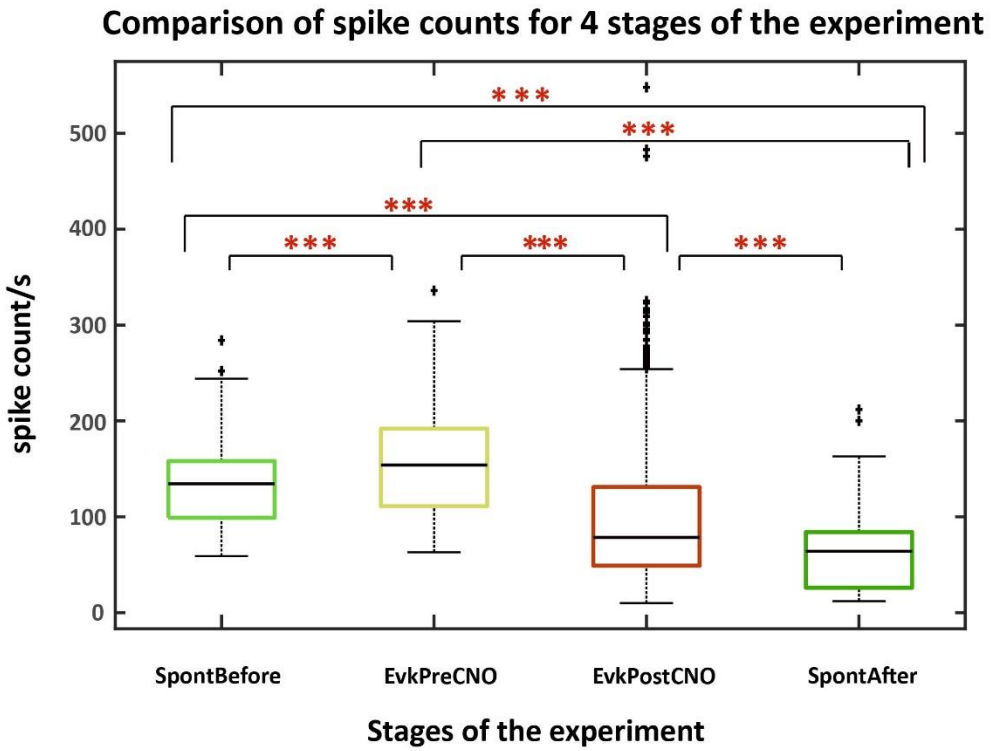
A: B – PreCNO block (50 trials) a- PostCNO blocks (total of 399 trials divided into 3 blocks of 133trials) a1- first 133 trials, a2- second 133 trials, a3- last 133 trials **B:** p-values for each sound intensity, post hoc test; in bold <0.05;

Data in panel B (Fig.5.8. B) shows statistical difference in results between B-a1 for 20,40,50 and 60dB, for all sound intensities between B-a2 and B-a3 as well for the vast majority of pairs from EvkPostCNO with exception of 0,10 and 30dB for the pair a2-a3.

5.4.2. Comparison of spike counts between 4 stages of the experiment

In the next step, differences between all four stages of experiment (5min of initial spontaneous activity, PreCNO, PostCNO, and 5 min of spontaneous activity at the end of recording) were compared (Fig.5.10., A). One-way ANOVA (p value = 7.44478×10^{-137}, F=224.6) was used for initial statistical analysis, followed by Tukey's test (Fig. 5.10., B). Here, the spike rates for evoked responses were combined across all intensities in PreCNO and PostCNO stages.

A



B

SpB-EvkPre	SpB-EvkPost	SpB-SpA	EvkPre-EvkPost	EvkPre-SpA	EvkPost-SpA
< 0.05	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**

Fig.5.10. Comparison of spike count across 4 blocks of data representing 4 stages of experiment.

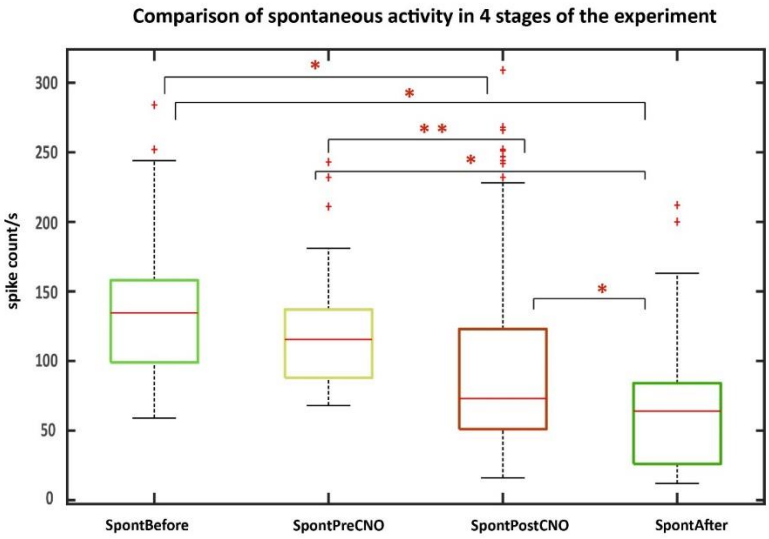
A: Comparison of spike counts between 4 stages of experiment (5min of initial spontaneous activity, PreCNO, PostCNO, and 5 min of spontaneous activity at the end of recording) **B:** p-values (Tukey's test) between each pair of experiment's stages

As shown in both panels of Fig.5.10., statistical significance was detected for difference in spike rate between all 4 stages of the experiment. The spike count/s increased after sound presentation, decreased after CNO injection and continued to decrease during last 5 min of spontaneous activity.

5.4.3. Comparison of neural activity between 4 stages of experiment during 'no-sound' periods

In the last step changes in neural activity during no sound presentation (defined as types of 'spontaneous' activity) were compared between experiment's stages. Below figure presents spike rates during 4 stages of experiment, while no sound was presented. SpontBefore: 5minutes of spontaneous activity at the beginning of the recording; SpontPreCNO: 50 repetitions of 1000ms period when no sound was presented (ITI) before DREADDs activation; SpontPostCNO: 410 repetitions of 1000ms ITIs after DREADDs activation; SpontAfter: last 5 minutes of recording without sound presentation (Fig.5.11., A).

A



B

SpB-SpPre	SpB-SpPost	SpB-SpA	SpPre-SpPost	SpPre-SpA	SpPost-SpA
0.1916	**≤ 0.05**	**≤ 0.05**	**0.0010**	**≤ 0.05**	**≤ 0.05**

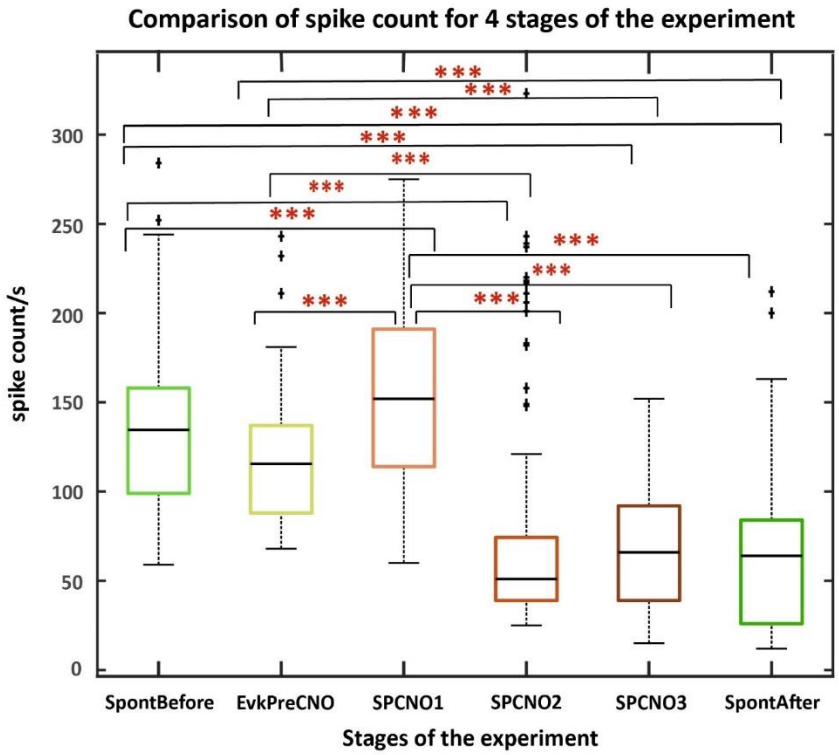
Fig.5.11. Comparison of spike counts for no-sound periods during 4 stages of the experiment

SpontBefore: 5minutes of spontaneous activity at the beginning of recording; SpontPreCNO: 50 repetitions of 1000ms period when no sound was presented(ITI) before DREADDs activation; SpontPostCNO: 410 repetitions of 1000ms it is after DREADDs activation, SpontAfter: last 5 minutes of recording without sound presentation

In panel B (Fig.5.11., B), p values obtained applying Tukey's post hoc test, after implementation of one-way ANOVA, are presented. Differences in spike counts were significant between all pairs with exception of 5 initial min of spontaneous activity vs activity in PreCNO during no sound periods (ITIs) pair. Similar to subsection 5.3.3., spontaneous activity was further compared including the division of SpontPostCNO into SPCNO1, SPCNO2, SPCNO3. One-way ANOVA (p value = $1.281102 \times 10^{-127}$, F= 162.24) was followed by Tukey's test.

The results (Fig.5.12, B) indicate that neural activity during no-sound periods did not change with sound presentation, initially increased after CNO injection (SPCNO1) and subsequently decreased in SPCNO2. This state was further maintained in SPCNO3 and SpontAfter.

A



B

SpB-SpPre	SpB-SPCNO1	SpB-SPCNO2	SpB-SPCNO3	SpB-SpA	SpPre-SpA
0.2254	**0.0006**	**0.0002**	**<0.0005**	**<0.0005**	**<0.0005**
SpPre-SPCNO1	SpPre-SPCNO2	SpPre-SPCNO3	SPCNO1-SpA	SPCNO2-SpA	SPCNO3-SpA
0.0001	0.9742	**<0.0005**	**<0.0005**	0.2097	0.3317
SPCNO1-SPCNO2	SPCNO1-SPCNO3	SPCNO2-SPCNO3			
<0.0005	**<0.0005**	0.9999			

Fig.5.12. Comparison of spike counts for no-sound periods during 4 stages of the experiment

A: Spike counts per second for each of stages of the experiment. SpontBefore: 5minutes of spontaneous activity at the beginning of recording; SpontPreCNO: 50 repetitions of 1000ms period when no sound was presented (ITI) before DREADDs activation; SPCNO1, SPCNO2 andSPCNO3: 690 repetitions of 1000ms it is after DREADDs activation, SpontAfter: last 5 minutes of recording without sound presentation

B: Post hoc analysis; p values between experiment stages pairs.

5.4.4. Histological data

Below, histological data is presented as described in detail in subsection 5.3.4.

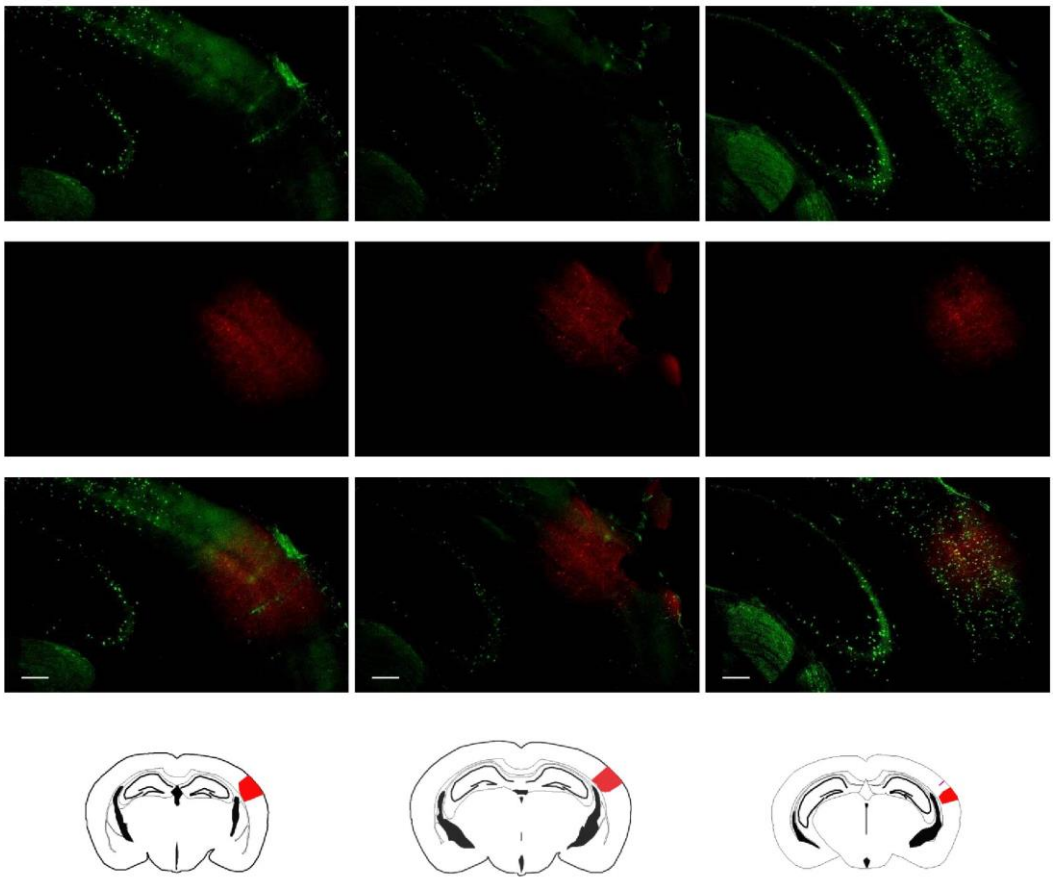


Fig.5.13. Histological analysis.

A) Green- PV^+ cells. **B) Red-** mCherry expression. **C)** PV^+ cells and virus overlay **D)** schemes of virus expression; probe location marked in the 3rd scheme. Bregma: -1.79mm, -2.15mm, -2.45mm . Scale bars 100µm.

In panel A PV^+ cells are visible, especially in the 3rd panel (Bregma: -2.45mm). In panel B expression of virus in anterior part of auditory cortex is depicted. Panel C marks overlay of PV^+ cells and virus. Probe was located rostrally to main area of virus expression as depicted in panel D.

5.5. Summary

In Chapter 5 two examples of electrophysiological recordings in anaesthetised mice were presented. In subsection 5.3. an animal previously used for optimisation of DREADDs technique, without behavioural training was tested. In section 5.4 results from an animal previously subjected to behavioural training are presented. Animals' multiunit activity was evaluated during 4 stages of the experiment (Spontaneous activity at the beginning of the recording, evoked activity before CNO administration, evoked activity after CNO administration and spontaneous activity at the end of the recording). Differences in spike count for each sound intensity were compared between EvkPreCNO and EvkPostCNO conditions. EvkPostCNO in both cases was divided into 3 segments due to duration of the recordings. Furthermore, overall differences between 4 stages of experiment were compared and spike rates during no-sound epochs were evaluated for each of 4 stages of the experiment with EvkPostCNO devided into 3 blocks. Finally, histological data was presented.

In subsection 5.3. (Fig.5.3.) the analysis of evoked responses before and after CNO administration was evaluated. The differences between EvkPreCNO and EvkPostCNO are mostly visible for the highest sound intensities what could have originated from the fact that in EvkPreCNO high spike rate was present only for 70 and 80dB, whereas for other intensities was much lower. This situation might be the effect of combination of the state of animal (some blood lose during craniotomy might deteriorated the cortex activity) and influence of urethane anaesthesia. The EvkPostCNO period showed dynamics over time leading to significant differences between sound intensities/segments. The highest increase, in the 3rd segment of EvkPostCNO may be due to CNO kinetics or improvement of activity but it could be as well a result of drifting of the electrode or change in the state of the animal, especially that final spontaneous activity is much higher that initial 5 min. Fig. 5.4. presents differences in spike counts between 4 stages of experiment. Sound presentation increased spike rate that further increased after CNO injection and during spontaneous activity. The difference between SpontAfter and EvkPostCNO, as well as between SpontBef and SpontAft indicates that the change was caused due to

CNO kinetics. Nevertheless, factors such as general state of the animal (improvement in general neural activity) could be considered. In Fig. 5.5. differences in spike rate between 'spontaneous' activity in 4 stages was presented. The lack of difference between SpB and SpontPreCNO could indicate that sound presentation did not impact baseline activity. Finally, Fig.5.7. shows histological data. The pictures of x4, x10 and x20 magnifications show expression of PV-Cre cells, expression of virus (mCherry) and their overlay, confirming expression of DREADDs in PV^+ cells. However, similar to results from Chapter 4, the distribution of virus covered only anterior part of auditory cortex allowing for only moderate influence of the activity of the structure. The data was collected from coordinates Bregma: -2.03mm.

In section 5.4. results from recording of animal previously subjected to behavioural training are presented. Fig.5.9. shows differences between EvkPreCNO and EvkPostCNO. As the drop of activity can be noticed only after 26min from CNO injection, the majority of differences can be seen between B-a2 and B-a3. The late impact of DREADDs activation may be related to the quality of injection or the metabolism of CNO further discussed in Chapter 6.

Fig.5.10. shows differences in spike count between all 4 experiment stages. There are significant differences between all groups. Spike count increased after sound presentation and decreased after CNO injection which state continued during SpontAfter.

In the figure 5.11, activity during no-sound epochs was compared between experiment stages. Apart from SpB-SpPre pair all other pairs showed significant difference in spike rate. Difference between SpB and SpPost could indicate effect of CNO on decrease in 'spontaneous' activity. In Fig. 5.13. histological data is presented. Electrode was located at Bregma: -2.45. Similar to results from subsection 5.3. and Chapter 4, although colocalisation of PV^+ cells and mCherry reflects expression of DREADDs in PV^+ cells of auditory cortex, only the anterior part of the structure expressed virus leading to moderate results.

5.6. Caveats and limitations

There are several caveats of techniques used in Chapter 5 that could have contributed to inconclusive results by demonstrating different results in two above-described animals.

The craniotomy, performed immediately before recording, could decrease the activity of cortex by blood loss or possible damage to a part of dura. Anaesthesia under urethane can also contribute to changes in neural activity especially when combined with blood lose during craniotomy. Further, the time spent on locating most appropriate recording site by penetrating various location can also contribute to changes in cortical activity by producing local damage and prolonging time of experiment. Moreover, as it's not possible to fully assess if the CNO injection was entirely correct, this step contributes to quality of changes after ip injection. Additionally, there are reports casting new light on metabolism of CNO as discussed in the next Chapter. Finally, as previously mentioned, the fact of not covering the whole area of auditory cortex during previous surgery contributes to moderate effects of DREADDs activation. Those limitations are further discussed in Chapter 6.

Chapter 6: Discussion

6.1. Overview

The study of correlation of behavioural outcomes and the mechanisms that may be guiding them remains one of the core topics in the present neuroscience. A very prominent branch of this topic is perceptual decision-making that studies how sensory inputs influence behaviour of animals and possible mechanisms underlying this process. The development and refinement of cutting-edge techniques for precise manipulation of neural activity that we have witnessed during last decade allows for more in detail research of this matter.

In this thesis I have focused on auditory decision-making in rodents and the importance of auditory cortex for the process of sound detection. By developing and implementing an auditory behavioural task in mice I have studied perceptual decision-making using the example of temporal expectations (Chapter 3). Further, a simplified version of this task was used to evaluate the necessity of auditory cortex for sound detection. For that purpose, chemogenetics was combined with formerly presented behavioural task (Chapter 4). As the last experiment, the effect of neural activity manipulation using chemogenetics on auditory cortical activity was studied in anaesthetised mice (Chapter 5). This series of experiments will lead to a further study of neural mechanisms underlying auditory decision-making.

In this chapter all obtained results were summarised and discussed (sections 6.2,6.3 and 6.4). Last but not least, further directions for this research were presented in section 6.5.

6.2. Development and optimisation of auditory behavioural task and its implementation in a study of temporal expectations

6.2.1. Development and optimisation of auditory behavioural task

To study perceptual decision-making, it's indispensable to base the experiment on a behavioural task that allows for precise control of both input and output parameters. By correlating the changes in a sensory input stimulus with changes in behaviour, conclusions can be made on the contribution of the stimulus or its particular characteristics to a decision.

Therefore, to fulfil the objectives of this thesis, a sound detection task was developed. This paradigm was used in a head-fixed condition, which eliminates movement-related artifacts and allows for precise delivery of stimulus and reward as well as fast response of the animal. It also allows for combination with techniques for neural recording and manipulation. However, as it causes immobility of the animal it often contributes to higher level of stress in the animal, especially at the beginning of training.

As the sensation of a stimulus by an animal is not observable per se, its response is necessary to evaluate this sensation. One approach to do so are tasks based on detection of a stimulus.

Although pinpointing neural underpinnings of a process is crucial to fully understand it, without behavioural part the obtained results are vastly incomplete. After acknowledging this fact, in last decades the balance of research moved from studies of purely neural sensitivity towards perceptual decision-making (Gold and Shadlen, 2007; Nienborg and Bruce, 2010).

An important matter to bear in mind is that psychometric sensitivity is always influenced by extra-sensory factors related, for example, to the task design (Stüttgen et al., 2011). The type of behavioural task selected obviously impacts psychometrics of stimulus detection. However, it's important to realise that there are other factors influencing the final effect (Stüttgen et al., 2011). In every task, it's favourable to analyse results from trained animals that achieved a certain level of hit rate, trying to avoid at the same time overtraining of the animals (Fine and Jacobs, 2002). However, even well-trained animals display variability in responses to the same stimulus. Therefore, as a source of motivation water of food deprivation is often implemented in the behavioural studies. Already a study from the sixties of last century showed that the hunger-satiety balance in Go/No-Go task changes over the duration of the session, as the animal by collecting the reward diminishes level of hunger, and that this change influences both probability to respond and the threshold of response (Boneau and Cole, 1967). A similar observation can be made when an animal gets

tired during the session, which means that the duration of training was too long (Stüttgen and Schwarz, 2008). As the behaviour variability can be seen not only between animals but also between sessions performed by the same animal, one way to tackle this issue is to cut out inactive trials from sessions at the stage of data analysis. This approach was used in Chapter 3 and Chapter 4 where during NDT, sessions were cut after 10 inactive trials. However, as shown in figure 4.12., the hit rate during sessions is dynamic, with possible restoration of good hit rate after momentary drop therefore, instead of terminating the session during the experiment, in an alternative approach a cut-off point is selected during the data analysis.

Go/No-Go task is a highly valuable paradigm for perceptual decision-making studies and it's easily learned by rodents. Some of its advantages are shorter learning time (comparing to yes-no or 2AFC tasks), presentation of only one type of stimulus and lack of pronounced working memory (Stüttgen et al., 2011). Nonetheless, as any other task it has various limitations. The most serious problem that occurs during the training is an issue with motivation related to attention. Animals, most probably, don't attend to the task during various epochs of a training session. Even though this fact is not directly related to psychophysics of the task, it has a serious negative impact on the results (Stüttgen et al., 2011). As the lack of attention leads to lower response ratio, the hit rate is decreasing and the threshold for detection is increasing. Additionally, in a Go/No-Go task it's impossible to state what was the cause of a failed response. Inability to detect a stimulus is equally probable as lack of attention and motivation. Although other paradigms force the animal to report choice on every trial, even yes-no tasks and two alternative forced choice tasks are not free from this caveat. In those paradigms, due to the lack of attention behavioural responses could be made randomly. The influence of attention can be even more significant as it can modulate neural sensitivity to a stimulus (Treue and Martinez Trujillo, 1999).

To sum up, although a head-fixed Go/No-Go Task is a paradigm with many advantages for detection task in mice, especially when combined with techniques for

neural activity recording and modulation, it's not free of its limitations. The head-fixed conditions provoke stress, especially in early stage of training. The scheme of task is also susceptible to animals' impulsivity and changes in attention and motivation. These problems were seen in results presented in Fig.3.3, A, B, Fig.3.4 and Fig.3.6., B in Chapter 3 where hit rate was average and variable, even though the RT of animals decreased between initial and final sessions. Similar trend was observed in Chapter 4. To deal with this effect in the future, stricter water deprivation schedules may be implemented, as long as it does not influence the level of health of the animals. To decrease the level of impulsivity, increasing the distance from licometer could be applied on more advanced sessions. An alternative is a 'black out time', a punishment pause increasing time of trial. However, this approach was tested in several sessions in various animals and led to decrease in motivation, therefore was abandoned. Additional option could be application of air-puff in the initial sessions of training to discourage the animal from impulsive licking. Nonetheless, being an aversive cue, this approach could also have negative influence on motivation.

6.2.2 Temporal Expectations

Incorporating time as an another dimension of action is crucial for executing various behaviours and was observed in a variety of species, from bees (Boisvert and Sherry, 2006) through fish (Talton et al., 1999) to rodents (Jaramillo and Zador, 2011) and humans (Doherty et al., 2005). Deciding when to decide often results as crucial as the decision itself. Although numerous studies have tackled this issue, vast majority was based on primates or human subjects. The development of new technologies allows however, more frequently, to test the significance of time in behaviour in rodents, using for example temporal expectations. Despite the interest in the topic, neither the mechanism (or mechanisms) nor detailed involvement of particular brain structures was fully discovered.

In Chapter 3, based on developed behaviour, temporal expectations in mice were tested using varied foreperiods at different presentation ratios or in a different number. The aim of this experiment was to look at dependency between FP and RT.

First, influence of 2FPs at various ratios on changes in RT was evaluated. Sessions with different parameters were combined over animals and FPs were assigned as either short or long. As application of 50:50 ratio did not yield significant results, sessions with uneven distribution of FPs were analysed. Obtained results suggested that it was the frequency of FP appearance rather than its duration that influenced RT (Fig.3.7.). Second, the impact of 4 FPs with even distribution was evaluated. As in the case of 2FPs, no significant difference in RT was observed (Fig.3.8.).. Therefore, the current overall results do not indicate dependency between FP and RT. Should the study be extended in the future, several issues need to be taken into account. An important factor is the number of animals. Although pooling data is important to obtain reliable results on a larger scale, it must be taken into account that differences in performance of individual animals, especially within small cohorts, can 'dilute' the final result (Buhusi et al., 2009). Furthermore, pooling data over animals trained under various FPs means that possibly different approaches could have been applied by different animals. Therefore, for further evaluation it would be worth to either probe the paradigms in higher number of animals or try out different paradigm in the same animal. In the first case, as discussed in subsection 6.2.1., different approach could be tried out to increase the hit rate of animals, therefore eliminating the doubt related to the cause of miss trials and increasing number of animals and sessions that meet inclusion criteria. The second alternative, which has been tried out in a subset of animals in experiments presented in this thesis brings additional caveats. It prolongs the time of training, increasing the possibility of head-post stability related issues and makes the training more complex as the animals need to switch to different parameters. The influence of task difficulty on behavioural results, causing often discrepancy between studies, was also discussed by Buhusi at al. (Buhusi et al., 2009) in a study looking at scalar property (showing that changes in the error of time estimation are modulated linearly with increase of timed interval) in C57Bl/6 mice. In this study, apart from confirming that mice, similar to humans, incorporate scalar property into their behaviour, a group of factors that may influence behavioural results was shown. Apart from aforementioned discrepancies between a single

animal and a cohort, mouse strain can also modulate outcome of timing related experiments, due to for example differences in dopaminergic system (Buhusi and Meck, 2005). Last but not least is the observation, here based on scalar property, about information that can be extracted from the errors made by animals in addition to those supplied by correct trials. This problem, although in a different paradigm and from different angle was examined in a study were false alarms' timing was evaluated in a temporal expectation task in mice. Chapuis and Chadderton (Chapuis and Chadderton, 2018) evaluated timing of actions, both hit and error, after biasing sound stimuli in short blocks of trials. This paradigm allowed to study behavioural responses and related to them activity in auditory cortex. It revealed that mice were able to tailor timing of their responses basing on timing of known biases. False alarms displayed a pattern aligned to target stream (out of 2 streams played simultaneously) and provided additional information about animals' strategy in the paradigm. Moreover, it showed differences of approaches between animals, highlighting the fact that various animals may apply different strategies under same paradigm and that valuable data can be extracted both from hit trials and 'failed' ones. All above data shows the multitude of factors that may influence the results of temporal expectations related experiments. As different strategies may occur under same paradigm, this probability is obviously much higher when discrepancies between parameters appear. The data presented in subsection 3.4. was based on FPs of various duration, spanning from 200 to 2000ms. This change in duration may incorporate different underlying mechanisms (Buhusi and Meck, 2005). Furthermore, applying FPs in non-block design and selecting short FPs durations might have led to inconclusive results on the dependency between FPs and RTs. The difference in RTs was observed while comparing ITI influence but as those are different parameters, results between FPs and ITIs can't be compared. The importance of timing for processes such as decision-making is hard to deny. Temporal expectations can increase the success rate of an animal in such a situation. However, the complexity and highly precise nature of time as a parameter provokes a need for broader series

of experiments, where multiple parameters could be modulated to fully elucidate underlying processes.

6.3. Modulations of behaviour using chemogenetics

In Chapter 4 the effects of modulation of the activity of auditory cortex on behaviour, using chemogenetics, were described. Due to the usage of DREADDs as modulating factor the kinetics of CNO is of high importance for the results of the presented experiments.

DREADD technology, used widely over last 11 years is an important and potent tool enabling both transient and remote changes in the activity of neurons. It was used in hundreds of studies providing new insights into variety of processes. Nonetheless, it's in vivo mechanism of action has not been thoroughly analysed before 2017 (Gomez et al., 2017) when Gomez *et al.* brought better understanding of processes involved in the technique, exposing caveats and limitations related to this tool and therefore revealed the need for more cautious analysis of results.

The study by Gomez revealed that systemic administration of CNO leads to its low levels in CSF and that drug kinetics is not satisfactory neither in in vivo nor in in vitro studies. They assumed that detected levels of CNO in both DREADD-expressing and naïve tissues were originating from their presence in blood vessels or CSF rather than brain itself (Pardridge, 2016). The detection of CNO in naïve animals suggests as well that CNO is not specifically binding to DREADDs as previously thought. At the same time, lack of detected clozapine in naïve animals suggests that, indeed, CNO is inert at low doses but this state changes when higher amounts of drug are delivered. All those statements lead to a conclusion that to see CNO-related effects, the concentration of the drug should be higher which would provoke various caveats such as confusion with off-target effects related to endogenous receptors or effects of converted clozapine that could interact with either DREADDs or, at high enough doses, clozapine binding sites. The behavioural effects were also assessed. In rats expressing DREADDs, contrary to controls, clozapine injections resulted in significant decrease in locomotor activity at the scale comparable to that induced by 100-fold higher dose of CNO. At a higher dose of clozapine effects on locomotor activity were

visible even in control animals.. In mice similar results were obtained. It was previously established, in nonhuman primate studies, that after conversion from CNO, clozapine reaches its peak concentration in cerebrospinal fluid after a period of 2-3h (Raper et al., 2017). This results were repeated in Gomez study (Gomez et al., 2017) both in DREADD-expressing and naïve animals . Therefore, an important issue to keep in mind is the time frame of the experiment as non-specific effects of CNO tend to have late onset.

Due to the fact that clozapine rather than CNO can cross BBB and activate DREADDs both in rats and mice, Manvich (Manvich et al., 2018) looked at possible conversion of CNO to clozapine in mice and if CNO administration results in clozapine-like effects in animals not expressing DREADDs.

This study showed that CNO is reverse-metabolised to clozapine in both rats and mice. In behavioural tests, where concentration of 1.25mg/kg was used, only 1/10 animals displayed drug-related behaviour. Whereas, while looking at the degree of drug conversion, administration of 10mg/kg CNO lead to appearance of, mainly, N-desmethylclozapine and to lower degree clozapine, both after 30 and 60min. Although this result looked contradictory to previous study (Guettier et al., 2009), claiming low convergence rate, more detailed studies revealed that actually in both cases the CNO to clozapine ratio was significant. It's also important to notice that the dynamics of conversion is different between mouse and rat. Whereas in rat clozapine can be again converted to CNO in mouse clozapine is metabolised above all to NDMC. The importance of that lies in the fact that NDMC can as well produce some of behavioural effects similar to those caused by clozapine (Wiebelhaus et al., 2012). An important observation from Manvich's study is that detected plasma levels of converted clozapine weren't announcing behavioural effects from CNO administration. It's assumed that this result comes from ease with which clozapine, even at low dosis, passes BBB to later be stored in the brain (Bender et al., 1994).

The abovementioned studies indicate that CNO administration can cause clozapine-like effects which is additionally characterized by variability between subjects

(Manvich et al., 2018). This fact does not discredit DREADDS as a highly useful tool in neurobiological research but instead it highlights the necessity for careful planning of the experiment, for example by comparing effects after CNO or vehicle injection within the same animal as well as incorporation of additional control where CNO is applied but only a fluorescent marker, in absence of DREADDs, is expressed

In this thesis, CNO was administrated at concentration of 1mg/kg in all experiments. This dose is slightly lower than the lowest one used in Manvich's studies. This would mean that any conversion to NDMC as well as clozapine would be low and not necessarily detected in blood when tested. However, the impact on behavioural results is still possible. As previously described, even though conversion of CNO to NDMC and clozapine is visible already after 30-60min, its peak occurs after 2-3h. Therefore, the experiments were ended before the peak time of conversion. Another important concern is the amount of CNO that crossed the BBB. This issue is additionally dependant on the quality of ip injection which cannot be objectively evaluated during the experiment. Further, the successful activity of DREADDs is dependent on the infected area. Despite targeting auditory cortex, as shown in subsection 4.5., the posterior part of auditory cortex did not express pAAV5-hSyn-DIO-hM3D (Gq)-mCherry, further contributing to the lack of confirmation of influence of DREADDs on behaviour. Additionally, one of the animals expressed DREADDs in one hemisphere.

As the behavioural design was based on the paradigm described in Chapter 3, similar caveats related to behaviour occurred. One of them was the level of impulsivity of the animal, which could alternatively be interpreted as selected strategy and which was visible in form of high level of catch trials under 3 presented conditions as shown both in subsections 4.4.1. and 4.4.2.

In subsection 4.4.1. where results from a single animal were presented, hit rate was higher for trials with higher sound intensity whereas RTs did not show a clear pattern under any of 3 conditions. This was especially visible in a very broad range of timing presented by the animal. Due to lack of differences in RTs after CNO administration

and high level of probability to go during catch trials in all 3 types of sessions, the influence of DREADDs on behaviour was not confirmed for this single animal.Therefore, in subsection 4.4.2. pooled data was analysed.Here, significant differences were observed in probability to go for 70dB between CTL and CNO groups and in reaction times for 60dB between CNO and SLN groups. Those results are quite surprising for two reasons. First, the results are not similar across sound intensities and significant changes are detected only for the highest sound intensities. However, similar trend was observed in Chapter 5 where spiking rate was affected only in the highest sound intensities. Second, the difference in results between CTL and SLN groups was unexpected. As SLN sessions were performed in trained animals, the expected results should have resembled CTL sessions. Partially, worse results could be associated to stress related to ip injections. However, as this condition was present in multiple sessions in both CNO and SLN sessions it was expected that the animals would get adjusted to this condition. Therefore, it's difficult to fully explain this variability in results. Altogether, similar to data from a single animal, no clear connection was detected between DREADDs application and behavioural changes.

While looking at the effects of modulation of the auditory cortex activity on behaviour, it is worth keeping in mind the discussion on the importance of this structure for the processing of simple sounds. This matter has been studied in numerous experiments, using different task designs, methods of auditory cortex activity inhibition as well as various animal models leading to, sometimes, contradictory results. The in-detail anatomical studies preceded experiments aiming at understating the functional role of this structure, that in their initial phase were mostly centred at A1 and lesion studies (Talwar et al., 2001).

Those experiments led to a conclusion that auditory cortex ablation had either none or moderate effects on sound detection depending on tested species. In rats, it was shown that ablation of all auditory cortex areas had almost no or no impact on the ability to localize sound (Kelly and Glazier, 1978), frequency discrimination thresholds or sound sensitivity (Kelly, 1970). This could lead to a simple conclusion that an intact auditory cortex is not necessary to perform simple sound processing. However, such

interpretation does not clearly explain the involvement of the structure in normal hearing. Due to the fact that lesion studies require a post-surgery recovery period, it is possible that a reorganization within the central auditory system allows for recovery of basic auditory functions (Talwar et al., 2001). Indeed, studies using acute methods found the importance of auditory cortex for simple sound processing. Reversible silencing of auditory cortex in ferrets using GABA-A antagonist, muscimol, showed the necessity of auditory cortex and especially of A1 for sound localization (Nodal et al., 2012). Similar results in the same species were obtained by cooling the structure (Wood et al., 2017). In rats, usage of muscimol during a task requiring sound detection and discrimination had profound but reversible effects on animals' ability to both detect and discriminate sounds. Application of that drug additionally altered auditory evoked potentials as confirmed using electrophysiology (Talwar et al., 2001). Therefore, the above results show that the selected method of study has a serious impact on obtained results and together with the usage of different animal species and task designs may lead to different conclusions. The complexity of the matter is further increased by regional differences in sound processing across auditory cortex (Nodal et al., 2012). Therefore, a detailed study of the importance of the auditory cortex to sound processing, in relation to perceptual decision-making in auditory-based tasks, is still required, with a special emphasis on the structure's sub-regions and their cell-type specific architecture.

Overall, although DREADDs modulated behaviour is a valid tool for evaluation of influence of various structures on decision-making and may help elucidate underlying mechanisms, several caveats and limitations were identified in presented experiments, which did not show expected correlations.. Overcoming those shortcomings would allow to obtain more detailed data that would contribute to our knowledge on perceptual decision-making in rodents.

6.4. Modulation of neural activity during sound presentations in anaesthetised mice

In Chapter 5, a study on effects of neural modulation using chemogenetics on activity in auditory cortex in anaesthetised mice was studied. As described in subsection 5.6., in DREADDs-based experiments the kinetics of CNO is important for the results. In the study by Manvich (Manvich et al., 2018) it was shown that the conversion of CNO takes place already after 30-60min while its peak occurs after 2-3h. Therefore, in the theory the last part of recording from the first presented animal could be influenced by NDMC or clozapine due to the duration of recording exceeding 2h, while the second experiment was terminated approximately after 1,5h, before the possible occurrence of peak concentration. Another important concern is the amount of CNO that crossed the BBB. This issue is additionally dependant on the quality of ip injection which cannot be objectively evaluated during the experiment. Further, the successful activity of DREADDs is dependent on the infected area. Despite targeting auditory cortex, as shown in Fig.5.7. and Fig.5.13., the posterior part of auditory cortex did not express pAAV5-hSyn-DIO-hM3D (Gq)-mCherry, what diminished the final effect.

In Fig.5.3. it's showed that for evoked responses the difference between EvkPreCNO and first 2 segments of EvkPostCNO is present only for 60dB, 70dB and 80dB, whereas the difference against 3rd segment is visible almost for all intensities. This effect potentially could be related to CNO conversions. In Fig.5.4., all comparisons between pairs of stages of the experiment resulted statistically significant. Sound presentation increased spike count/s when compared to initial spontaneous activity, while after CNO injection the activity kept on increasing both during EvkPostCNO and SpontAfter stages. The fact that spike counts during SpontAfter were much higher than during SpontBefore is therefore probably related to activation of chemogenetics agent. This issue was further studied as shown in Fig.5.5. and Fig.5.6. From Fig.5.6., where PostEvkCNO was divided in 3 blocks, it can be seen that during no-sound epochs introduction of sound did not influence spike count, whereas CNO injection resulted in initial drop in activity after which neural activity was steadily increasing till the end of the experiment. Those results are in accordance with Fig.5.4. The unexpected result, mainly lack of increase in spike rate after sound presentation (both in overall

and no-sound period), most probably comes from the fact that from the beginning of the experiment higher spike count was seen only for higher sound intensities.

In the second presented example, as shown in Fig.5.8., the recording was shorter and the decrease in activity, again for higher sound intensities, occurred after almost 30min from CNO injection (after short decrease following the CNO injection) which corresponds to the time of drug conversion. This fact was further visualised in Fig. 5.9. where statistically significant differences in spike count occurred mostly between conditions B and a2, a3. Comparison of all stages of experiment (Fig.5.10.) showed increase in spike counts after sound presentation and decrease in neural activity after CNO injection both during EvkPostCNO and SpontAfter stages. In no-sound epochs throughout the experiment (Fig.5.11, Fig5.12) difference between SpontBefore and EvkPreCNO was not observed. However, after CNO injection spike count increased in SPCNO1 (Fig.5.12.), then decreased during SPCNO2 and maintained this state till the end of the recording. Therefore, similarly to previous example, the effect of modulation of neural activity by chemogenetics was visible for both evoked and spontaneous activity. Nonetheless, both examples differed from each other to some extent.

There are several additional elements, apart from CNO kinetics, that can influence the quality of recorded neural activity. First of them is the effect of urethane. Even though the drug's amount is same for all animals, calculated by their weight, its effects may vary depending on the quality of craniotomy, duration of the experiment and general health state of the animal. Another factor is the craniotomy where limited blood lose may occur which further contributes to effects of anaesthesia and condition of the cortex. The condition of the cortex may also deteriorate with subsequent lowering of the probe in search of the final recording site. All those elements together may influence the differences in results seen between various animals. The last factor is the time between surgery and sacrifice. As one of presented animals was previously subjected to behavioural training the incubation period of DREADDs was longer. Despite the observed differences between animals, or because of them, it would be worth to continue this study on more numerous cohort of

animals. More importantly, this study should be upgraded to electrophysiological recordings in awake animals what would eliminate the issues related to anaesthesia and more importantly would provide a fuller picture on decision-making in sensory cortices. Although for a long time the activity of auditory cortex was related in vast majority to processing of auditory signals, nowadays we have multiple evidence proving otherwise (Rodgers and DeWeese, 2014; Bagur et al., 2017; Chapuis and Chadderton, 2018; Francis et al., 2018). Therefore, continuation of studies of changes evoked both in neural activity of auditory cortex and in behaviour post chemogenetics activation would most certainly provide important body of data related to the phenomenon in question.

6.5 Final conclusions and future directions

In this thesis, perceptual decision-making in rodents was studied using behaviour, chemogenetics and electrophysiology. The combination of those methods allowed to access the effects of chemogenetics modulation of activity of auditory cortex on both behavioural outcomes and neural activity as well as test behavioural implications on their own, when implementing paradigm with temporal expectations. The developed behavioural paradigm proved being a valid tool for decision-making assessment on the example of temporal expectations. The shortcomings of the paradigm were identified, pointing the direction of refinement of the method. Further, the necessity of auditory cortex for the process was estimated using both behavioural test and neural recordings in anaesthetised animals. Here, again, the potential of used methods was shown and limitations influencing the results were observed. Modifications to the methods based on gathered data would allow to collect more reliable and detailed data and expand current findings. In the future, after refining used approaches behavioural paradigm should be combined with electrophysiological recordings in awake animals. This would allow, in the future, to assess the role of auditory cortex in perceptual decision-making, and further the contribution of primary auditory cortex to the process.

Appendix

Script 1 –Pretraining 1, all animals

```
%% access folders

Path = 'xxx';

nLines = 5; % ignore text lines

nVars = 6; % number of variabes

 %% scan folders

FoldersInfo = dir(Path);

FoldersInfo(1:2) = []; % ignore irrelevant items (. , ..)

 nAnimals = length(FoldersInfo); %number of animals

BigMat = []; %Big Matrix including: animal#, session#org, session#ordered, lick rate (#/min)

for a = 1:nAnimals

   MyAnimal = FoldersInfo(a).name;

   fprintf([MyAnimal, '\n']);

 %% scan files

   Files = dir([Path, '\', MyAnimal, '\Pre1\*.txt']); % obtain text file information

   nSessions = length(Files);% number of sessions for an animal

   %% core

   for s = 1:nSessions

      %% open file

      MyFname = Files(s).name;

      MySessionNum = str2double(MyFname(10:12));% session number

      fid = fopen([Path, '\', MyAnimal, '\Pre1\', MyFname]);

      tmp = textscan(fid, '%s', nLines, 'delimiter', '\n'); % scan first 5 lines

      tmp = fscanf(fid, '%d')';

      bMat = reshape(tmp, nVars, length(tmp)/nVars)'; % 6 variables (time(hr, min, sec), lick#, interval, time stamp

      fclose(fid);

      %% calculate

      LickNum = bMat(end, 4);% lick number

      TotalTime = (bMat(end, end) - bMat(1, end))/60000; % session duration in min

      %% update

      BigMat(end+1, :) = [str2double(MyAnimal), MySessionNum, s, LickNum/TotalTime];%update for each session

   end

end
```

Script2 –Pretraining 2, all animals

```
%% access folders

Path = 'xxx';

nLines = 3; % ignore text lines

nVars = 9; % number of variables

%% inclusion criteria

Threshold.nTrials = 100; % min nr of trials per session

%% scan folders

FoldersInfo = dir(Path);

FoldersInfo(1:2) = []; % ignore irrelevant items (. , ..)

nAnimals = length(FoldersInfo);% number of animals

BigMat = []; %BigMatrix: animal#, session#org, session#ordered, hit rate (#/min), meanRT, medianRT, hit rate (/session)

for a = 1:nAnimals

   MyAnimal = FoldersInfo(a).name;

   fprintf([MyAnimal, '\n']);

   %% scan files

   Files = dir([Path, '\', MyAnimal, '\Pre2\*.txt']); %obtain text file information

   nSessions = length(Files);

   ActSessionNum = 0;

   %% core

   for s = 1:nSessions

      %% open file

      MyFname = Files(s).name;

      MySessionNum = str2double(MyFname(10:12));% session number

           fid = fopen([Path, '\', MyAnimal, '\Pre2\', MyFname]);

      tmp = textscan(fid, '%s', nLines, 'delimiter', '\n'); % scan first 5 lines

      tmp = fscanf(fid, '%d')';

      bMat = reshape(tmp, nVars, length(tmp)/nVars)';

      fclose(fid);

 nTrials = size(bMat,1);% number of trials

 %% when inclusion criterion is met

        if nTrials >= Threshold.nTrials

            %% assessment: hit rate (/min)

        HitNum = bMat(end, 5);

        TotalTime = (bMat(end,end) - bMat(1, 6))/60000;

        HitRate = HitNum/TotalTime;

        %% assessment: hit rate (/session)
```

```
        HitRatePerSe = HitNum/nTrials;
        %% assessment: RT
        RTs = bMat(bMat(:,7)~=-1,7);
        MeanRT = mean(RTs);
        MedianRT = median(RTs);
        %% update after each session
        ActSessionNum = ActSessionNum + 1;
        BigMat(end+1,:) = [str2double(MyAnimal), MySessionNum, ActSessionNum, ...
          HitRate, MeanRT, MedianRT, HitRatePerSe];
      end
    end
end
```

Script 3- Temporal Expectations FPs vs RTs

```
%% access folders
Path = 'xxx';
nLines = 13; %ignore text lines
nVars = 13; % variable number: 1. trial#, 2. correctionPara, 3. ITI, 4. PrestimWin, 5. StimType,
  % 6. Outcome, 7. RT, 8-13, time info
  %%inclusion criteria
Threshold.nTrials = 100;%minimum number of trials
Threshold.Hit = 0.6; % mimimum P(hit) at the highest intensity
Threshold.FPtypes = 4; % number of FPs per session
%% scan folders
FoldersInfo = dir(Path);
FoldersInfo(1:2) = []; % ignore irrelevant items (. , ..)
%%%info%%%
nAnimals = length(FoldersInfo); %number of animals
HitMat = [];
BigMat = []; % animal#, session#org, session#ordered, StimType, P(hit), meanRT, medianRT
FP_1 = [];
FP_2 = [];
FP_3 = [];
FP_1a = [];
FP_2a = [];
FP_3a = [];
meanFP_1 = [];
meanFP_2 = [];
```

```
meanFP_3 = [];
meanFP_4 = [];
FPRTMat4 = [];
FPAlter = []; %final data matrix
for a = 1:nAnimals
   MyAnimal = FoldersInfo(a).name;
   fprintf([MyAnimal, ':']);
   %% scan files
   Files = dir([Path, '/', MyAnimal,  '/NDT/NDT*.txt']); % obtain text file information
      nSessions = size(Files,1);
      endoftrials=0; % for cutting inactive 10 sessions at file end
      ActSessionNum=0; % for included sessions if criteria met
   %% core
   for s = 1:nSessions
      %% open file
      MyFname = Files(s).name;
      MySessionNum = str2double(MyFname(9:11));%session number
     fid = fopen([Path, '/', MyAnimal, '/NDT/', MyFname]);
      tmp = textscan(fid, '%s', nLines, 'delimiter', '\n'); % scan first 5 lines
      tmp = fscanf(fid, '%d')';
      bMat = reshape(tmp, nVars, length(tmp)/nVars)';
      fclose(fid);
      %% reshape
      bMat(bMat(:,2)~=0, :) = []; % ignore correction trials
      nTrials = size(bMat,1);%number of trials

      %% cut session after 10 inactive trials
      % idle trials
      t_idle = -1;
      counter=0; % counting inactive trial
      endoftrials = nTrials;
      for t=1:nTrials
         if bMat(t,7) == t_idle
            counter = counter + 1;
         else
            counter = 0;
```

```
        end
        if counter==10
            endoftrials = t-10; % where session ends now
            break
        end
    end
    bMat = bMat(1:endoftrials,:);
     FPtypes = unique(bMat(:,4)); % find different foreperiods
    if length(FPtypes) == Threshold.FPtypes % inclusion criteria
        FPtypes = unique(bMat(:,4));
        HitNum = length(find(bMat(:,6)==1));
        HitRatePerSession = HitNum/nTrials;
        if (length(FPtypes) == Threshold.FPtypes) && (HitRatePerSession >= Threshold.Hit)&&
(length(bMat)>=Threshold.nTrials) % inclusion criteria
            ActSessionNum = ActSessionNum + 1;
            % HitRate update
            HitMat(end+1,:) = [str2double(MyAnimal), MySessionNum, HitRatePerSession];
            % Hit rate for diffrent FPs
            fp=1:length(FPtypes);   % diffrent Foreperiods
            %% 4 FP types Reaction Times
            if length(FPtypes) == 4 % inclusion criteris
                bMat(bMat(:,7)==-1, :) = [];
                RT= (bMat(:,7));

                for i=1:length(bMat)
                    if bMat(i,4) == FPtypes(fp(1))
                        FP_1(i, 1) = bMat(i,7);
                    end
                    if bMat(i,4) == FPtypes(fp(2))
                        FP_2(i, 1) = bMat(i,7);
                    end
                     if bMat(i,4) == FPtypes(fp(3))
                        FP_3(i, 1) = bMat(i,7);
                     end
                    if bMat(i,4) == FPtypes(fp(4))
                        FP_4(i, 1) = bMat(i,7);
                    end
```

```
            end
            meanFP_1 = mean(FP_1);
            meanFP_2 = mean(FP_2);
            meanFP_3 = mean(FP_3);
            meanFP_4 = mean(FP_4);
            FPRTMat4(end+1,:) = [str2double(MyAnimal), MySessionNum, FPtypes(fp(1)), meanFP_1];
            FPRTMat4(end+1,:) = [str2double(MyAnimal), MySessionNum, FPtypes(fp(2)), meanFP_2];
            FPRTMat4(end+1,:) = [str2double(MyAnimal), MySessionNum, FPtypes(fp(3)), meanFP_3];
            FPRTMat4(end+1,:) = [str2double(MyAnimal), MySessionNum, FPtypes(fp(4)), meanFP_4];
            FPAlter(end+1,:) = [str2double(MyAnimal), MySessionNum, FPtypes(fp(1)), FPtypes(fp(2)),FPtypes(fp(3)),
FPtypes(fp(4)), meanFP_1, meanFP_2, meanFP_3, meanFP_4];
          end
        end
      end
  end
end
allVal1 = [200 500];
allVal2 = [500 1000];
allVal3 = [1500];
allVal4 = [1900 2000];
for val1 = allVal1
  for val2 = allVal2
    for val3=allVal3
      for val4 = allVal4
      sameFPs = [];
      for p=1:size(FPAlter, 1)
        if (FPAlter(p, 3) == val1) && (FPAlter(p, 4) == val2) && (FPAlter(p, 5) == val3) && (FPAlter(p, 6) == val4)
          sameFPs(p, :) = [FPAlter(p,7), FPAlter(p,8), FPAlter(p,9), FPAlter(p,10)];
        end
      end
      if isempty(sameFPs) == 0
        sameFPs = sameFPs(any(sameFPs ~= 0,2),:);
        nRows = size(sameFPs,1);
        nCols = size(sameFPs,2);
        if (nRows > 1)
         fprintf('nRows=%6.4f and nCols=%6.4f \n', [nRows, nCols].' );
         end
```

```
        end
      end
      end
   end
end
```

Script 4- Chemogenetics

```
Path = 'xxx';

nLines = 13; %ignore lines with text only (paradigm info)

nVars = 13; %variables: 1. Trial#, 2. Correction Trial, 3. ITI, 4. FP, 5. StimType (dB),

%6. Outcome, 7. RT, 8-13, Time Info

maxdBstim = 70; % highest sound intensity used


% inclusion criteria

Threshold.nTrials = 150; % min nr of trials per session

Threshold.nTrials2 = 100;

Threshold.HitAtHigh = 0.6; % Mimimum P(hit) at the highest intensity

Threshold.nStypes = 3; % min nr of StimTypes


% scan folders

FoldersInfo = dir(Path);

FoldersInfo(1:2) = []; % ignore irrelevant items (. , ..)


%%%%%%%%%%%%%%%%%%%%%%%%

nAnimals = length(FoldersInfo); % detect number of animals

BigMat = [];% for CTL(control) sessions: animal#, session#org, session#ordered, StimType, P(hit), meanRT, medianRT,
CNOon/off

BigMatCNO = []; % for CNO sessions(under activated DREDDs,CNO injections)

BigMatSLN = []; % for SLN sessions (saline injections)

for a = 1:nAnimals

   MyAnimal = FoldersInfo(a).name;

   fprintf([MyAnimal, ':']);


      %% Control (CTL sessions, no injections)

      %% scan files

      Files = dir([Path, '\', MyAnimal,  '\NDT\NDT*.txt']); % obtain text file informaton

      nSessions = size(Files,1);
```

```
endoftrials=0; % for cutting session after 10 inactive trials
ActSessionNum=0;

for s = 1:nSessions
   %% open file
   MyFname = Files(s).name;
   MySessionNum = str2double(MyFname(9:11)); % extract session number

   fid = fopen([Path, '\', MyAnimal, '\NDT\', MyFname]);
   tmp = textscan(fid, '%s', nLines, 'delimiter', '\n');
   tmp = fscanf(fid, '%d')';
   bMat = reshape(tmp, nVars, length(tmp)/nVars)'; % organise variables in 13 columns
   fclose(fid);
   %% reshape the matrix
   bMat(bMat(:,2)~=0, :) = []; % ignore correction trials
   bMat(bMat(:,5)==0, :) = []; % ignore mistake 0 dB traials
   nTrials = size(bMat,1); %number of trials

   %% check if there are enough trials
   if nTrials >= Threshold.nTrials
      %% cut session if there are 10 inactive trials in a row for all dB

      % find maximum Stimulus
      StimTypes = unique(bMat(:,5));

      % inactive trials
      t_idle = -1;

      counter=0; % counting inactive trials
      endoftrials = nTrials; % new number of trials
      for t=1:nTrials
         if bMat(t,7) == t_idle
            counter = counter + 1;
         else
            counter = 0;
```

```
    end
    if counter==10
        disp('10 trials idle mouse Control');
        endoftrials = t-10; % where session ends now
        break
    end
end
bMat = bMat(1:endoftrials,:);

change=bMat(:,6);  % change values to adjust to scale: 999(catch trial) to 30dB
%  disp(change);
for ch=1:length(change)
    if change(ch)==3
        change(ch)=2;
    end
    if change(ch)==4 %FA is hit(go),allows to plot hit rate and RT
        change(ch)=1;
    end
end
%  disp(change);
bMat(:,6)=change;
            catchTrial =bMat(:,5);
for cT=1:length(catchTrial)
    if catchTrial(cT)==999
        catchTrial(cT)=30;
    end
end
bMat(:,5)=catchTrial;
             %% core assessment
%% hit rate for highest dB(70) and all other dBs

if length(StimTypes) >= Threshold.nStypes
    StimTypes = unique(bMat(:,5));
    HitNumAtHigh = length(find(bMat(:,5)== maxdBstim & bMat(:,6)==1));
    nTrialsAtHigh = length(find(bMat(:,5)== maxdBstim));
```

```
            HitRatePerSession = HitNumAtHigh/nTrialsAtHigh;

            display(HitRatePerSession);

            if length(StimTypes) >= Threshold.nStypes && HitRatePerSession >= Threshold.HitAtHigh &&
(length(bMat)>=Threshold.nTrials2) % inclusion criteria

              ActSessionNum = ActSessionNum + 1;


              for st = 1:length(StimTypes)

                %% P(hit)

                nHit = length(find(bMat(:,5)== StimTypes(st) & bMat(:,6)==1));

                MyNtrials = length(find(bMat(:,5)==StimTypes(st)));

                MyPhit = nHit/MyNtrials;

                                %% assessment: Reaction Time

                idx = find(bMat(:,5)==StimTypes(st) & bMat(:,6)==1);

                if ~isempty(idx)

                  RTs = bMat(idx,7);

                  MeanRT = mean(RTs);

                  MedianRT = median(RTs);

                else % no hit

                  MeanRT = -1;

                  MedianRT = -1;

                end

                %% update the matrix

                BigMat(end+1,:) = [str2double(MyAnimal), MySessionNum, ...

                  StimTypes(st), MyPhit, MeanRT, MedianRT, 0];

              end

                disp(BigMat);

            end

          end

        end

    %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% repeat for CNO and SLN)
```

Script 5 –Electrophysiology

```
%    Filebase = 'xxx'; %rec name (animal name)

%    nCh = 49; % 32channels +16 ch EEG + 1 sync channel
```

```
%    SynCh = 1;
%% 1. event detection
 %% make sync dat
if fopen('sync.dat') == -1
   fprintf('creating sync.dat file ... \n');
   MakeSyncDat(Filebase, nCh, SynCh); %%%%%%%%%%%%%%%%%
end

%% down sampling
if fopen('sync.eeg')==-1
   fprintf('down-sampling sync.dat file...\n');
   DwnSampleSync('sync.dat'); %%%%%%%%%%%%%%%%%%
end

% %% event detection (block #1)
BlockNum = 1;
if fopen([Filebase,'.ndbs.',num2str(BlockNum),'.syn.evt']) == -1
   EvtData1 = MyEvtDetection(Filebase, BlockNum);
else
   EvtData1 = load([Filebase,'.ndbs.',num2str(BlockNum),'.syn.evt']);
end
%
% %% event detection (block #2)
BlockNum = 2;
if fopen([Filebase,'.ndbs.',num2str(BlockNum),'.syn.evt']) == -1
   EvtData2 = MyEvtDetection(Filebase, BlockNum, EvtData1(end,1)+1000);
else
   EvtData2 = load([Filebase,'.ndbs.',num2str(BlockNum),'.syn.evt']);
end



%% 3-1. spike train analysis I (OVERALL FIRING)
%% some prep
KwikFileName = [Filebase, '.kwik'];
SR = 20; %  sampling rate in kHz
```

```
%% load spk info
Res = double(hdf5read(KwikFileName,'/channel_groups/0/spikes/time_samples'))/SR; % all rec spike timesin ms
Clu = hdf5read(KwikFileName, '/channel_groups/0/spikes/clusters/main');
Clu(1) = []; % 1st element tells how many number of clusters the file contains.
SpkTime = Res(Clu > 1); % all rec times in msec

%% making histogram
MaxTime = ceil(max(SpkTime)/60000); % sort of recording duration in minute
Time = 0:1:MaxTime; % in min (bin size ... 1 min)

%% counting spikes in each bin
nSpks = histc(SpkTime/60000, Time);% spike time/1000 to sec/60 to min

%% get spontaneous spike rates
MaxTime2 = ceil(max(SpkTime)/1000); % sort of recording duration in seconds
Time2 = 0:1:MaxTime2; % in sec (bin size ... 1 sec)

nSpks2 = histc(SpkTime/1000, Time2);% spike times per sec in sec bins
SpkRateFirst5M=nSpks2(1:5*60)';
SpkRateLast5M=nSpks2(end-5*60:end-1)';

%% converting into spike rate (spk/sec)
SpkRate = nSpks/60;

%% stim event
EvtTime1 = EvtData1([1, end],1)/60000; % in min pre CNO
EvtTime2 = EvtData2([1, end],1)/60000; % in min post CNO

%% ploting a barchart
M = 1.1*max(SpkRate);
figure(5);subplot(211);
fill([EvtTime1; flipud(EvtTime1)],[zeros(2,1); M*ones(2,1)],'c','FaceColor',[0.95 0.95 1],'LineStyle','none'); hold on;
fill([EvtTime2; flipud(EvtTime2)],[zeros(2,1); M*ones(2,1)],'m','FaceColor',[1 0.95 0.95],'LineStyle','none');
bar(Time, SpkRate, 'k'); % ploting a barchart
hold off;
axis([Time(1)-0.5, Time(end)-0.5, 0, M]);
```

```
xlabel('min');ylabel('spikes/sec'); % labeling axes
box off; % get rid of the box
title(Filebase); % putting the title

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%% 3-2. spike train analysis II (tracing evoked responses)
Twin = 50; % in msec
nStim = max(EvtData1(:,2)); % # of intensities
nReps1 = size(EvtData1,1)/2/nStim; % # of repetitions in pre-drug period
nReps2 = size(EvtData2,1)/2/nStim; % # of repetitions in post-drug period

SpkMat1 = zeros(nReps1, nStim);
SpkMat2 = zeros(nReps2, nStim);
%% get spikes between sets of stimulations
% pre
TwinITI=1000;
Evtinterval=diff(EvtData1(:,1)); % get interval between events
Evtinterval=[Evtinterval;1500];
MyON=EvtData1(Evtinterval>1000);% only take intervals that are >1000ms
MyON=MyON+500; % start 500ms after end of stimulation
for r = 1:nReps1
   SpkRatePre(r) = length(find(SpkTime > MyON(r) & SpkTime <= MyON(r) + TwinITI));
end
% post
Evtinterval=diff(EvtData2(:,1));
Evtinterval=[Evtinterval;1500];
MyON=EvtData2(Evtinterval>1000);
MyON=MyON+500; % start 500ms after end of stimulation
for r = 1:nReps2
   SpkRatePost(r) = length(find(SpkTime > MyON(r) & SpkTime <= MyON(r) + TwinITI));
end

%% find mean spontaneous spike rates
meanSpkRates(1)=mean(SpkRateFirst5M);
meanSpkRates(2)=mean(SpkRatePre);
meanSpkRates(3)=mean(SpkRatePost);
```

```
meanSpkRates(4)=mean(SpkRateLast5M);
figure(4);
bar([1:4],meanSpkRates)
%% constructing SpkMats
for s = 1:nStim
   MyON = EvtData1(find(EvtData1(:,2) == s),1);
   for r = 1:nReps1
      SpkMat1(r,s) = length(find(SpkTime > MyON(r) & SpkTime <= MyON(r) + Twin));
   end
end

for s = 1:nStim
   MyON = EvtData2(find(EvtData2(:,2) == s),1);
   for r = 1:nReps2
      SpkMat2(r,s) = length(find(SpkTime > MyON(r) & SpkTime <= MyON(r) + Twin));
   end
end

BigSpk = [SpkMat1; SpkMat2]/(Twin/1000); % spk/sec

%% display (for indiv)
figure(6)
X = [-nReps1:-1, 1:nReps2];
M = 1.1*max(BigSpk(:));
for s = 1:nStim
   subplot(3,3,s);
   plot([0 0],[0 M], 'r:');hold on;
   plot(X, BigSpk(:,s),'k.-');hold off;
   axis([-nReps1-1 nReps2+1 0 M]);
   box off;
   xlabel('repetition#');ylabel('spikes/sec');
   title([num2str(10*(s-1)), ' dB']);
end

set(gcf, 'PaperUnits', 'inches');
set(gcf, 'PaperSize', [15 10]); % width, height (in inches)
```

```
set(gcf, 'PaperPosition', [0 0.1 15 9.8]); %[left, bottom, width, height]
print('-djpeg',[Filebase, '_evoked_raw.jpg'],'-r300');

%%%%%%%%%%%%%%%
%% averaging
Tblock = 10; % every 10 trials, evoked responses are computed in Twin
nReps = size(BigSpk,1);
nBs = floor(nReps/Tblock);

AvgRate = zeros(nBs, nStim);
for b = 1:nBs
   AvgRate(b,:) = mean(BigSpk(Tblock*(b-1)+1:Tblock*b,:));

end

%% display
figure(5);subplot(212);
CO = jet(nStim);
X = [-nReps1/Tblock:-1, 1:floor(nReps2/Tblock)];
M = 1.1*max(AvgRate(:));
plot([-0.9 -0.9],[0 M], 'r:');hold on;
for s = 1:nStim
   plot(X, AvgRate(:,s), '.-', 'Color', CO(s,:));
end
hold off;
axis([X(1)-1 X(end)+1 0 M]);
box off;
xlabel('block #');ylabel('spikes/sec');
title(['average for each ', num2str(Tblock),' trials']);

set(gcf, 'PaperUnits', 'inches');
set(gcf, 'PaperSize', [15 10]); % width, height (in inches)
set(gcf, 'PaperPosition', [0 0.1 15 9.8]); %[left, bottom, width, height]
print('-djpeg',[Filebase, '_summary.jpg'],'-r300');

%%%%NEW COMPARE 4 STAGES  SPIKES PER SEC
```

```
SpkF5 = nSpks2(1:y,1); %first 5 min spont SPK/SEC IN SEC BINS

SpkL5 =  nSpks2(x:y,1);% last 5 min spont SPK/SEC IN SEC BINS

Pre=nSpks2(x:y,1); %one column all spk/sec PreCNO 450 +50ITI=500 entries

Post=nSpks2(x:y,1); % oe column spk/sec 6300+700ITI=7000 entries

% make all newSpk2 size so add till  7000

addFL5= NaN(x,1);

newSpkF5=[SpkF5;addFL5];

newSpkL5=[SpkL5;addFL5];

addSpkMat1=NaN(x,1);

new2SpkMat1=[Pre;addSpkMat1];

CompareAllStages=[newSpkF5,new2SpkMat1,Post,newSpkL5];

boxplot(CompareAllStages)


[p,tbl,stats] = anova1(CompareAllStages)

c = multcompare(stats)


%%%% compare spont

SpkF5 = nSpks2(x:y,1); %first 5 min spont SPK/SEC IN SEC BINS

SpkL5 =  nSpks2(x:y,1);% last 5 min spont SPK/SEC IN SEC BINS

Pre=nSpks2(x:y,1); %one column all spk/sec PreCNO 450 +50ITI=500 entries

PreITI=Pre(x:y:end,:); %take last sec from each trial ='spont'

Post=nSpks2(x:y,1);

PostITI=Post(x:y:end,:);

addBA=NaN(x,1);

sSpkF5=[SpkF5;addBA];

sSpkL5=[SpkL5;addBA];

addPreITI=NaN(x,1);

newPreITI=[PreITI;addPreITI];

CompareSpont=[sSpkF5,newPreITI,PostITI,sSpkL5];

boxplot(CompareSpont)

[p,tbl,stats] = anova1(CompareSpont)

c = multcompare(stats)


%%%% compare spont withEVOKED POST divided into 3

SpkF5 = nSpks2(x:y,1); %first 5 min spont SPK/SEC IN SEC BINS

SpkL5 =  nSpks2(x:y,1);% last 5 min spont SPK/SEC IN SEC BINS
```

```
Pre=nSpks2(x:y,1); %one column all spk/sec PreCNO 450 +50ITI=500 entries

PreITI=Pre(x:y:end,:); %take last sec from each trial ='spont'

Post=nSpks2(x:y,1);

PostITI=Post(x:10:end,:);

addPreITI2=NaN(x,1);

newPreITI=[PreITI;addPreITI2];

a1ITI=PostITI(x:y,:);

a2ITI = PostITI(x:y,:);

a3ITI = PostITI(x:y,:);

addITI=NaN(x,1);

a1=[a1ITI;addITI];

a2=[a2ITI;addITI];

a3=[a3ITI;addITI];

CompareSpont=[SpkF5,newPreITI,a1,a2,a3,SpkL5];

boxplot(CompareSpont)

[p,tbl,stats] = anova1(CompareSpont)

c = multcompare(stats)



%% main plot Evoked Pre Post through db


% for stats

addNaN= NaN(x,9);

FirstPre=BigSpk(x:y,:);

EvokPreCNO= [FirstPre;addNaN];

EvokPostCNO= BigSpk(x:y,:);


%divide Post into blocks as it's long (700trials=3x230 exclude last block

   % compare firts POST blocks then with pre)ANOVA

   %1step make 4 bigblocks each 100trials so 10 blocks

   E1stset = EvokPostCNO(x:y,:);

   E2stset = EvokPostCNO(x:y,:);

   E3stset = EvokPostCNO(x:y,:);


%%%% stats compare each dB PreCNO with all 3 bigbocks(3x230trials) PostCNO

%CompareMat = [];
```

```
CompareMat = [EvokPreCNO(:,1),E1stset(:,1),E2stset(:,1),E3stset(:,1)...
        EvokPreCNO(:,2),E1stset(:,2),E2stset(:,2),E3stset(:,2)...
        EvokPreCNO(:,3),E1stset(:,3),E2stset(:,3),E3stset(:,3)...
        EvokPreCNO(:,4),E1stset(:,4),E2stset(:,4),E3stset(:,4)...
        EvokPreCNO(:,5),E1stset(:,5),E2stset(:,5),E3stset(:,5)...
        EvokPreCNO(:,6),E1stset(:,6),E2stset(:,6),E3stset(:,6)...
        EvokPreCNO(:,7),E1stset(:,7),E2stset(:,7),E3stset(:,7)...
        EvokPreCNO(:,8),E1stset(:,8),E2stset(:,8),E3stset(:,8)...
        EvokPreCNO(:,9),E1stset(:,9),E2stset(:,9),E3stset(:,9)];
```

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