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[ELECTROPHYSIOLOGICAL CHARACTERIZATION OF P300 EVENT-RELATED POTENTIALS IN A RAT MODEL OF ALZHEIMER'S DISEASE]

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TITLE: **ELECTROPHYSIOLOGICAL CHARACTERIZATION OF P300 EVENT-RELATED POTENTIALS IN A RAT MODEL OF ALZHEIMER'S DISEASE**

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Abstract:

Background: Alzheimer's disease (AD) is an incurable and debilitating neurodegenerative disease characterized by a progressive loss of cognitive and bodily functions. The disease is associated with accumulation of toxic peptides and cholinergic degeneration in the brain. Event-related potentials (ERPs) are measured by electroencephalography (EEG) and have been widely studied in AD patients, since ERPs represent an objective measure of brain processing. The P300 ERP is of particular interest because a compromised P300 is commonly associated with the loss of cognitive function in AD. A rodent model of P300 would be useful in developing new pharmacological compounds that affect the cognitive system.

Methods: ERPs were recorded by EEG from hippocampus, auditory cortex, parietal cortex, and frontal/prelimbic cortex during an auditory discrimination paradigm in rats. Three experiments were conducted using two different experimental setups. A total of three sessions were analyzed. **1:** Sensory paradigm with no discrimination of tones, **2:** discrimination paradigm, and **3:** scopolamine (0.1mg/kg) / saline treated rats in the discrimination paradigm for each experiment.

Results: No P300 could be identified during experiment 1 (Three tone discrimination). A clearly defined P300 developed in hippocampus in experiment 2+3 and prelimbic cortex in experiment 3 with a latency of 170-180ms during discrimination training of the rats in experiment 2 and 3 (Two tone discrimination). Scopolamine increased latency in hippocampus in experiment 2 similar to what is seen in humans providing evidence that the P300 observed in the rats are translatable to the P300 seen in humans.

Conclusion: A method to evoke a P300 in rats was established. The P300 identified in rat hippocampus shows similar features to the human P300 and would be useful in studying new compounds that affect the cognitive function in AD. The prelimbic cortex also showed a trend towards mimicking human P300 but further studies are required to validate this.

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Preface

This master thesis in Medicine with Industrial Specialization - Translational Medicine, is based on experimental work performed at H. Lundbeck A/S, Copenhagen, in the period of August 2011 to June 2012.

The study consists of three experiments. The first experiment was conducted in the time period of September 2011 to November 2011. The second experiment was conducted in the time period of January 2012 to April 2012. The third experiment was started in May 2012 and is expected to end by June 2012.

The second experiment had to be postponed due to a viral infection in the animal facilities requiring disinfection of the entire site from end of November to medio January. The third experiment was conducted in the late stage of the project period to validate findings from experiment 2. Data retrieved from experiment 3 are currently being analyzed and only key findings are shown in this report.

Acknowledgement

Huge thanks to my advisors Parisa Gazerani, Associate Professor at Aalborg University, Jesper Frank Bastlund, Principal Scientist at H. Lundbeck A/S, and Bettina Clausen, Ph.D. Student at H. Lundbeck A/S, for their support and guidance throughout the project. Also thanks to Steven C. Leiser, Senior Research Scientist at H. Lundbeck A/S US division and Søren Ellegaard, owner of Ellegaard Systems A/S for helping me developing the procedures and experimental setup that was used. Also thanks to H. Lundbeck A/S for giving me the opportunity to conduct this project. Further thanks to all my colleagues at H. Lundbeck A/S for being open-minded, helpful, and allowing me to join in their meetings, seminars, and other projects.

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List of abbreviations:

Aβ	Amyloid-β
ACh	Acetylcholine
AChEI	Acetylcholine Esterase Inhibitor
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's disease Assessment Scale – Cognitive subscale
APOE	Apolipoprotein E
APP	Amyloid Precursor Protein
CSF	Cerebrospinal Fluid
EEG	Electroencephalography
ERP	Event-Related Potential
FDG-PET	Fludeoxyglucose Positron Emission Tomography
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
NFT	Neurofibrillary Tangles
NMDA	N-Methyl-D-Aspartate
qEEG	Quantitative Electroencephalography
S.C.	Subcutaneously

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1 Introduction to Alzheimer's disease

Alzheimer's disease (AD) is the most common type of dementia[1]. It was first identified in 1907 by Alois Alzheimer who described a female patient with severe cognitive deficits and in the post-mortem examination noticed an intracellular accumulation of tangled neurofibrils and the accumulation of an unidentified pathological metabolite[2], later identified to be amyloid-β (Aβ)[3]. AD is an incurable and debilitating neurodegenerative disease characterized by a progressive loss of cognitive and bodily functions such as memory loss, changes in mood and personality, and confusion[4]. AD has characteristic pathological changes also known as hallmarks of AD. These are deposition of extracellular Aβ plagues, intracellular neurofibrillary tangles (NFT) and loss of cholinergic synapses in regions of the brain associated with higher mental function such as the neocortex and hippocampus[5, 6].

The current treatment options offer only symptomatic relief and no curative treatment is available at the moment[7], and require care from family and professional caregivers, especially in its later stages[1]. In 2011, an estimated worldwide population of 30 million had the disease[8, 9], primarily those of age 65 and above[1]. This number is expected to be quadrupled in 2050 making it a global health issue[8], both for the individual people inflicted and for the society as a whole[1]. Hence, better elucidation of the mechanisms underlying AD which can potentially lead to novel and more efficient therapy for this disease is warranted.

1.1 The cause of Alzheimer's disease

The exact cause and pathophysiology of AD is not yet fully understood[10-12]. A proposed theory of the development of AD focuses on the accumulation of Aβ. Mutations in various genes in the amyloid pathway increase the risk of developing AD. In healthy humans, Aβ is cleaved by the amyloid precursor protein (APP) into $A\beta_{1-40}$ or $A\beta_{1-42}$. The ratio between these two isoforms are determined by α, β, and γ secretases. The $A\beta_{1-42}$ accumulates more readily into plagues than $A\beta_{1-40}$ [9, 13].

Mutations in APP, or two genes that are part of the α-secreatase complex, Presenilin 1 and 2, have shown to increase the production of $A\beta_{1-42}$ and people with these mutations have shown to develop AD early in life[14]. People with Trisomy 21, also known as down syndrome, who have and extra copy of APP almost always exhibit AD at an age of 40[14]. Another gene that is a major risk factor for AD is apolipoprotein E (APOE). APOE is part of the trafficking and clearance of Aβ and people carrying two APOE ε4 alleles have higher risk of developing AD than individuals with the APOE ε3 alleles[15]. The accumulation of Aβ plagues is believed to trigger the formation of the intracellular NFT. NFT are composed of tau, a microtubule stabilizing protein, that when hyperphosphorylated may accumulate into tangles. These NFT are disrupting the cells and can induce degeneration[15].

Some risk factors for AD are mutations in the genes mentioned above, older age, cerebrovascular diseases such as hypertension, hypercholesterolemia, and diabetes. It has also been shown that environmental factors such as head trauma can increase the risk of mild cognitive dementia (MCI) and AD dementia[6]. The relatively long time period between AD pathology and onset of clinical symptoms are believed to be due to a "brain reserve" in which the brain can withstand some amount of injury before clinical symptoms occur[6]. A summary of the interplay between these pathophysiological factors can be seen in figure 1.

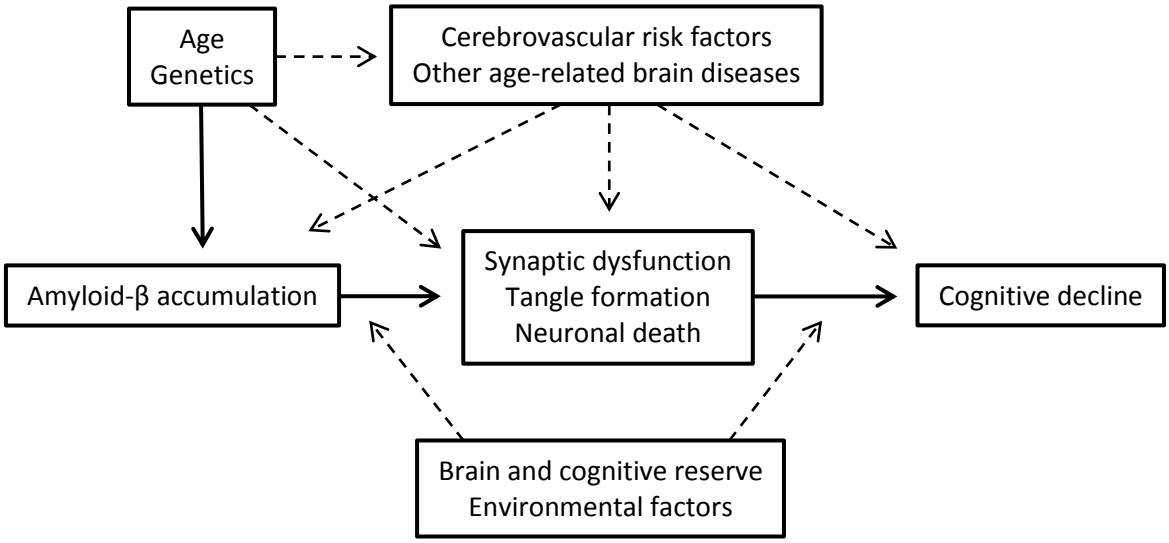

Figure 1: The interplay between the pathophysiological factors that can influence the development of the cognitive decline seen in AD. The straight lines indicate what is believed to be the primary pathway to the development of AD, dotted lines indicate factors that can influence on this pathway. (Adopted with few modifications)[6].

The synaptic dysfunctions are primarily associated to the cholinergic system in the brain. This have been linked to cognitive function, learning, and memory[16] and is mediated by the neurotransmitter acetylcholine (ACh) binding to nicotinic ACh receptors and muscarinic ACh receptors[17]. The dysfunctions in this cholinergic system have been linked to the cognitive deficits seen in AD[18]. These deficits of the cholinergic system seen in AD could be due to a decreased synthesis of ACh, decreased reuptake of ACh, decreased release of ACh, and/or loss of cells in nucleus basalis of Meynert which in healthy humans have many cholinergic projections to the neocortex[19].

1.2 Symptoms and diagnosis

AD can be divided into three stages. A non-symptomatic preclinical stage[6], a symptomatic pre-dementia stage, also known as mild cognitive impairment (MCI)[20], and a dementia stage[21]. The difference between MCI and dementia are whether the cognitive impairment influences daily activities or not[20]. The two symptomatic stages, MCI and dementia, are primarily diagnosed clinically by patient history and cognitive assessment by the physician[20, 21].

The assessment of the cognitive function is done using various neurophysiological tests such as the mini-mental state examination (MMSE) or the Alzheimer's disease assessment scale – cognitive subscale (ADAS-Cog). The MMSE is a short 11 question questionnaire which is used to quickly access the cognitive state of patients with dementia[22]. The ADAS-Cog are a more comprehensive test that consists of 11 tasks for testing disturbances in memory, language, and other cognitive abilities[23].

However, it is difficult to establish a confident diagnosis of AD as other diseases such as frontotemporal lobar degeneration, cerebrovascular diseases, lewy body disease, and prion disease share symptoms and disease biomarkers with AD[24].

1.3 Medical management of Alzheimer's disease

Currently, five different medications are used in management of AD, four which are acetylcholine esterase inhibitors (AChEIs) (Donepezil, Rivastigmine, Galantamine, and Tacrine) and one N-methyl-d-aspartate

(NMDA) receptor antagonist (Memantine)[25]. Tacrine is only used as last resort as it has been linked to hepatotoxicity[26].

The AChEIs work by inhibiting the enzyme acetylcholine esterase resulting in a decrease of degradation of ACh in the synaptic cleft leading to an increase in the concentration and duration of action of ACh. This can lead to improvements in cognitive function and improvements in function and daily activities in some patients[25].

The NMDA receptor, an ionotropic glutamate receptor, is activated by NMDA. In AD, the NMDA receptors are believed to be disrupted by Aβ leading to increased calcium influx resulting in excitotoxicity. This can be reduced by inhibiting the NMDA receptor with a NMDA antagonist[25].

The current pharmacological treatments are symptomatic with improvements in cognition but there are no evidence of slowing the progression of the disease[7]. Therefore, new treatments that are capable of delaying the disease progression is highly sought after[9].

1.4 Biomarkers of AD

Biomarkers can be used to increase the certainty of the diagnosis of AD, to follow the progression of the disease, and to monitor the effect of treatment[24]. Examples of various AD biomarkers can be seen in table 1.

Disease hallmark	Biomarker
Amyloid-β	- ↓CSF-$A\beta_{1-42}$ - High PET amyloid tracer retention
Neurodegeneration	- Hypometabolism by FDG-PET - ↑CSF-Tau - ↑CSF-Phosphorylated Tau - Gray matter loss by volumetric MRI - Hippocampal atrophy by volumetric MRI

Cognitive deficit	- EEG*
	  ○ qEEG changes
	  ○ ERP changes

Table 1: Biomarkers that can be used for increasing the certainty of AD in patients[20, 21, 24, 27, 28]. *The application of EEG as a biomarker for AD is primarily used in clinical trials and research[27]. **CSF:** Cerebrospinal Fluid, **Aβ:** Amyloid-β. **FDG-PET:** Fludeoxyglucose Positron Emission Tomography. **MRI:** Magnetic Resonance Imaging. **EEG:** Electroencephalography. **qEEG:** Quantitative EEG. **ERP:** Event-related potentials

Identifying people in the non-symptomatic preclinical stage are very difficult as no or only very poor cognitive symptoms are present and one has to rely on biomarkers. The use of biomarkers in this preclinical stage is problematic as the pathophysiology of AD is not yet fully understood[6]. The progress of different biomarkers throughout the preclinical, MCI and dementia stage was proposed by Jack, C.R. et al.[29] and can be seen in figure 2.

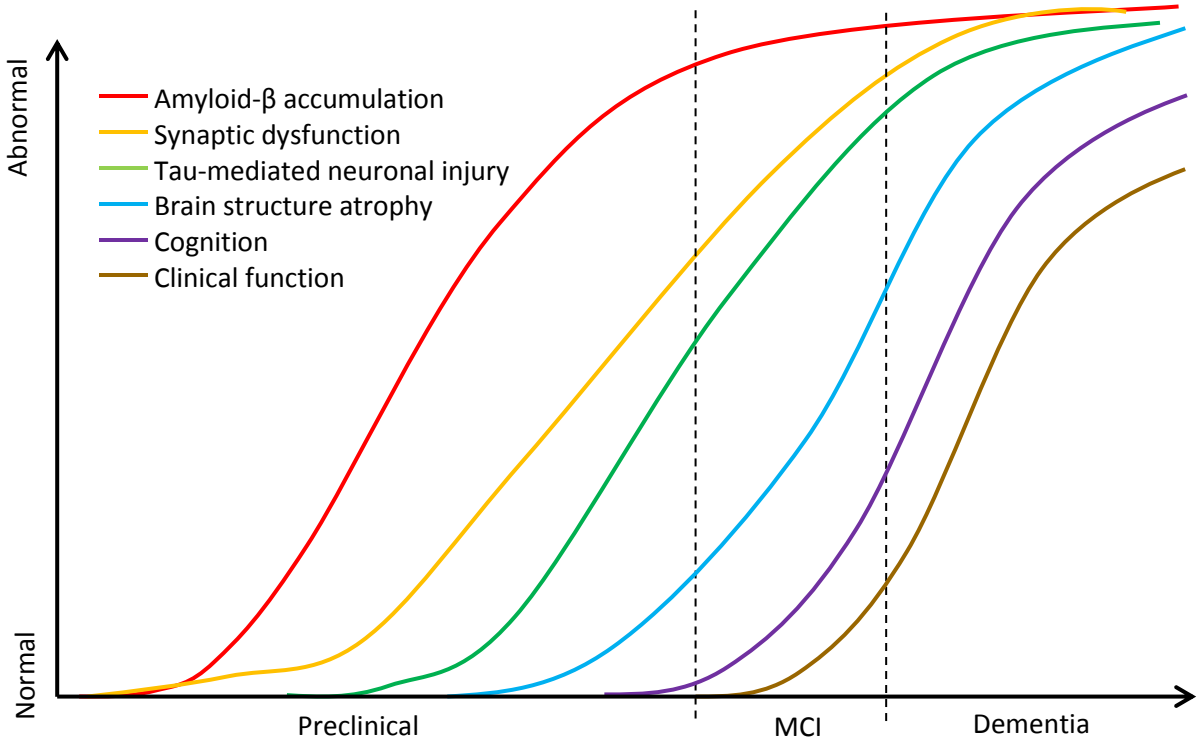

Figure 2: A hypothetical model of biomarkers throughout the different stages of AD (Adopted with few modifications)[6, 29]. **MCI:** Mild Cognitive Impairment

Even though electroencephalography (EEG) is a non-invasive and cheap method of brain imaging, only limited attention has been paid to its use in AD. Over the last decade, major improvements in the technology have led to an increased interest in developing and validating EEG biomarkers in AD[27].

1.5 Electroencephalography

EEG is the recording of the electric activity in the brain. It measures the voltage fluctuations generated within neurons of the brain that have become electrically charged by transporting ions across membranes. It cannot measure the electrical activity of a single neuron but measures the synchronized electrical bursts of multiple neurons[30].

1.5.1 Event-related potentials

Event-related-potentials (ERPs) are being used as a noninvasive clinical marker for brain function in human patients. ERPs are voltage changes specified to a physical or mental occurrence that can be recorded by EEG[31].

In Figure 3, an example of an ERP signal can be seen. The signal can be divided into two parts, a pre-stimuli section consisting of a baseline with no clear potentials and a post-stimuli section consisting of various potentials. The first positive potential is called P1, followed by a negative potential N1, then P2, N2, and so forth. The latency of these potentials is measured from onset of stimuli to the peak of the potential. Sometimes the peaks are named using the latency, e.g. if N1 occur at a latency of 40ms it is named N40 or if P3 occur at a latency of 300ms it is named P300. The baseline amplitude is the difference between the peak of a potential and the mean of the pre-stimulus baseline[32, 33].

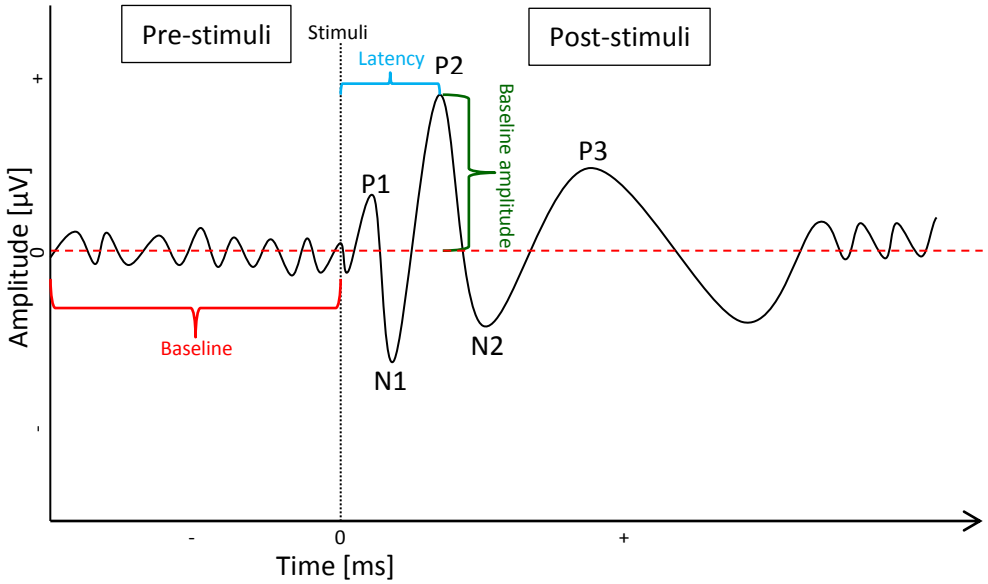

Figure 3: Illustration of a possible ERP signal. On the X-axis the time is shown with 0 at the stimuli. The Y-axis is the amplitude with 0 at the baseline. In the pre-stimuli window a baseline is visible from which a horizontal average can be calculated shown by the red dotted line. In the post-stimuli window the various potentials can be seen with the first positive peak named P1, the next negative named N1 etc. Note that the orientation on the Y-axis can be reversed in some cases[34] and the signal presentation varies from where in the brain recording is performed in the subject.

The EEG data consist of the actual ERP waveform and random noise created from random brain activity or muscle activity and/or environmental noise such as lighting or mechanics[35]. To retrieve a clearer ERP waveform it is necessary to remove or reduce the random noise by averaging multiple trials together. The theory behind this is that the ERP waveform is always the same after each stimulus as it is time-locked to the stimuli and would therefore not be altered by averaging. The noise on the EEG occurs randomly and is not time-locked to any stimuli. The noise would then even itself out as more trials are averaged together[36]. Thus the averaging process of the EEG signal increases the visibility of the ERP by reducing the random occurring noise.

ERPs can be divided into three different classes; somatosensory, visual and auditory, according to which type of stimuli is used[34]. The auditory class is the one in focus of this project. A commonly used method to measure auditory ERPs is by use of the active oddball paradigm where the subject have to distinguish an infrequent stimulus (target stimulus) from a frequent stimulus (non-target stimulus) by e.g. pressing a button when the target stimuli occur[32]. If a non-target stimulus occurs, a sensory complex consisting of the P1, N1, P2 and N2 peaks can be recorded by EEG with no or only a small P300 visible[32, 37]. When the target stimuli occur, the sensory component and a P300 potential can be seen[32]. Hence, the P300 potential can be defined as a time-locked response to rare, response-relevant stimuli. This is illustrated on figure 4 below.

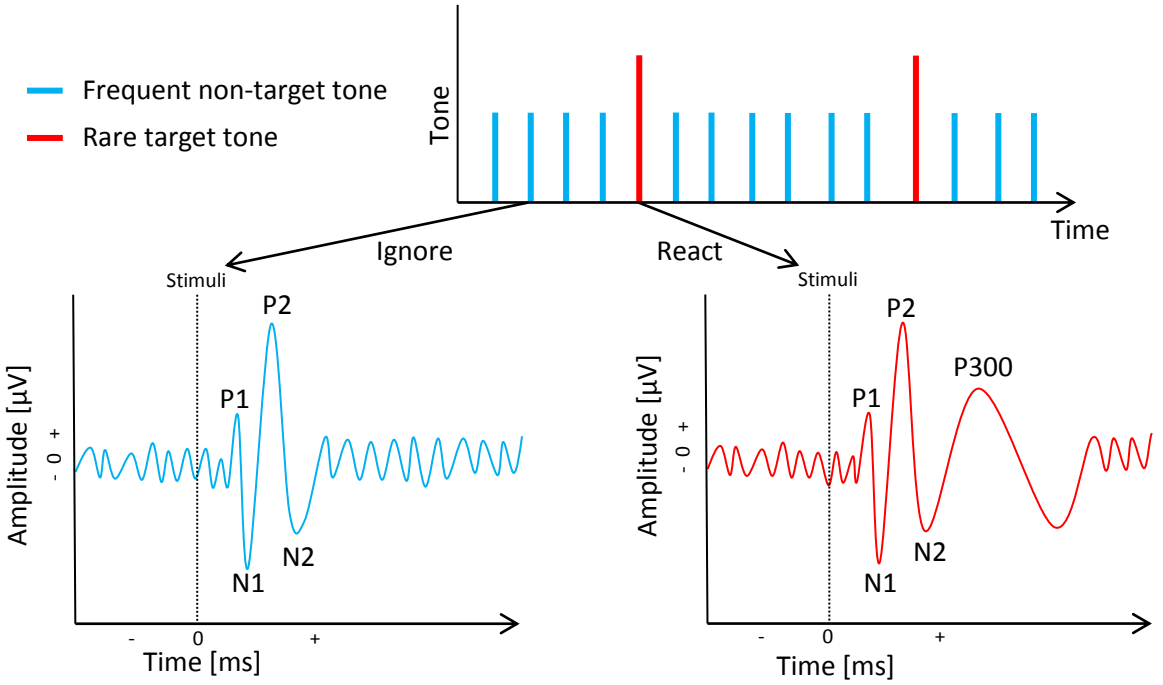

Figure 4: Example on how a P300 signal can be evoked. A subject is exposed to two tones, a frequent non-target tone which must be ignored, and a rare target tone which the subject have to react to, e.g. press a button. On the frequent non-target tone only a sensory component is evoked (in this example P1, N1, P2 and N2). On the rare target tone, the same sensory component is evoked together with a later P300 potential[32].

The P300 potential commonly occurs at a latency of 300 – 1000ms in humans and is associated with attention, stimulus evaluation, decision making, and is believed to originate from the frontal cortex and medial/parietal brain regions[32]. The amplitude of the signal is a measure of CNS activity[28]. Shorter latency is linked to superior cognitive function[38]. The latency can increase if the task increases in difficulty, e.g. the difference in hertz of the non-target and target tone is small[28, 39].

The P300 signal can be divided into two subgroups, P3a and P3b. The P3a signal is seen in engagement of attention towards unexpected changes in the environment. The P3b signal is seen in tasks where a person has to identify an improbable event, e.g. identifying a target tone from a non-target tone like the example above on figure 4. P3b is commonly referred to as P300 as it is the most studied[32, 40].

1.5.2 P300 in Alzheimer's disease

In dementia diseases such as AD where cognitive function is reduced, it has been shown that the latency of P300 is increased[37] and the amplitude decreased[41] compared to similarly aged healthy people[41]. As the disease progress, a latency increase and an amplitude decrease of the P300 can be observed[28, 40]. Figure 5 illustrates the difference in P300 between healthy subjects and AD patients.

Few studies have been conducted on the currently approved drugs for AD and their effect on P300. The drugs Donepezil[42-46], Rivastigmine[42], Tacrine[47], and Memantine[48] have been tested on P300 paradigms in AD patients all showing a shortening of the P300 latency when compared to control group. Only one study showed an increase of the P300 amplitude[42].

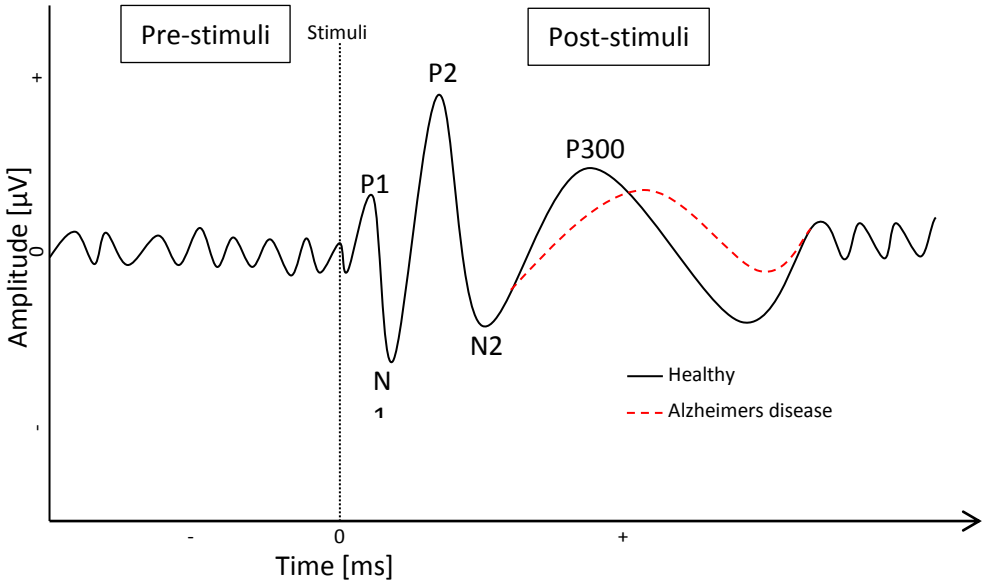

Figure 5: The difference between the P300 in healthy subjects and subjects with AD. The latency is increased and amplitude is decreased in the AD subjects illustrated by the red dotted line.[37, 41].

These auditory potentials can be studied in rodents and a rodent model for P300 would be very useful when developing drugs that influence the cognitive system as those desired for treatment of AD. This is due to easy access of subjects and the various transgenic models that can mimic the disease. But further studies on the translatability between rodent P300 and human P300 are required to ensure that the signals observed are comparable.

1.5.3 Rodent models of P300 ERPs

The latency of ERP signals in rodents is smaller than in humans[49, 50]. This reduced latency is due to the fact that the signal has to travel a shorter distance in the much smaller brain size of rodents[49]. This seems to correlate fine with the sensory component of the paradigm (P1, N1, P2, and N2) which is about 40-50 % shorter in rodents than in humans[49, 50].

In table 2, a comparison of the latencies between human, mouse, and rat can be seen. It is important to note that the latency components in the rodents are not well defined and vary from study to study and electrode placement in the brain.

Only a limited amount of auditory evoked P300 studies have been conducted in rats and a summary of these publications and posters can be seen in table 3 below.

Component	Human latency [ms][49]	Mouse latency [ms][50]	Rat latency [ms][49]
P1	last positive peak before N1, ≈50	20	10-30
N1	90-150	40	41-80 (30-50)*
P2	160-250	80	80-130 (50-100)*
N2	240-350	Not described	130-200*
P3	320-450	120	Not well defined

Table 2: Latency intervals of the different components of auditory evoked potentials in human, mouse, and rat. *The intervals can vary between studies. Some define them to appear earlier than described (e.g. N1: 30-50ms, P2: 50-100ms[51]).

Study	Year	Sample Size	Brain regions implanted	Paradigm	Latency of P300 [ms]
Hurlbut, B. J. et al[52]	1987	10	Cortical midline	Passive discrimination task	≈300*
Yamaguchi, S. et al.[53]	1993	8	Frontal cortex Vertex Visual cortex Posterolateral dorsal skull	Passive discrimination task	240
Ehlers, C. L. et al[54]	1994	26	Enterohinal cortex Hippocampus Frontal cortex Parietal cortex	Active discrimination task	300-400
Iwanami, A. et al[55]	1994	4	Auditory cortex	Active discrimination task	290
Jodo, E. et al[56]	1995	14	Medical forebrain bundle	Active discrimination task	260-580
Shinba, T. et al[57]	1996	4	Hippocampus	Active discrimination task	452.0±30.7
Brankačk, J. et al[58]	1996	16	Frontal cortex Border of Parietal and temporal cortex Occipital cortex Retrosplenial cortex	Active discrimination task	274
Shinba, T.[59]	1997	6	Frontal cortex Temporal cortex Parietal cortex	Passive discrimination task Active discrimination task	≈275* 450
Shinba, T.[60]	1999	3	Hippocampus	Active discrimination task	463.7±103.7
Galicia, O. et al[61]	2000	5	Vertex	Passive discrimination task	200-250
Sambeth, A[49]	2003	12	Vertex	Active discrimination task Passive discrimination task	380
Hattori, M. et al[62]	2010	14	Hippocampus	Active discrimination task	200-500
Leiser, S. et al[63]	2010	N/A	Vertex	Active discrimination task	120
Clausen, B. et al[64]	2011	30	Hippocampus Prefrontal cortex	Active discrimination task	≈400*

Table 3: A summary of the different studies on P300 in rats. *This value is measured from a figure in the article. The exact latency is not described in the text.
The passive discrimination task is when the rat does not have to perform any operant procedure during the experiment (P3a). The active discrimination task is when the rat has to do an operant procedure during the experiment (P3b).

Due to lack of information about first sensory component in the majority of published articles and as no guidelines are available for the latency window, comparisons across studies are difficult to achieve. Another issue regarding the sensory component is that the presentation of the signal can vary from each recording site.

By performing a simple experiment in which the rat only have to listen to the tones used in the actual experiment, the composition of the sensory component can be properly identified at each electrode position and used as a baseline when identifying the sensory component in the actual experiment in which increased amount of EEG noise is visible, such as artifacts from the rats movement.

It is interesting that the latency of the P300 component in rats is not shorter compared with humans, which could indicate that the P300 component identified in many of the studies might be some later cognitive process and the actual P300 component occurs much earlier at around 128-180ms (Human = 320-450ms) if the 40 % reduction is applicable[65]. This is supported by Leiser, S. et al who found a latency of P300 at 120ms in rats[63].

1.6 Rodent models of Alzheimer's disease

Rodents do not develop AD naturally and the disease therefore has to be artificially induced. This can be done be either use of transgenic models in which certain genes related to AD can be inserted to the rodent genome and induce the development of pathological features such as Aβ or NFT accumulation[66]. Another method is by inducing injuries in the brain with either lesions or chemicals that destroy specific AD related areas of the brain e.g. 192-IgG-saporin, a toxin that destroy cholinergic neurons in the basal forebrain, thereby mimicking the cholinergic degeneration of AD[67]. Alternatively, one can also induce a chemical that disrupts the function of certain neurons, e.g. the drug scopolamine, a non-selective muscarinic receptor antagonist, which blocks the effect of ACh in the synaptic cleft leading to a cognitive deficit similar to the one seen in AD[68].

Two studies have tried to measure P300 in an active auditory paradigm in AD rats, one using the 192-IgG-Saporin toxin[64], and one using scopolamine[63]. Both studies identified an increase of P300 latency in the AD rats compared to the control rats. The study by Leiser, S. et al. also showed that donepezil could restore the P300 latency back to normal in the scopolamine induced AD in rats[63].

Further studies of the P300 signaling in AD rodent models are required to get a better understanding on the generation and function of the signal and would be useful in testing the cognitive effects of future compounds that could be used in treatment of cognitive deficits.

2 Aim

In the following experiments the main goal was to establish a method to examine the P300 signal in rats during an active auditory oddball paradigm, in which a rat has to respond to an auditory stimuli by either performing a task or ignore the stimuli. Electrodes are positioned in the frontal cortex, parietal cortex, auditory cortex and hippocampus. The cholinergic deficit in AD was mimicked by the drug scopolamine, a competitive muscarinic ACh receptor antagonist, and its effect on the P300 signal was measured. It was expected that the sensory peaks are in the range described in table 2 and the P300 to occur at a latency shorter than what is seen in humans.

The specific aims of the present study were:

- To develop a method to evoke a P300 signals in the rat brain during an active auditory oddball paradigm similar to what is seen in humans.
- To identify if scopolamine have similar effect on P300 signal as seen in human studies. E.g. reduced amplitude and increased latency.

We aimed at developing a valid model of P300 in rats and a scopolamine-induced model of AD-like changes in the P300. Such a rodent model would be very valuable in evaluating potential cognition-enhancing AD drug candidates due to the high translatability of P300 as a biomarker.

3 Methods

All experiments were performed in accordance with Danish legislation, and animals were treated in adherence to guidelines for the care of experimental animals (License 2009/561-1596).

All animals were housed individually in the H. Lundbeck Animal facilities in Copenhagen, Denmark, in Macrolon (type III) cages with standard sawdust bedding and environmental enrichment (transparent house and wooden chew blocks), under constant temperature and humidity in a 12/12 light/dark cycle (Lights on at 6AM). Food (Altromin 1324) and water was given ad libitum prior to surgery and during the recovery period after surgery. During experimental procedures they were kept on 80% of free-feeding weight. The animals were rewarded with food pellets (Dustfree Purified Rodent tablets - 5TUL, standard 45mg tablet, Research Diets & P J Noyes) when they performed correctly during the experiments. All animals were handled daily by the experimenter. Animals were euthanized following the experiments using carbon dioxide gas (80 % CO_2 and 20 % O_2).

3.1 Experiment 1

In the first experiment, a rat had to press a lever when a lever tone was played. A target tone indicated that the rat has pressed enough times and that a food reward has been released. Non-target tones were played between lever tones. At the end of the study scopolamine was induced in half of the rats to see whether this can affect the P300 signal.

The experiment consisted of two protocols. The first protocol consisted of training the rat to press the lever. The second protocol consisted of the actual paradigm, see appendix for details.

3.1.1 Subjects

A total of 12 male Sprague-Dawley rats were obtained from Charles River (Germany). They weighed between 250g and 325g at the day of surgery. No rats were excluded during the experiment.

3.1.2 Surgical preparation

Each rat was anesthetized with a 1:1 mixture of Hypnorm® (0.25mg/kg fentanyl and 12.5 mg/kg fluanisone, H. Lundbeck A/S) and Dormicum® (6.25 mg/kg midazolam, Roche) subcutaneously (S.C.). When the rat was fully anesthetized, the hair was shaved from neck to nose. The skin was sterilized with Iobac© Vet. (1.6% Iodine solution, Novartis Agri A/S), and eye ointment (200mg paraffin oil and 800mg petroleum jelly, Optha A/S) was applied to prevent excision of the cornea. The rat was fixed in a steotaxic frame and given a local analgesia, ≈0.7mL Marcain® (2.5 mg/mL bupivacaine, AstraZeneca) S.C. at the site of incision. 5 surface electrodes (E363/20, Plastics One) and 1 depth electrode (E363/1, Plastics One) were inserted stereotaxically according to Paxinos & Watson[69]. **1:** Frontal cortex, 4.7mm anterior to bregma, 0.5mm lateral. **2:** Parietal association cortex, 3.8mm posterior to bregma, 3.0mm lateral. **3:** Secondary auditory cortex, 4.8mm posterior to bregma, 6.4mm lateral. **4:** Ventral hippocampus CA3, 5.3mm posterior to bregma, 4.6mm lateral, 2.6mm ventral to dura. **5:** Reference, 8.0mm anterior to bregma, 1.0mm lateral. **6:** Ground, 4.5mm posterior to bregma, 4.0mm medial. All electrodes were inserted into a 6-channel pedestal (MS363, Plastics One) which was then attached to the skull with dental cement (RelyX™ Unicem, 3M ESPE and GC Fuji PLUS™, GC America INC.) and the skin was sutured. The rats were allowed to recover for at least 10 days after surgery. The rats received analgesia, Rimadyl vet® (5mg/kg Carprofen, Orion Pharma Animal Health) S.C., and antibiotics, Baytril vet® (2.5mg/kg Enrofloxacin, Bayer) S.C., 30min prior to surgery and for 5 days after the surgery. Sutures were removed after 5-7 days. See appendix for in depth description of the surgery procedure.

3.1.3 Behavioral procedures

Experiments were conducted during the light phase. The rats were placed in a test chamber inside a soundproof box. An audio speaker was attached to the side of the box. At one end a retractable lever and a pellet receptacle together with lighting were attached, see figure 6. See appendix for details.

All mechanics in the experiment were controlled with EGGO-Lab, Ellegaard Systems A/S, V. 1.0.1.6

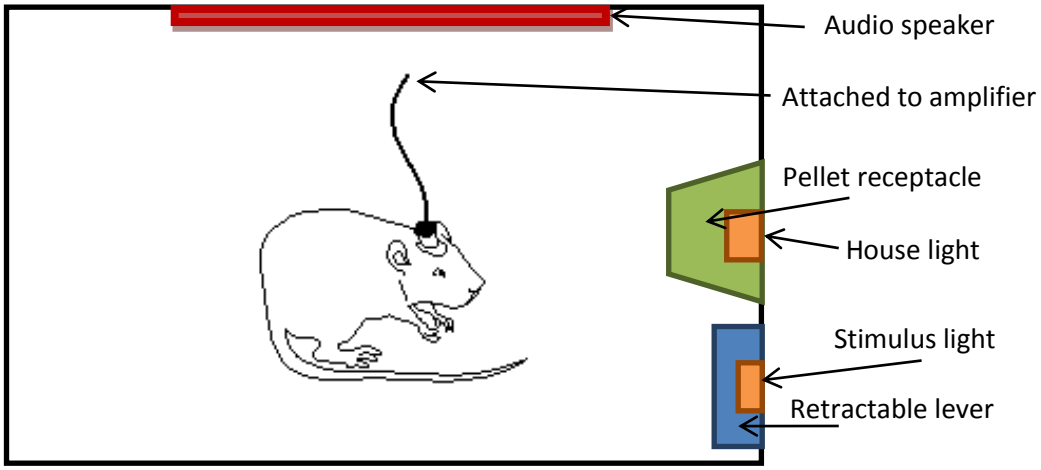

Figure 6: Setup of the test chamber for experiment 1. Red = Audio speaker, Green = Pellet receptacle, Orange = Lighting, Blue = retractable lever. The cable attached to the head of the rat is attached to an amplifier positioned outside of the test chamber.

The rats were trained to press the lever for a food reward. The number of correct presses required for releasing a food reward increased during the training sessions of the first protocol with a final requirement of 2, 4, 6, 8 or 10 presses (mean = 6 presses). A frequent non-target tone (2000Hz, 90dB, 20ms duration) was played every 6, 7, 8, 9 or 10sec (mean = 8sec). A rare target tone (4000Hz, 90dB, 20ms duration) was played when the rat had pressed the correct number of times on the lever and indicated that a food reward was released.

When a rat could perform 60 correct responses in less than 30min it was introduced to the second protocol. The second protocol had the same setup as the one just described but the lever would be retracted and a new lever tone (3000Hz, 90dB, 20ms duration) indicated the introduction of the lever to the test chamber. The rat then had 5sec to start pressing the lever and 10sec to press the required number of lever presses to release a food reward and the lever would retract. If the rat failed to press the required amount of times the lever was retracted with no food reward being released and the lighting in the box was turned off for 10sec.

The procedure can be seen on figure 7. See appendix for in depth description of the protocols.

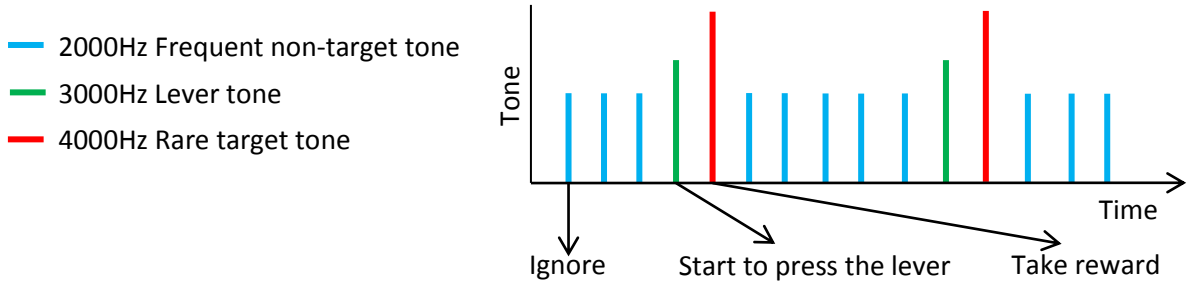

Figure 7: Example of the procedure in experiment 1. The lever was introduced when the lever tone (3000Hz) was played. The rat then had to press 2, 4, 6, 8 or 10 times for a food reward to be released indicated by the target tone (4000Hz). A series of non-target tones (2000Hz) was played in between.

At the end of the study 6 rats were injected with scopolamine (0.1mg/kg Scopolamine[63, 68], Sigma-Aldrich®, dissolved in 0.9 % NaCl) S.C. and the remaining 6 rats were given saline (9mg/mL Sodium Chloride, Fresenius Kabi) of equal volume S.C. The drug was given twice in two days. On the first day no experiment was performed due to high sedation induced by scopolamine. On the following day the rats were exposed to the experiment 30min after injection, as the high sedative effect only was present after the first injection on the previous day.

An auditory sensory paradigm was conducted on the last day. Rats were placed in a test chamber with only the audio speakers installed. The 2000Hz tone was played every 6sec for 20min, this was followed by the 3000Hz, and 4000Hz. also every 6sec for 20min for a total of 60min. Each tone was played at 90dB with 20ms duration.

The timeline of experiment 1 can be seen in figure 8 below.

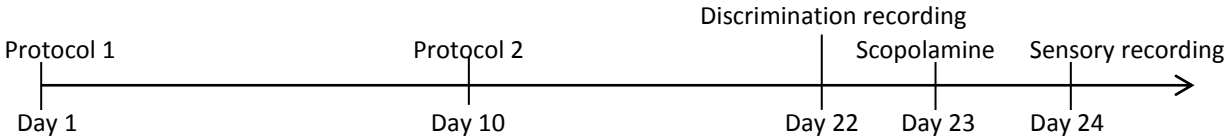

Figure 8: Timeline for experiment 1. A rat was exposed to and completed a protocol on each trial day. Resting days are not included in the timeline. A day was only counted if an experiment was conducted.

3.1.4 Recording

All recording instruments were electrically shielded in a Faraday cage. An EEG cable was inserted to the socket on the scalp of the rat. All EEG signals were amplified using an amplifier (Brownlee Precision Model 440 Amplifier) with a bandpass of 1.0 – 100Hz, gain of 5000, digitized with a sampling rate of 1000 samples/second (CED Power1401 mk 2), and recorded on a computer with SPIKE2 V. 6.09. (Cambridge Electronic Devices, United Kingdom).

3.1.5 Data analyses

All signal analyses were done in Spike2 V.7.07d. (Cambridge Electronic Devices, United Kingdom) All tones were averaged in the time interval 200ms pre-stimuli to 500ms post-stimuli. Tones that occurred on top of major artifacts or noise were excluded from averaging. The baseline was calculated by averaging the signal in the 200ms window pre-stimuli. All events were included in the averaging process.

A total of three sessions were analyzed: A sensory paradigm with no task requirement of the rat, a discrimination paradigm session, and a scopolamine/saline treated session.

Latency was measured from onset of stimuli to the tip of a peak. The amplitude was calculated as the difference from the tip of the peak to the baseline. If double peaks occurred within the expected range, the highest (or lowest) were measured.

The mean values were compared statistically by two-way ANOVA with tones as one factor and paradigm as the other factor. If a peak was missing for both tones in a paradigm, one-way ANOVA was used instead for statistic comparison. Post hoc analysis was performed by Bonferroni t-test. Means are considered to be statistically different if $P<0.05$.

3.2 Experiment 2

The method used in experiment 1 to evoke a P300 did not function properly, see discussion, and a new method to evoke a P300 had to be established. In the second experiment, a rat had to distinguish between two tones, a target tone and a non-target tone. A correct response released a food reward. An incorrect response initiated a timeout period where all lighting and audio were turned off for 30sec. At the end of the study a cross-over study with scopolamine was conducted to see whether it could affect the P300 signal.

The experiment consisted of two protocols. The first protocol consisted of training the rat to press the lever when a screening tone was played. The second protocol consisted of the discrimination paradigm.

3.2.1 Subjects

A total of 14 male Sprague-Dawley rats were obtained from Charles River (Germany). They weighed between 275g and 325g at day of surgery. 5 were rats lost following surgery due to complications.

3.2.2 Surgical preparation

Same procedure as was described for experiment 1. Electrode coordinates. **1:** Frontal cortex, 4.7mm anterior to bregma, 0.5mm lateral. **2:** Parietal association cortex, 3.8mm posterior to bregma, 3.0mm lateral. **3:** Secondary auditory cortex, 4.8mm posterior to bregma, 6.4mm medial. **4:** Ventral hippocampus CA3, 5.3mm posterior to bregma, 4.6mm lateral, 2.6mm ventrally to dura. **5:** Reference, 8.0mm anterior to bregma, 1.0mm lateral. **6:** Ground, 2.3mm anterior to bregma, 2.7mm medial.

3.2.3 Behavioral procedures

Experiments were conducted during light phase. The rats were placed in a test chamber inside a soundproof box. An audio speaker was attached to the side of the box. At the left side of the test chamber two retractable levers and two stimuli light above each lever were attached. At the right side of the test chamber a pellet receptacle together with house lighting were attached. The bottom lever was always the correct one which the rat had to press, see figure 9. See appendix for details.

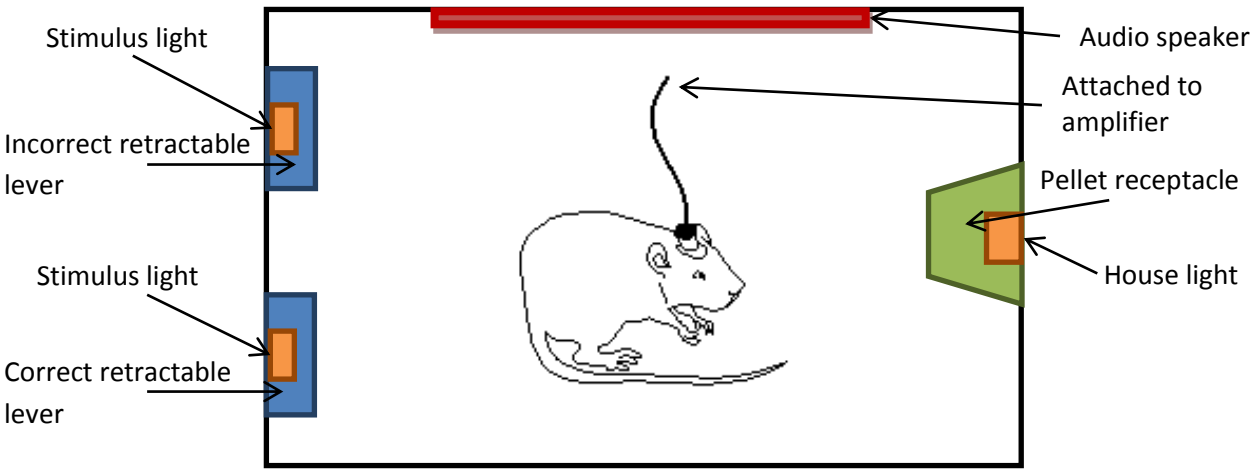

Figure 9: Setup of the test chamber for experiment 2. Red = Audio speaker, Green = Pellet receptacle, Orange = Lighting, Blue = retractable lever. The cable attached to the head of the rat was attached to an amplifier positioned outside of the test chamber.

Following recovery, the rats were exposed to an auditory sensory paradigm to **A:** record sensory components and **B:** identify best frequency and intensity to use in the discrimination paradigm. The tones in the sensory paradigm were played consecutively with 6 second between each tone. The protocol was 2000Hz(80dB, 20ms), 2000Hz(90dB, 20ms), 2000Hz(100dB, 20ms), 3000Hz(80dB, 20ms), 3000Hz(90dB, 20ms), 3000Hz(100dB, 20ms), 4000Hz(80dB, 20ms), 4000Hz(90dB, 20ms), 4000Hz(100dB, 20ms), repeated 50 times for a total of 45min. The same protocol was performed with the frequency 6000Hz, 7000Hz and 8000Hz as well.

Based on results from the sensory paradigm it was decided to use 6000Hz, 7000Hz, and 8000Hz at 90dB for the discrimination paradigm, see the discussion. Rats were trained to press the correct lever when a training tone (7000Hz, 90dB, 20ms) was played. The training tone was played every 8-16sec (mean = 12sec). When training tones were played, stimuli light above the correct lever turned on with a 0.75sec delay. The rat had 5sec to press the correct lever. If the rat pressed the correct lever a food reward was released. If the rat pressed the incorrect lever or no lever at all, a 30sec timeout session was initiated in which all lighting was turned off and no audio was played. When a rat had >80% correct responses within in 15min it was introduced to the second protocol.

In the second protocol the rat had to distinguish between two tones, a target tone (8000Hz, 90dB, 20ms) and a non-target tone (6000Hz, 90dB, 20ms). If a target tone was played, the rat must press the correct lever. If a non-target tone was played, the rat must ignore it and not press any lever. On the first two days the target tone was played 50% of the times with stimulus light guiding. On following two days, target tone was played 40% of the times with stimulus light guiding. This was followed by another two days where target tone was played 30% of the times with stimulus light guiding. Last, target tone was played 30% of the times with no stimulus light guiding for the rest of the experiment.

An example of this can be seen in figure 10. See appendix for in depth description of the protocols.

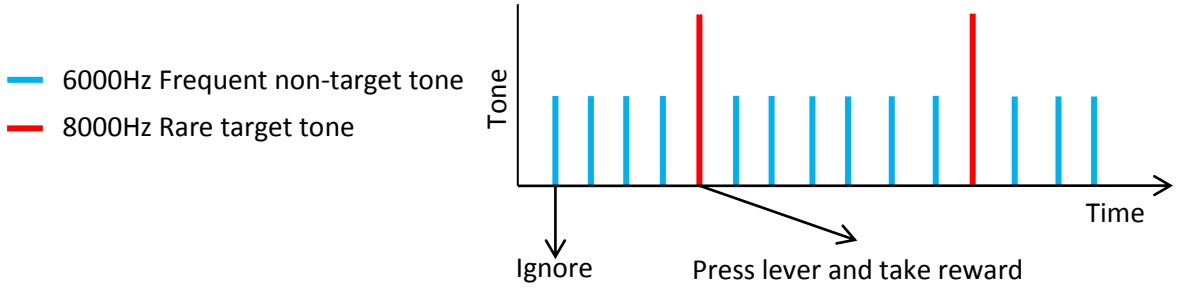

Figure 10: Example of the procedure in experiment 2. The rat had to ignore the frequent non-target tone (6000Hz) and react to the rare target tone (8000Hz) where it had to press the correct lever once for a food reward to be released.

A cross-over study with scopolamine was performed with 7 days between each treatment. 5 rats received scopolamine (scopolamine (0.1mg/kg Scopolamine[63, 68], Sigma-Aldrich®, dissolved in 0.9 % NaCl) S.C. and 4 rats received saline (9mg/mL Sodium Chloride, Fresenius Kabi) S.C. on the first session and after the 7 days washout period the rats received the opposite compound. The rats were exposed to one training session in-between the two scopolamine sessions to keep them up to date in the paradigm.

A timeline of experiment 2 can be seen in figure 11.

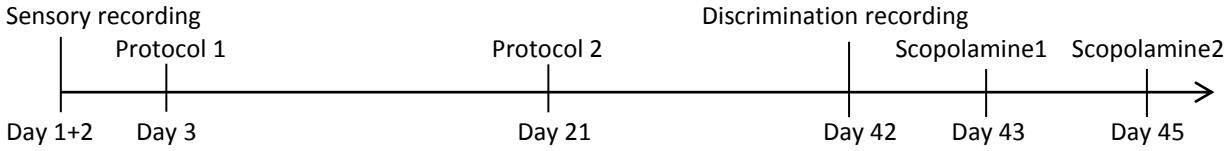

Figure 11: Timeline for experiment 2. A rat was exposed to and completed a protocol on each trial day. Resting days are not included in the timeline. A day was only counted if an experiment was conducted.

3.2.4 Recording

Same recording setting as described in experiment 1.

3.2.5 Data analyses

Same data analysis as described in experiment 1, except that only correct responses to the tones were analyzed.

3.3 Experiment 3

The third experiment was similar to experiment 2. The only difference was that rats were screened prior to surgical implantation and the stimulus light cue was removed early in the discrimination paradigm. This study was conducted to follow up on results from experiment 2 and to further study the ERP in frontal cortex.

3.3.1 Subjects

A total of 20 male Sprague-Dawley rats were obtained from Charles River (Germany). They were all screened in the training paradigm as explained for experiment 2. 8 rats had a >80% correct response at day 10 of training and were picked for surgery. They weighed between 275g and 325g at the day of surgery.

3.3.2 Surgical preparation

Same procedure was followed as described for experiment 2, except that the secondary auditory cortex electrode was moved to the primary auditory cortex, which required the temporal muscle to be detached to allow for the electrode to be positioned beneath. The frontal electrode was changed to a depth electrode into the prelimbic cortex. Electrode coordinates. **1:** Prelimbic cortex, 3.2mm anterior to bregma, 0.8mm lateral, 2.2mm ventrally to dura. **2:** Parietal association cortex, 3.8mm posterior to bregma, 3.0mm lateral. **3:** Primary auditory cortex, 4.5mm posterior to bregma, 7.4mm medial. **4:** Ventral hippocampus CA3, 5.3mm posterior to bregma, 4.6mm lateral, 2.6mm ventrally to dura. **5:** Reference, 8.0mm anterior to bregma, 1.0mm lateral. **6:** Ground, 2.3mm anterior to bregma, 2.7mm medial.

3.3.3 Behavioral procedures

After recovery, the rats were exposed to an auditory sensory paradigm to record sensory components for 6000Hz (20ms, 90dB) and 8000Hz (20ms, 90dB). As rats had already been trained to press the lever during screening they were only given 1 day of training paradigm with light stimulus guiding, and 3 days of training paradigm without light stimulus guiding. On the following two days they were introduced to the 50% discrimination paradigm without light stimulus guiding. This was followed by two days where the target tone was played 40% of the times without stimulus light guiding. The rest of the experiment period the target tone was played 30% of the times without stimulus guiding.

3.3.4 Recording

Same recording setting as described in experiment 1 and 2.

3.3.5 Data analyses

Same data analysis as described in experiment 2.

3.4 Methodological considerations and validation

3.4.1.1 Surgery

In experiment 1, the secondary auditory cortex and parietal cortex electrodes were located so close to each other that they almost were in contact. In experiment 2, the secondary auditory cortex electrode was therefore moved to medial side of the midline instead of the lateral. The ground electrode was moved more anterior to make room for the secondary auditory cortex electrode.

3.4.1.2 Sound

When calibrating the tones used in the experiments, it was observed that the acoustic level varied from one place to other inside the test chamber. This could not be fixed without major changes to the test chamber. The sound was therefore calibrated near the lever for all tones as the rat was expected to sit here for most of the time throughout the experiments.

The air suction connected to the experiment boxes was observed to be very powerful in experiment 1 which created great amount of background acoustic noise inside the boxes. This was a critical issue as it potentially could mask the different tones used in the experiment. This was fixed by reducing the suction flow of the air condition to the minimum level allowed, thus reducing the background noise to almost silence.

3.4.1.3 EEG

Large EEG artifacts were observed when the rat reached to its food reward. This was due to the fact that the pedestal attached to the rat hit the top of the cavity wall. Unfortunately no short-term solution to this

was possible at the time of experiment 1. The long-term solution was to replace the enclosed cavity with one with a larger hole to give headroom for the pedestal. This modification was implemented for experiment 2 and 3.

It was observed that every time the pellet dispenser was activated in one test chamber it created an artifact on the EEG trace recorded for both rats. These artifacts were created by the power supply to the pellet dispenser. A solution to this issue during experiment 1 was to implement a delay of 500ms from the onset of the tone, so the artifact was pushed beyond the theoretical area of the P300 signal on the EEG. No solution could be carried out for the artifact created in the opposite box. However, this was not considered to cause any problems as the risk of a food pellet being released at the exact same time as a tone was played was considered to be very small.

The retractable lever also created an artifact in the EEG readout when it moved in and out, so a 500ms delay was also added here as well for experiment 1. In experiment 2+3 the retractable lever was kept out at all time to eliminate this artifact.

In experiment 2 it was observed that during the training phases with light cues, the turning on/off of the house light and stimulus light created an artifact on the EEG. To solve this, a delay of 750ms from tone onset was implemented for turning off the house light and turning on the stimulus light.

The EEG signal throughout the first 20 days of experiment 1 showed a high amount of noise on all channels. This was reduced by replacing the EEG cables with shorter and more noise resistant shielded versions.

3.4.1.4 Behavioral issues

During the experiment some rats started to bite and destroy the cable attached to their head. This problem was fixed by wrapping a metal spring around the wire. This increased the amount of noise seen on the raw EEG trace, but no direct visible effect could be observed on the ERPs. In experiment 3, the metal sheeting was grounded, reducing the noise.

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4 Results

4.1 Experiment 1

Below, a comparison of the auditory sensory paradigm vs. the auditory discrimination paradigm and saline treatment vs. scopolamine treatment are shown for the hippocampal electrode. The secondary auditory cortex, parietal association cortex, and frontal cortex electrodes were excluded as no clearly defined peaks could be identified during the discrimination paradigm, see figure 42 in appendix for example. The ERPs during the auditory sensory paradigm for secondary auditory cortex, parietal association cortex and frontal cortex were visible, see figure 41 in appendix for example.

In the first part (4.1.1), two paradigms were compared, an auditory sensory paradigm in which the rats only had to listen to the tones and not perform any task, and an auditory discrimination paradigm in which the rat had to: **A:** ignore the 2000Hz tone, **B:** start to press a lever on a 3000Hz tone, and **C:** Receive a reward following a 4000Hz tone. ERPs elicited following a 3000Hz tone had to be excluded due to artifacts created by the introduction of the retractable lever to the test chamber, see figure 43 in appendix for example.

In the second part (4.1.2), the effect of scopolamine treated rats vs. a saline control group on the ERP was tested.

The rats quickly learned to press the lever for food at day 3. After further training they were introduced to protocol 2 (introduction of lever tone) on day 11 and the last protocol (protocol 2D) on trial day 18. Scopolamine was given on trial day 23. Figure 12 illustrate the progress of the rats during the experiment.

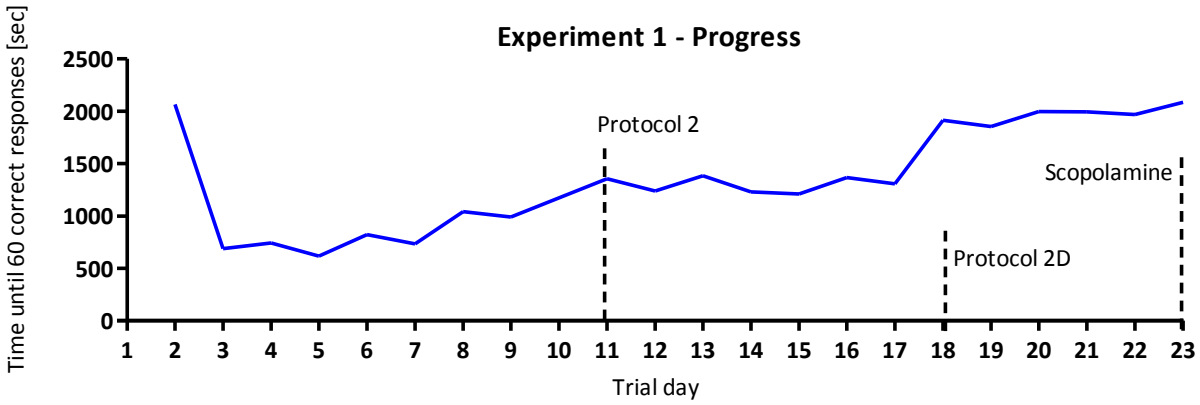


Figure 12: Progress of the rats. The time spent until performing 60 correct responses for each experiment day are shown. No data were recorded on the first day.

During the discrimination paradigm in experiment 1, the rats did not seem to use the auditory stimuli as cue for their task. Instead of the 3000Hz lever tone they used the visual cue of the lever moving into the test chamber. For the food reward, instead of listening for the 4000Hz reward tone they checked the pellet receptacle multiple times during pressing to see if a food reward had been released or waited till the lever retracted. It seemed that the rats were so focused on pressing the lever that they ignored the auditory stimuli. The rats were highly motivated in the paradigm and almost all completed 60 tasks in less than half an hour. The scopolamine treated rats were slightly worse in performing the tasks than the saline group.

4.1.1 Sensory vs. normal paradigm

4.1.1.1 Hippocampus

The ERPs for hippocampus consisted of a N1, P2, N2 and P3 peak. The P3 were only visible during the discrimination task, figure 13, A+B.

P1: No P1 was visible

N1: A N1 at 20-23ms was observed for both tones in both the sensory and discrimination paradigm. No significant difference could be observed in latency or amplitude.

P2: A P2 at 50ms was observed for both tones in both the sensory and discrimination paradigm. No significant difference could be observed in latency or amplitude.

N2: A N2 at 80ms could only be observed during the 4000Hz tone in both sensory and discrimination paradigm. No significant difference could be observed in latency or amplitude.

P3: A P3 at 110-127ms could only be observed during the discrimination task. A significantly (One-way ANOVA: $F(1,21)=5.74$, $p=0.026$, post hoc: $t=2.40$, $p=0.026$) longer latency was observed for the 2000Hz vs. 4000Hz tone, figure 14, A. Both peaks were significantly larger (Two-way ANOVA: $F(1,44)=28.93$, $p<0.001$, post hoc: $t=3.96$, $p=0.002$ for 2000Hz. Two-way ANOVA: $F(1,44)=28.93$, $p<0.001$, post hoc: $t=3.65$, $p=0.004$ for 4000Hz) than the amplitude in the same region of the sensory paradigm, figure 14, B.

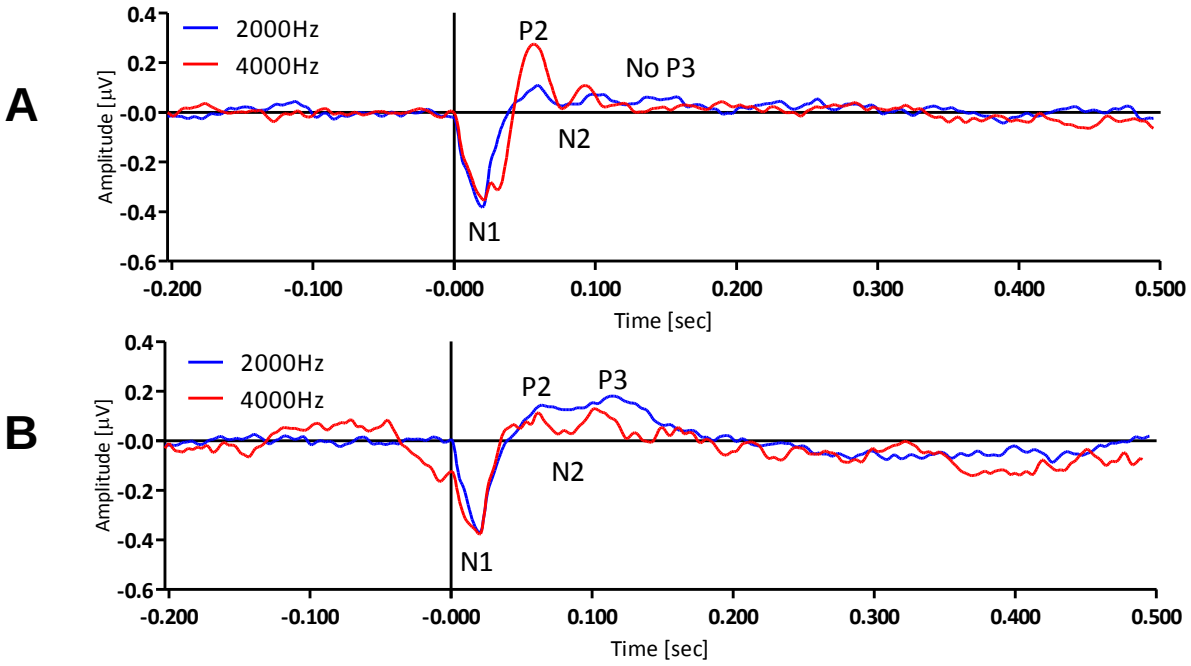


Figure 13: ERP for Hippocampus for all rats (N=12). Stimuli onset at time = 0 sec. **2000Hz** = Non-target tone. **4000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

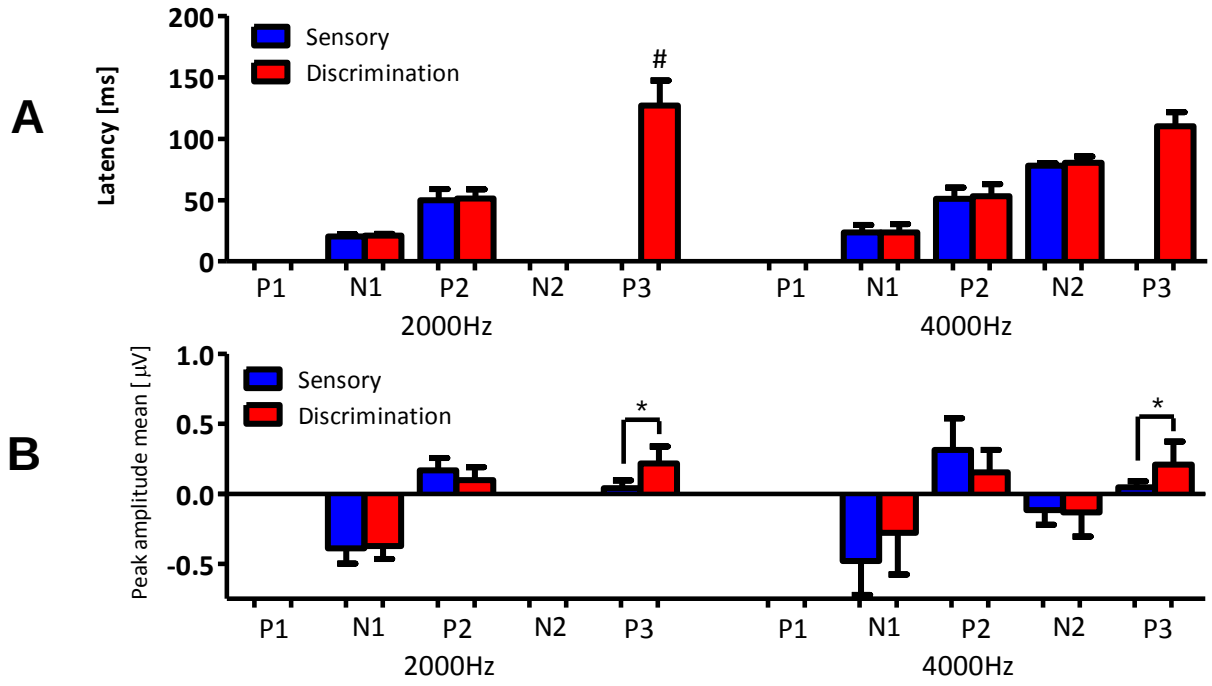


Figure 14: Latency (**A**) and amplitude (**B**) for hippocampus in sensory vs. discrimination paradigm. *=P<0.05. **#:** Indicates if statistic difference (P<0.05) between the 2000Hz and 4000Hz tone for the same paradigm

4.1.2 Saline vs. Scopolamine treatment

4.1.2.1 Hippocampus

The ERP for hippocampus consisted of a N1, P2, N2 and P3 peak. The P3 were visible on both saline and scopolamine groups, figure 15, A+B.

P1: No P1 was visible

N1: A N1 at 21ms was observed for both tones in both treatment groups. No significant difference could be observed in latency or amplitude.

P2: A P2 at 50-60ms was observed for both tones in both treatment groups. Significant larger (Two-way ANOVA: $F(1,14)=6.95$, $p=0.009$, post hoc: $t=3.75$, $p=0.013$) amplitude was observed for the discrimination vs. sensory paradigm on the 4000Hz tone, figure 16, B. A significant smaller (Two-way ANOVA: $F(1,14)=13.14$, $p=0.003$, post hoc: $t=4.40$, $p=0.004$) amplitude was observed for 2000Hz vs. 4000Hz in the saline treated group, figure 16, B. No significant difference could be observed in latency

N2: A N2 at 75-80ms could only be observed during the 4000Hz tone in both treatment groups. No significant difference could be observed in latency or amplitude.

P3: A P3 at 128-140ms could be observed in both treatment groups. No significant difference could be observed in latency or amplitude.

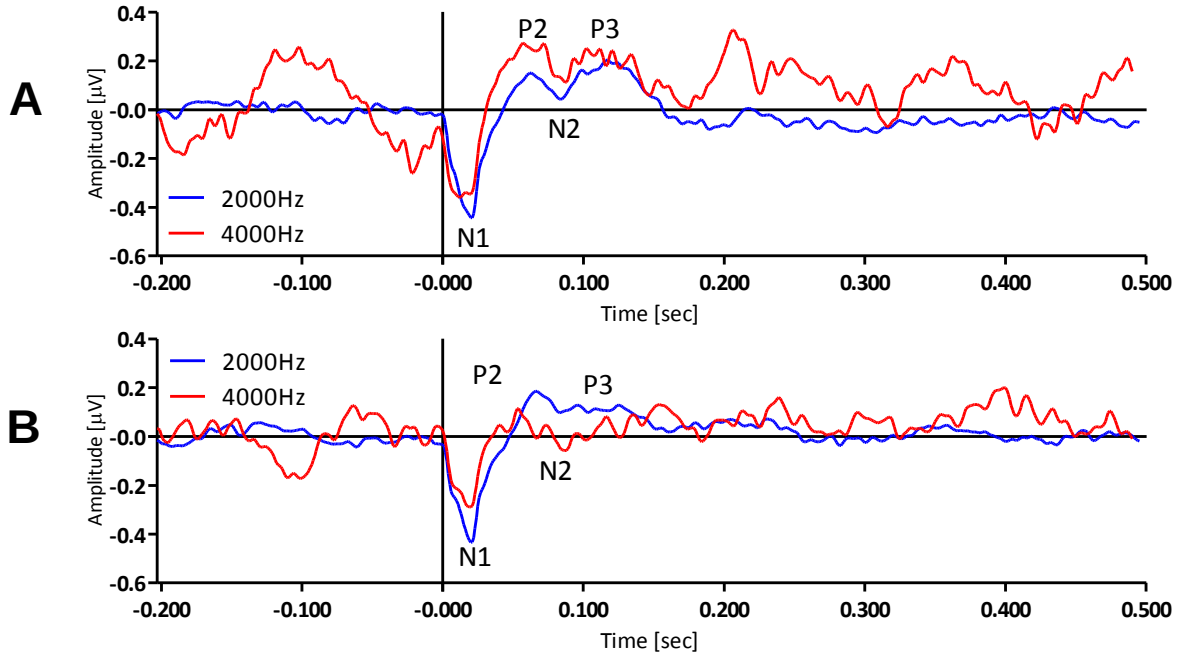


Figure 15: ERP for Hippocampus for all rats (N=12). Stimuli onset at time = 0 sec. **2000Hz** = Non-target tone. **4000Hz** = Target tone **A:** Saline treated group **B:** Scopolamine treated group.

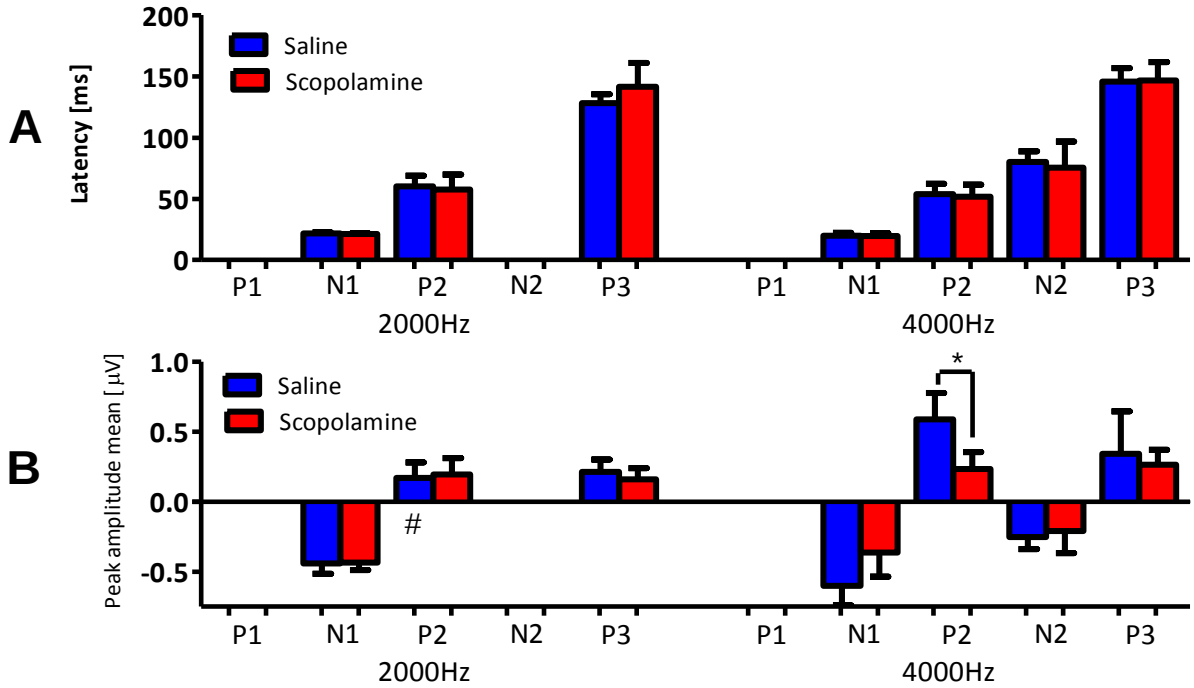


Figure 16: Latency (**A**) and amplitude (**B**) for hippocampus in saline vs. scopolamine treatment. *=P<0.05. **#:** Indicates if statistic difference (P<0.05) between the 2000Hz and 4000Hz tone for the same paradigm

4.2 Experiment 2

Below, a comparison of the auditory sensory paradigm vs. the auditory discrimination paradigm and saline treatment vs. scopolamine treatment are shown for the hippocampal electrode, secondary auditory cortex, parietal association cortex and frontal electrodes

The first part (4.2.1) consists of an auditory sensory paradigm to identify the ideal tone frequency and intensity to use in the discrimination paradigm. The second part (4.2.2) shows the peak development during training over several days. The third part (4.2.3) compares the auditory sensory paradigm with the auditory discrimination paradigm in which the rat had to ignore the non-target tone and react on the target tone. The fourth part (4.2.4) compares the effect of scopolamine on the ERPs.

During the discrimination paradigm in experiment 2, the rats had difficulty distinguishing between the two tones when stimulus light cue was removed. Due to bad performance, the stimulus light cue was added again for two consecutive days to remind the rats of the paradigm, see figure 17.

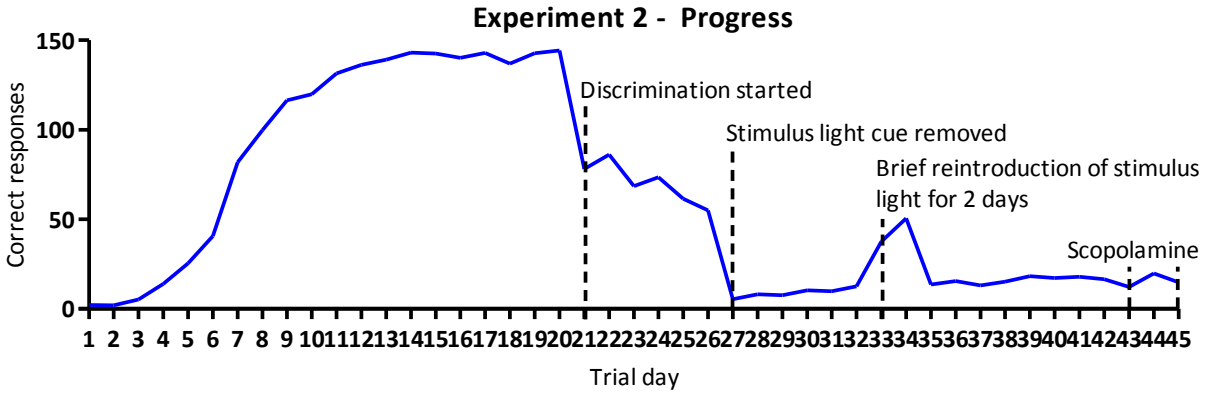


Figure 17: Progress of the rats. The amount of correct responses in 1 hour for each day is shown.

4.2.1 Validation of tones

The ERP components appeared more uniform for the 6, 7, and 8000Hz tones vs. the 2, 3, and 4000Hz tone. The higher the intensity, the more stable the amplitudes appeared. See figure 44, 45, 46 and 47 in appendix.

4.2.2 Peak development

The ERPs for the auditory sensory paradigm and trial day 21, 23, 25, 30 and 40 for both 6000Hz and 8000Hz tones in the discrimination paradigm are shown on the figure 18 and 19. These illustrate the development and alterations in the peaks as the performance of the rats improved over time. The ratio of the target / non-target tone is shown in each figure. The focus is on the P3 peak.

4.2.2.1 Hippocampus:

In the hippocampal electrode a P3 peak appeared at trial day 30 for the target tone (8000Hz) and was consistently visible during the rest of the trials. No P3 peak appeared to the non-target tone (6000Hz) during any trial. See figure 18, A+B.

4.2.2.2 Secondary auditory cortex:

In the secondary auditory cortex electrode a P3 peak was visible in both the non-target and target tone. No clearly defined development or alteration was visible for P3 for any tone. See figure 18, C+D.

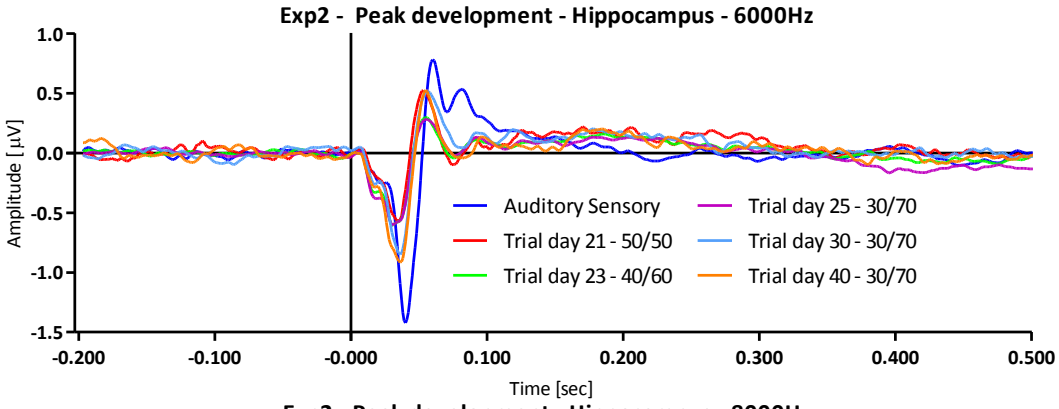
4.2.2.3 Parietal association cortex:

In the parietal association cortex electrode a P3 peak was evident to both the non-target and target tone. No clearly defined development or alteration was visible for P3 for any tone. See figure 19, A+B.

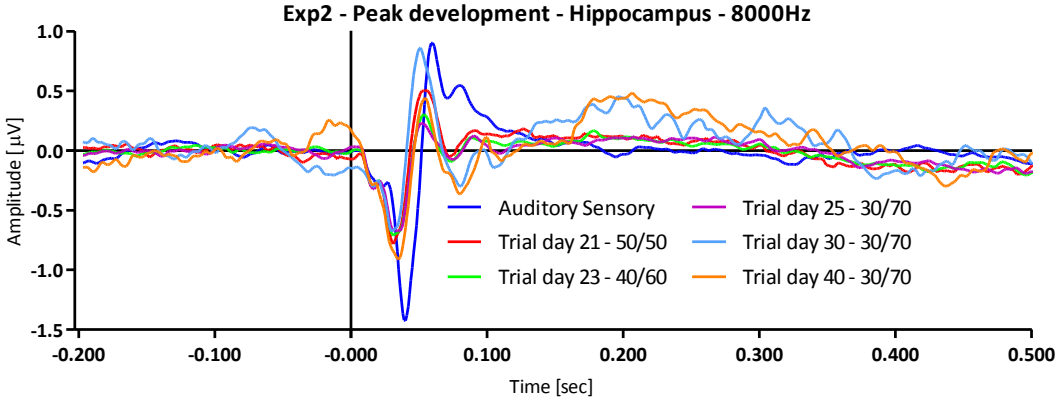
4.2.2.4 Frontal cortex:

In the parietal cortex electrode a P3 peak was visible in both the non-target and target tone. The waveform was larger for trial 30 and 40 at 200ms for the 6000Hz tone. No clearly defined development or alteration was visible as a response to P3 for the target tone. See figure 19, C+D.

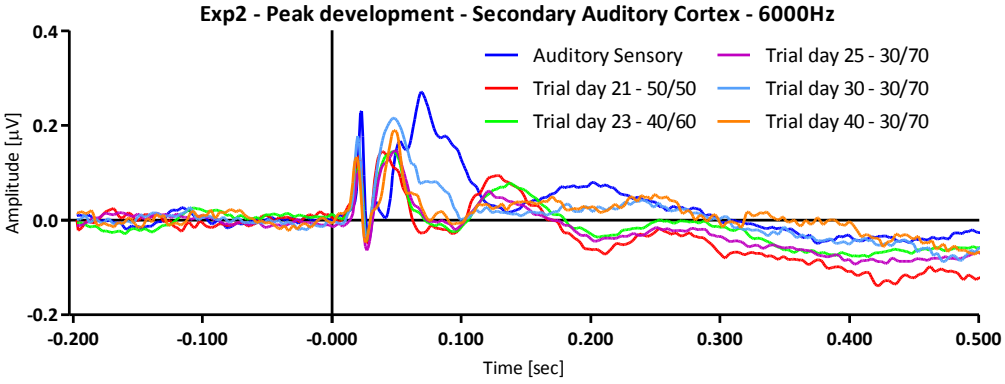
A



B



C



D

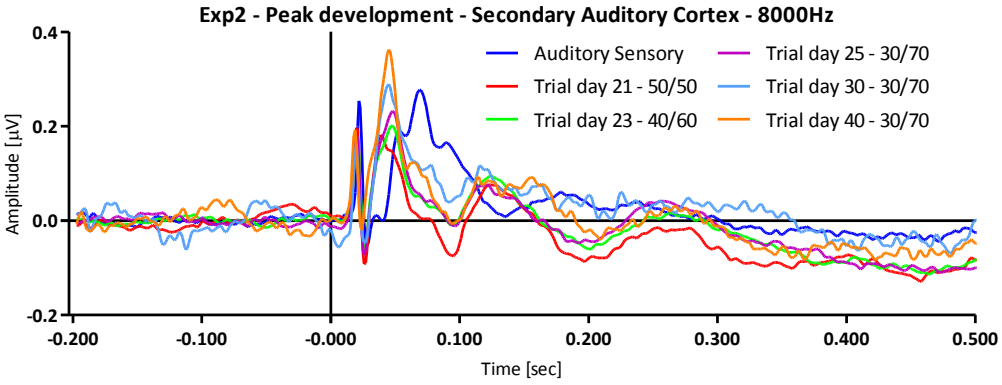


Figure 18: Peak development for hippocampus (A+B) and secondary auditory cortex (C+D). Trial day 1 was first day in discrimination paradigm. Stimuli onset at time = 0 sec. Ratio of target/non-target tone is illustrated for each day.

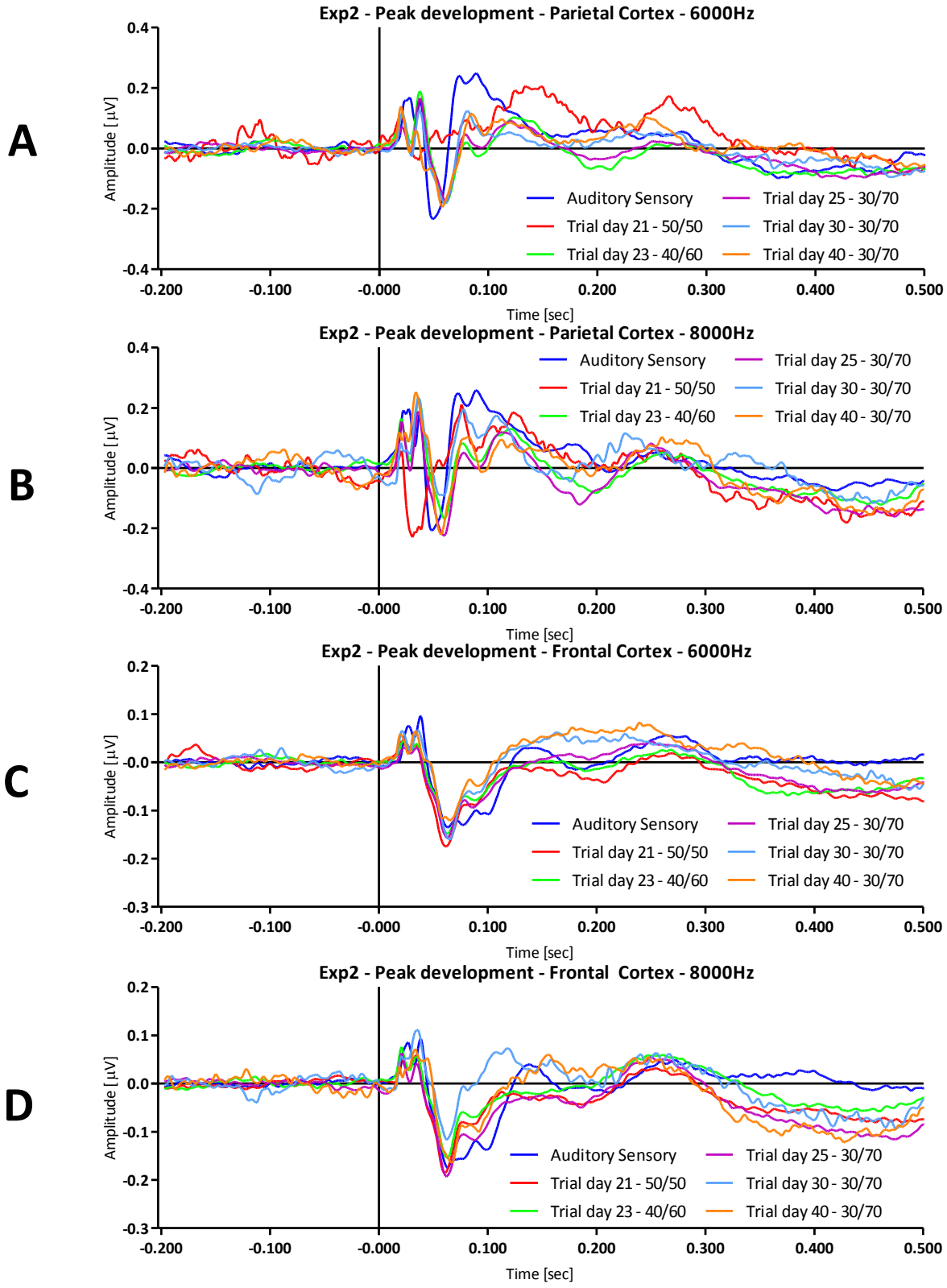


Figure 19: Peak development for parietal cortex (A+B) and frontal cortex (C+D). Trial day 1 was first day in discrimination paradigm. Stimuli onset at time = 0 sec. Ratio of target/non-target tone is illustrated for each day.

4.2.3 Sensory vs. Discrimination paradigm

4.2.3.1 Hippocampus

The ERP for hippocampus consisted of a N1, P2, N2 and P3 peak. The P3 was only visible during the discrimination task, figure 20, A+B.

P1: No P1 was visible

N1: A N1 at 30-40ms was observed for both tones in both the sensory and discrimination paradigm. No significant difference was observed in latency or amplitude.

P2: A P2 at 50-60ms was observed for both tones in both the sensory and discrimination paradigm. No significant difference was observed in latency or amplitude.

N2: A N2 at 80-90ms was observed for both tones in both the sensory and discrimination paradigm. A significant (Two-way ANOVA: $F(1,29)=16.54$, $p<0.001$, post hoc: $t=4.72$, $p<0.001$) lower amplitude was observed during the 8000Hz tone, figure 21, B. A significant larger (Two-way ANOVA: $F(1,29)=7.16$, $p=0.012$, post hoc: $t=4.02$, $p=0.002$) amplitude for 8000Hz vs. 6000Hz tone on the discrimination paradigm was observed, figure 21, B. No significant difference was observed in latency of the peak.

P3: A P3 at 170-180ms could only be observed during the discrimination task. No significant difference in latency was observed, but both P3 peak amplitudes were significantly larger (Two-way ANOVA: $F(1,38)=93.52$, $p<0.001$, post hoc: $t=4.33$, $p<0.001$ for 6000Hz. Two-way ANOVA: $F(1,38)=93.52$, $p<0.001$, post hoc: $t=9.29$, $p<0.001$ for 8000Hz) than the amplitude in the same region of the sensory paradigm, figure 21, B. A significantly (Two-way ANOVA: $F(1,38)=16.68$, $p<0.001$, post hoc: $t=5.00$, $p<0.001$) larger P3 amplitude was observed for the discrimination paradigm vs. sensory paradigm during the 8000Hz, figure 21, B.

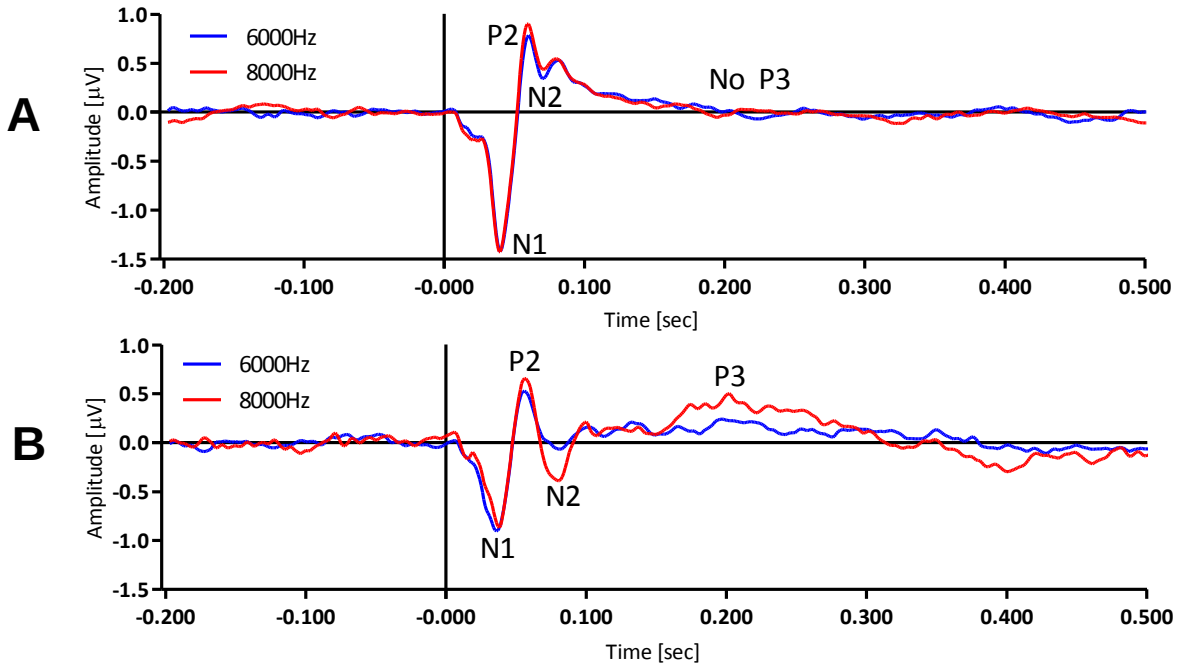


Figure 20: ERP for Hippocampus for all rats (N=9). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

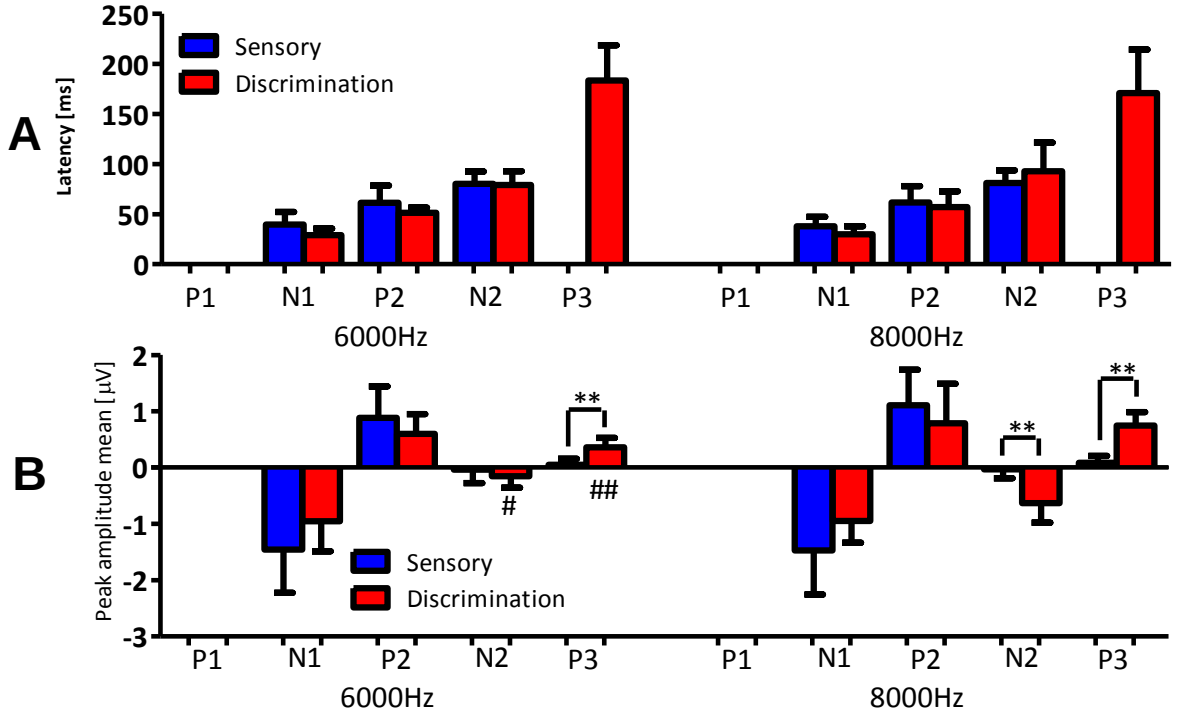


Figure 21: Latency (**A**) and amplitude (**B**) for hippocampus in sensory vs. discrimination paradigm. *=P<0.05, **=P<0.001. **#:** Indicates if statistic difference (#=P<0.05, ##=P<0.001) between the 6000Hz and 8000Hz tone for the same paradigm.

4.2.3.2 Secondary auditory cortex

The ERP for the secondary auditory cortex consisted of a P1, N1, P2, N2 and P3 peak. A P3 peak was visible in the data obtained during the discrimination paradigm and a P3-like peak was evident to the tones in the sensory paradigm, figure 22, A+B.

P1: A P1 at 13-18ms was observed for both tones in both the sensory and discrimination paradigm. The latency was significantly (Two-way ANOVA: $F(1,38)=37.47$, $p<0.001$, post hoc: $t=4.38$, $p<0.001$, for 6000Hz. Two-way ANOVA: $F(1,38)= 37.47$, $p<0.001$, post hoc: $t=2.48$, $p<0.001$ for 8000Hz) shorter during the discrimination vs. sensory paradigm for both tones, figure 23, A. No significant difference was observed for the amplitude.

N1: A N1 peak at 20-32ms was observed for both tones in both the sensory and discrimination paradigm. The latency was significantly (Two-way ANOVA: $F(1,38)=48.91$, $p<0.001$, post hoc: $t=5.23$, $p<0.001$, for 6000Hz. Two-way ANOVA: $F(1,38)= 48.91$, $p<0.001$, post hoc: $t=4.66$, $p<0.001$ for 8000Hz) shorter during the discrimination paradigm for both tones, figure 23, A. No significant difference was observed for the amplitude of the peak.

P2: A P2 peak at 40-60ms was observed for both tones in both the sensory and discrimination paradigm. The latency was significantly (Two-way ANOVA: $F(1,38)=63.72$, $p<0.001$, post hoc: $t=5.80$, $p<0.001$, for 6000Hz. Two-way ANOVA: $F(1,38)= 63.72$, $p<0.001$, post hoc: $t=5.49$, $p<0.001$ for 8000Hz) shorter during the discrimination paradigm for both tones, figure 23, A. A significant significantly (Two-way ANOVA: $F(1,38)=6.23$, $p=0.017$, post hoc: $t=3.28$, $p=0.014$) larger amplitude was observed for the 8000Hz vs. 6000Hz tone in the discrimination paradigm, figure 23, B.

N2: A N2 at 80-130ms was observed for both tones in both the sensory and the discrimination paradigm. The latency was significantly (Two-way ANOVA: $F(1,38)=47.70$, $p<0.001$, post hoc: $t=5.19$, $p<0.001$, for 6000Hz. Two-way ANOVA: $F(1,38)= 47.70$, $p<0.001$, post hoc: $t=4.59$, $p<0.001$ for 8000Hz) shorter during the discrimination paradigm for both tones, figure 23, A. No significant difference was observed for the amplitude.

P3: A P3 at 205-250ms was observed for both tones in both the sensory and the discrimination paradigm. The latency was significantly (Two-way ANOVA: $F(1,37)=30.59$, $p<0.001$, post hoc: $t=3.71$, $p=0.004$, for 6000Hz. Two-way ANOVA: $F(1,37)= 30.59$, $p<0.001$, post hoc: $t=4.11$, $p=0.001$ for 8000Hz) larger during the discrimination paradigm for both tones, figure 23, A. No significant difference was observed for the amplitude.

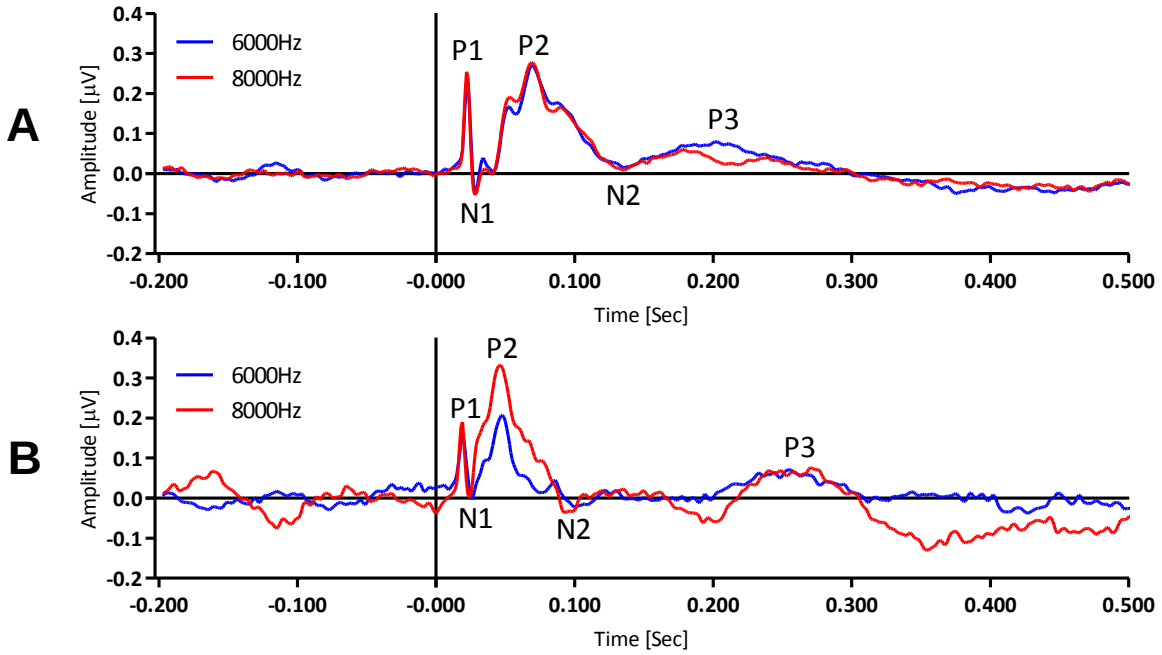


Figure 22: ERP for secondary auditory cortex for all rats (N=9). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

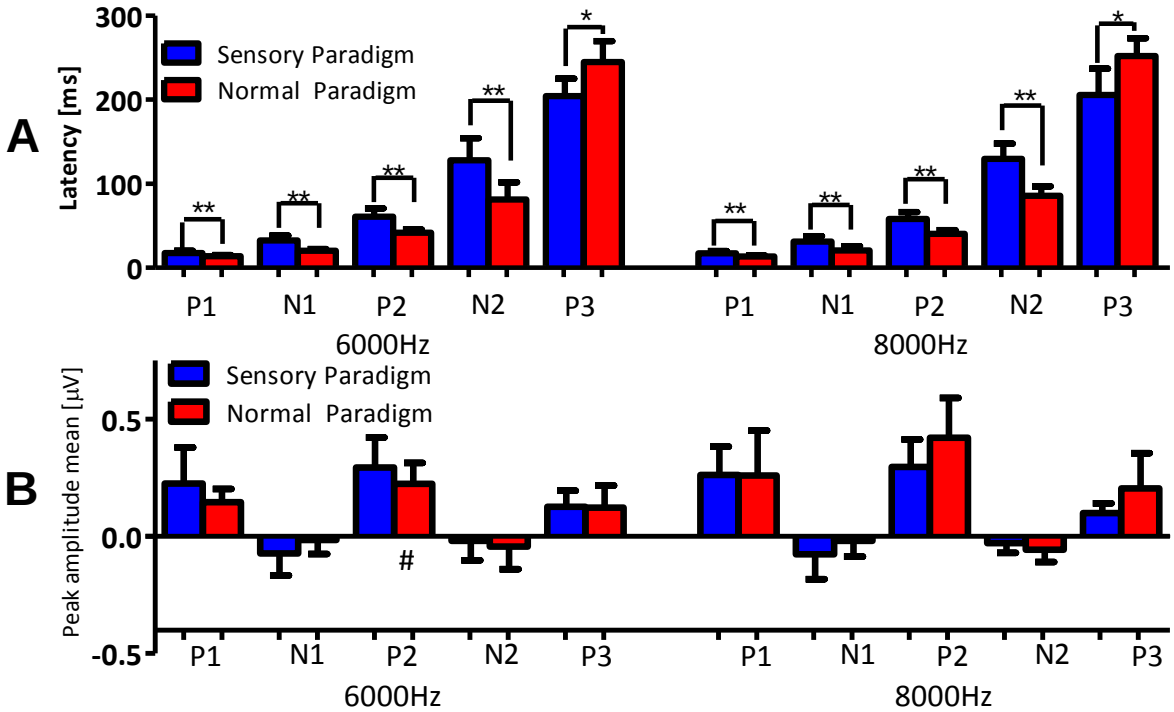


Figure 23: Latency (**A**) and amplitude (**B**) for secondary auditory cortex in sensory vs. discrimination paradigm. *=P<0.05, **=P<0.001. **#:** Indicates if statistic difference (P<0.05) between the 6000Hz and 8000Hz tone for the same paradigm

4.2.3.3 Parietal cortex

The ERP for the parietal cortex consisted of a P1, N1, P2, N2 and P3 peak. A P3 peak was visible in the data obtained during the discrimination paradigm and a P3-like peak was evident to the tones in the sensory paradigm, figure 24, A+B.

P1: A P1 at 26-29ms was observed for both tones in both sensory and discrimination paradigm. A significantly (Two-way ANOVA: $F(1,38)=7.27$, $p=0.010$, post hoc: $t=2.97$, $p=0.031$) larger amplitude was observed for the 8000Hz vs. 6000Hz tone during the discrimination paradigm, figure 25, B. No significant difference was observed for the latency.

N1: A N1 peak at 45-50ms was observed for both tones in both the sensory and discrimination paradigm. No significant difference was observed in latency or amplitude.

P2: A P2 peak at 73-85ms was observed for both tones in both the sensory and discrimination paradigm. A significantly (Two-way ANOVA: $F(1,38)=12.93$, $p<0.001$, post hoc: $t=3.15$, $p=0.019$) lower amplitude was observed for the discrimination paradigm during the 6000Hz tone, figure 25, B. No significant difference was observed for the latency.

N2: A N2 at 150-180ms was observed for both tones in both the sensory and discrimination paradigm. A significantly (Two-way ANOVA: $F(1,38)=8.69$, $p=0.005$, post hoc: $t=2.86$, $p=0.042$) lower amplitude was observed for discrimination vs. sensory paradigm during the 8000Hz tone, figure 25, B. No difference was observed for the latency.

P3: A P3 at 230-250ms was observed for both tones in both sensory and discrimination paradigm. No significant difference was observed for both latency and amplitude.

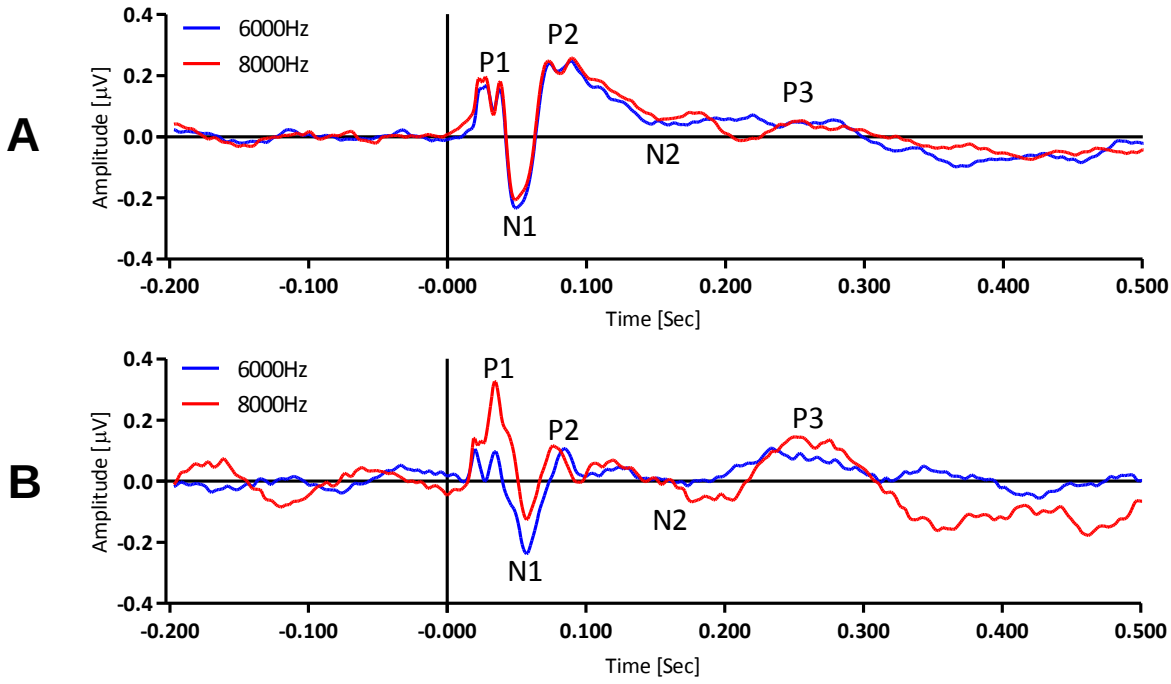


Figure 24: ERP for parietal cortex for all rats (N=9). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

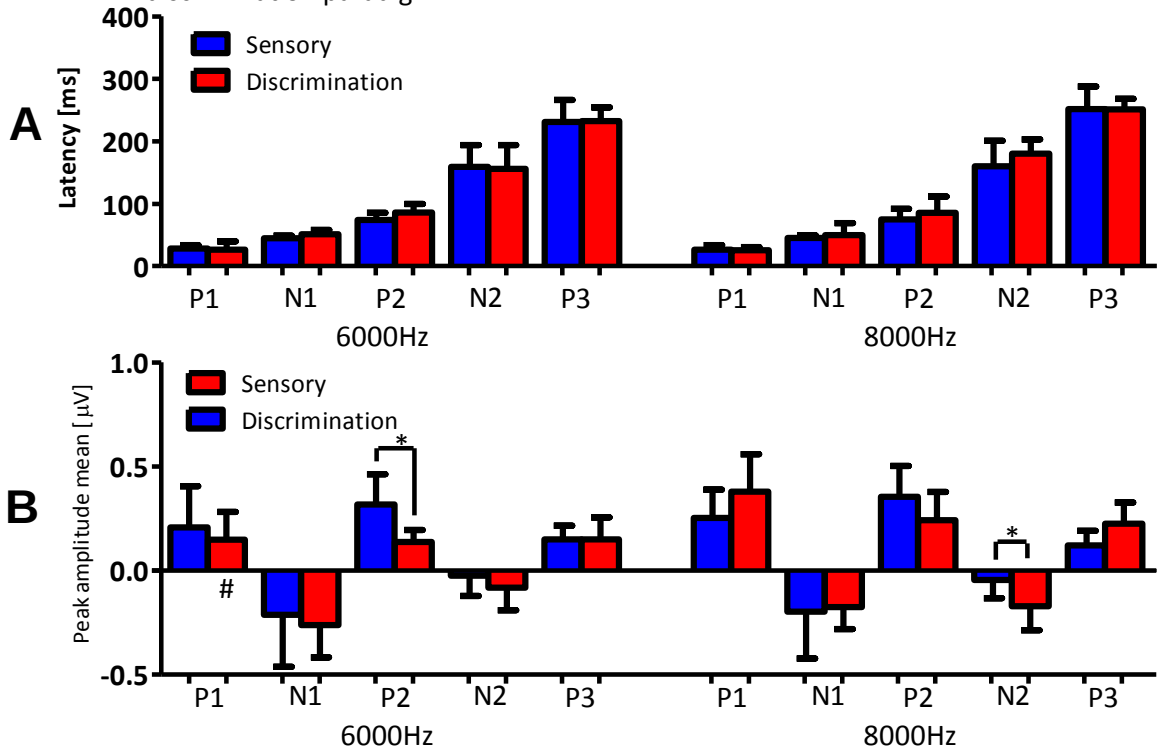


Figure 25: Latency (**A**) and amplitude (**B**) for parietal cortex in sensory vs. discrimination paradigm. *=P<0.05, **=P<0.001. **#:** Indicates if statistic difference (P<0.05) between the 6000Hz and 8000Hz tone for the same paradigm

4.2.3.4 Frontal cortex

The ERP for the frontal cortex consisted of a P1, N1, P2, N2 and P3 peak. A P3 peak was visible in the data obtained during the discrimination paradigm and a P3-like peak was evident to the tones in the sensory paradigm, figure 26, A+B.

P1: A P1 at 26-28ms was observed for both tones in both sensory and discrimination paradigm. A significantly (Two-way ANOVA: $F(1,34)=4.26$, $p=0.047$, post hoc: $t=4.07$, $p=0.002$) larger amplitude was observed for the discrimination vs. sensory paradigm during the 8000Hz tone, figure 27, B. A significantly (Two-way ANOVA: $F(1,38)=13.49$, $p<0.001$, post hoc: $t=4.84$, $p<0.001$) larger amplitude was observed for the 8000Hz vs. 6000Hz tone during the discrimination paradigm, figure 27, B. No significant difference was observed for the latency.

N1: A N1 peak at 50-75ms was observed for both tones in both sensory and discrimination paradigm. A significantly (Two-way ANOVA: $F(1,35)=6.84$, $p=0.013$, post hoc: $t=2.98$, $p=0.031$) shorter latency was observed for the discrimination vs. sensory paradigm during the 6000Hz tone, figure 27, A. No significant difference was observed for the amplitude.

P2: A P2 peak at 85-140ms was observed for both tones in both the sensory and discrimination paradigm. A significantly (Two-way ANOVA: $F(1,34)=23.92$, $p<0.001$, post hoc: $t=5.57$, $p<0.001$) shorter latency was observed for the discrimination vs. sensory paradigm during the 6000Hz tone, figure 27, A. A significantly (Two-way ANOVA: $F(1,34)=7.34$, $p=0.010$, post hoc: $t=3.80$, $p=0.003$) longer latency was observed for the 8000Hz vs. 6000Hz tone during the discrimination paradigm, figure 27, A. No significant difference was observed for the amplitude.

N2: A N2 peak at 155-180ms was observed for both tones in both the sensory and discrimination paradigm. No significant difference was observed for the latency and amplitude.

P3: A P3 at 230-260ms was observed for both tones in both the sensory and discrimination paradigm. A significantly (Two-way ANOVA: $F(1,34)=6.18$, $p=0.018$, post hoc: $t=3.80$, $p=0.003$) larger amplitude was observed for the discrimination vs. sensory paradigm during the 8000Hz tone, figure 27, B. A significantly (Two-way ANOVA: $F(1,38)=6.54$, $p=0.015$, post hoc: $t=3.58$, $p=0.006$) larger P3 amplitude was observed for the 8000Hz vs. 6000Hz tone during the discrimination paradigm, figure 27, B. No significant difference was observed for the latency.

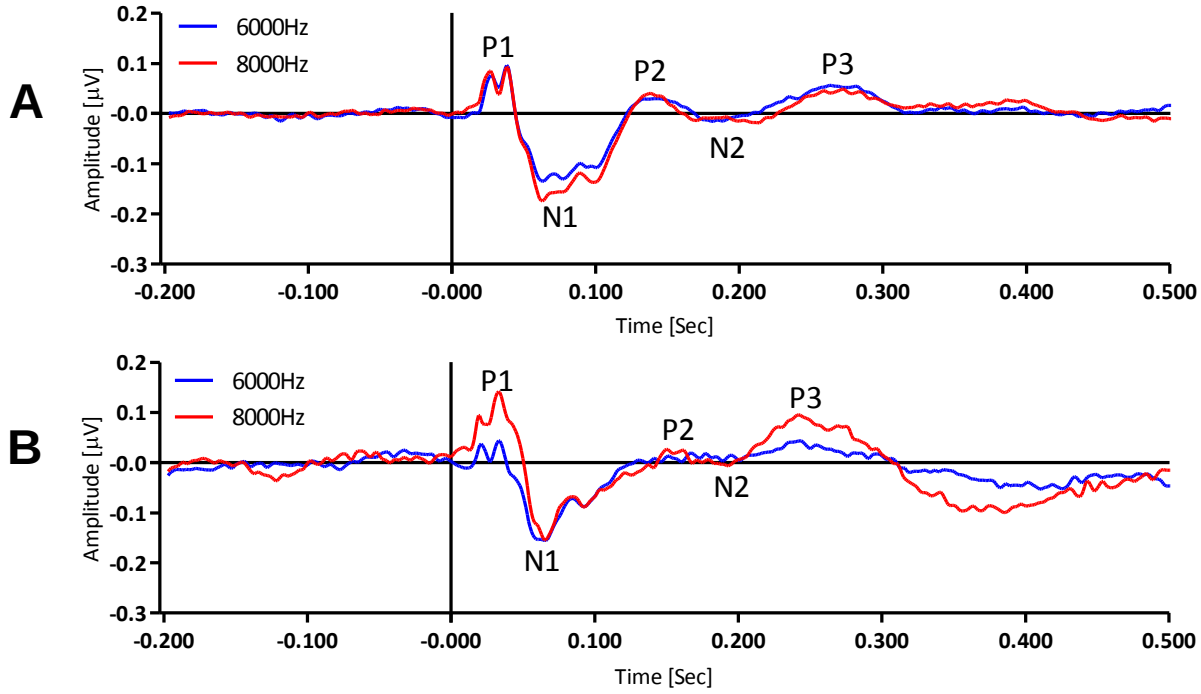


Figure 26: ERP for frontal cortex for all rats (N=9). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

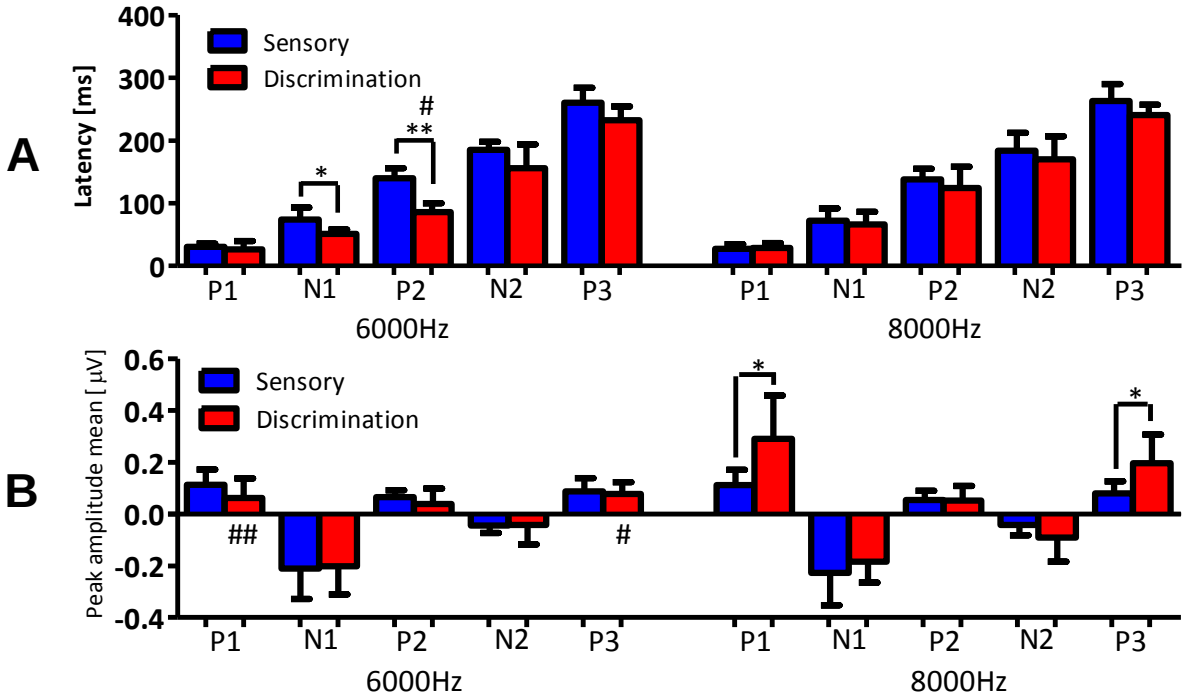


Figure 27: Latency (**A**) and amplitude (**B**) for frontal cortex in sensory vs. discrimination paradigm. *=P<0.05, **=P<0.001. **#:** Indicates if statistic difference (#=P<0.05, ##=P<0.001) between the 6000Hz and 8000Hz tone for the same paradigm.

4.2.4 Saline vs. Scopolamine treatment

4.2.4.1 Hippocampus

The ERP for the hippocampus consisted of a N1, P2, N2 and P3 peak. The P3 was visible in both treatment groups, figure 28, A+B.

P1: No P1 was visible.

N1: A N1 peak at 31ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

P2: A P2 peak at 48-58ms was observed for both tones in both treatment groups. A significantly (Two-way ANOVA: $F(1,30)=6.83$, $p=0.014$, post hoc: $t=2.85$, $p=0.049$) longer latency was observed for scopolamine vs. saline on the 6000Hz tone, figure 29, A. No significant difference was observed for the amplitude.

N2: A N2 peak at 70-83ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

P3: A P3 at 163-236ms was observed or both tones in both treatment groups. A significantly (Two-way ANOVA: $F(1,30)=0.87$, $p=0.360$, post hoc: $t=3.28$, $p=0.016$) longer latency was observed for scopolamine vs. saline on the 8000Hz tone, figure 29, A. A significantly (Two-way ANOVA: $F(1,30)=2.43$, $p=0.130$, post hoc: $t=3.84$, $p=0.004$) longer latency was observed for the 8000Hz tone vs. 6000Hz tone in the scopolamine treated group, figure 29, A. A significant (Two-way ANOVA: $F(1,30)=16.70$, $p<0.001$, post hoc: $t=3.14$, $p=0.023$) larger amplitude was observed for the saline treatment between the 6000Hz and 8000Hz tone, figure 29, B.

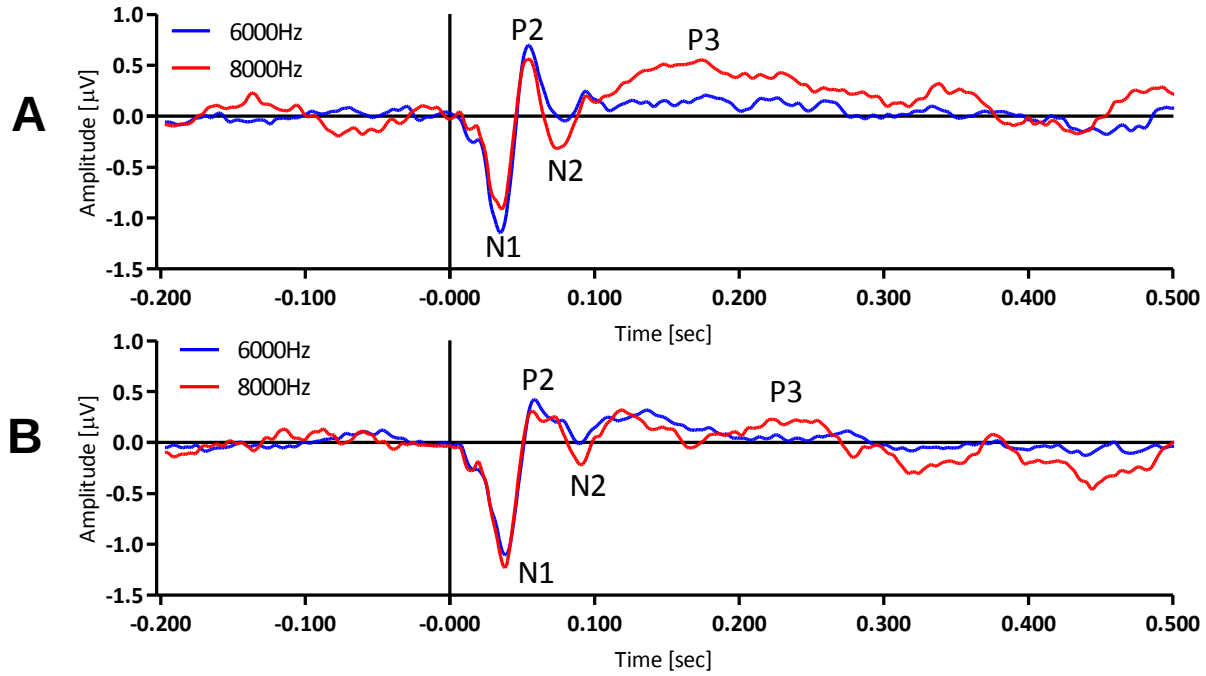


Figure 28: ERP for Hippocampus for all rats (N=9). **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Saline treated group **B:** Scopolamine treated group.

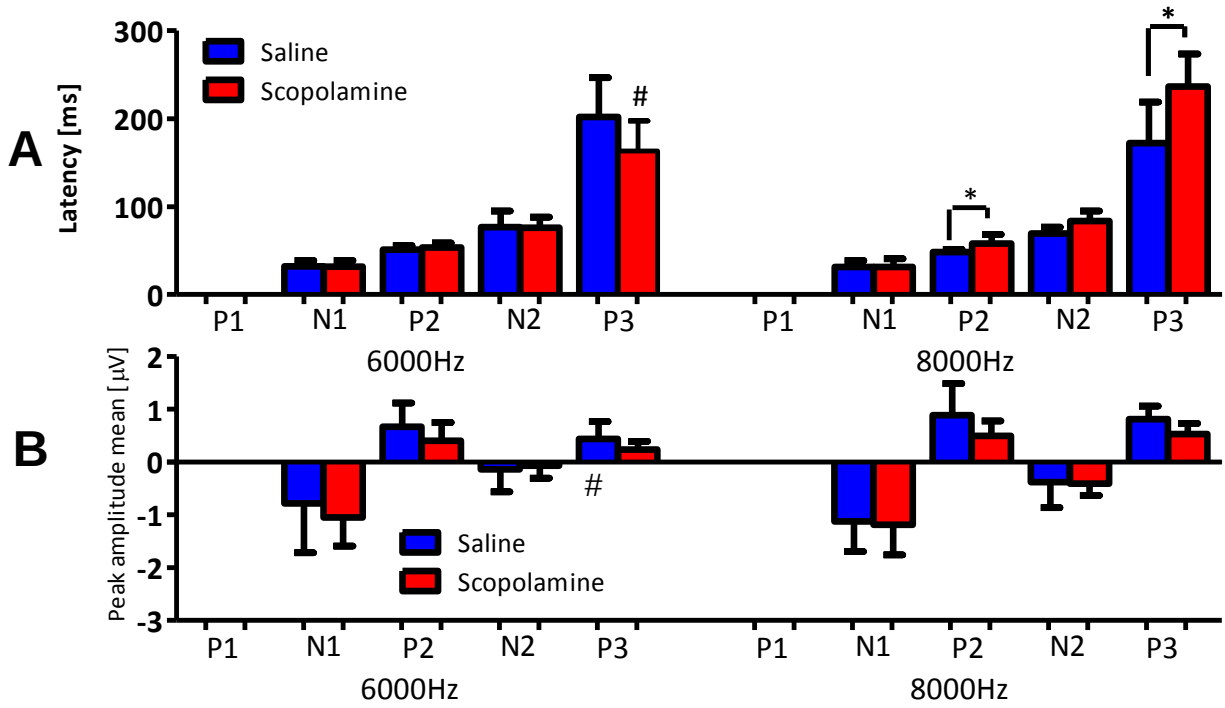


Figure 29: Latency (**A**) and amplitude (**B**) for hippocampus in saline vs. scopolamine treatment. *=P<0.05. **#:** Indicates if statistic difference (P<0.05) between the 6000Hz and 8000Hz tone for the same paradigm.

4.2.4.2 Secondary auditory cortex

The ERP for the secondary auditory cortex consisted of a P1, N1, P2, N2 and P3 peak. The P3 was visible in both treatment groups, figure 30, A+B.

P1: A P1 peak at 14-17ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

N1: A N1 peak at 22-23ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

P2: A P2 peak at 38-43ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

N2: A N2 peak at 80-135ms was observed for both tones in both treatment groups. A significantly (Two-way ANOVA: $F(1,32)=27.77$, $p<0.001$, post hoc: $t=3.69$, $p=0.005$ for 6000Hz. Two-way ANOVA: $F(1,32)=27.77$, $p<0.001$, post hoc: $t=3.77$, $p=0.004$ for 8000Hz) longer latency was observed for scopolamine vs. saline on both tones, figure 31, A. A significantly (Two-way ANOVA: $F(1,32)=14.55$, $p<0.001$, post hoc: $t=3.71$, $p=0.005$) lower amplitude was observed for scopolamine vs. saline on the 8000Hz tone, figure 31, B.

P3: A P3 peak at 241-271ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

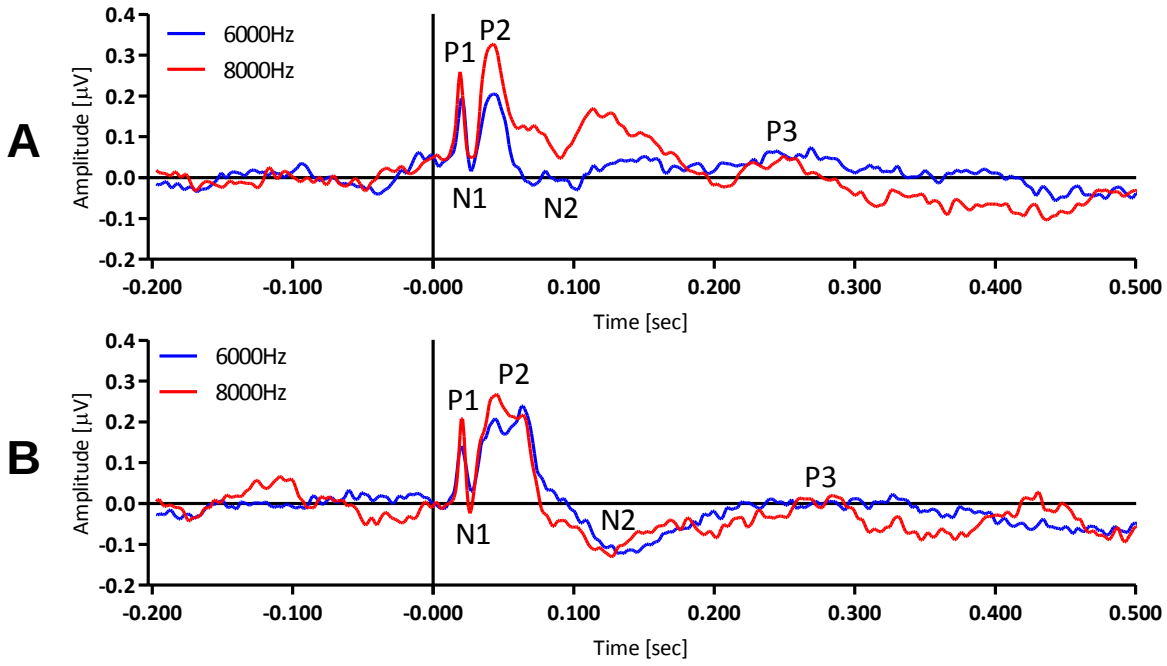


Figure 30: ERP for secondary auditory cortex for all rats (N=9). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Saline treated group **B:** Scopolamine treated group.

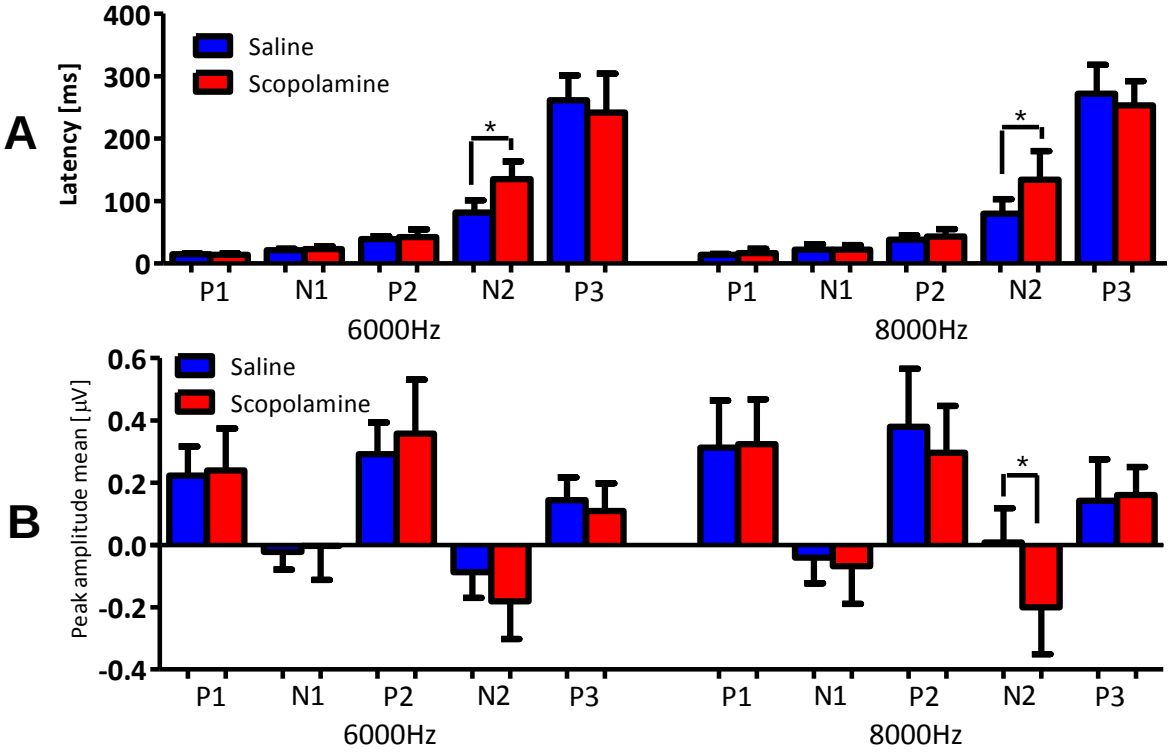


Figure 31: Latency (**A**) and amplitude (**B**) for secondary auditory cortex in saline vs. scopolamine treatment. *=P<0.05.

4.2.4.3 Parietal cortex

The ERP for the parietal cortex consisted of a P1, N1, P2, N2 and P3 peak. The P3 was visible in both treatment groups, figure 32, A+B.

P1: A P1 peak at 24-27ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

N1: A N1 peak at 48-53ms was observed for both tones in both treatment groups. A significantly (Two-way ANOVA: $F(1,32)=10.67$, $p=0.003$, post hoc: $t=3.33$, $p=0.013$) lower amplitude was observed for saline vs. scopolamine on the 6000Hz tone, figure 33, B. No significant difference was observed for the latency.

P2: A P2 peak at 74-113ms was observed for both tones in both treatment groups. A significantly (Two-way ANOVA: $F(1,32)=14.39$, $p<0.001$, post hoc: $t=3.46$, $p=0.009$) shorter latency was observed for scopolamine vs. saline on the 6000Hz tone, figure 33, A. No significant difference was observed for the amplitude.

N2: A N2 peak at 148-166ms was observed for both tones in both treatment groups. A significantly (Two-way ANOVA: $F(1,32)=13.47$, $p<0.001$, post hoc: $t=2.84$, $p=0.047$) larger amplitude was observed for scopolamine vs. saline group on the 8000Hz tone, figure 33, B. No significant difference was observed for the latency.

P3: A P3 peak at 227-297ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

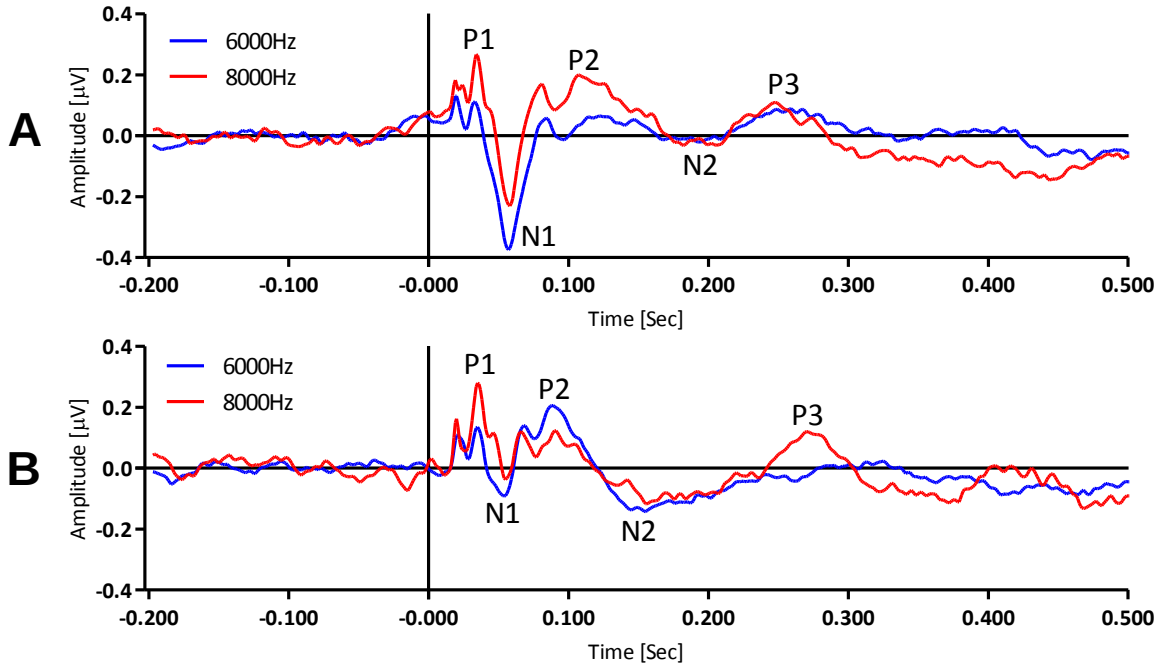


Figure 32: ERP for parietal cortex for all rats (N=9). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Saline treated group **B:** Scopolamine treated group.

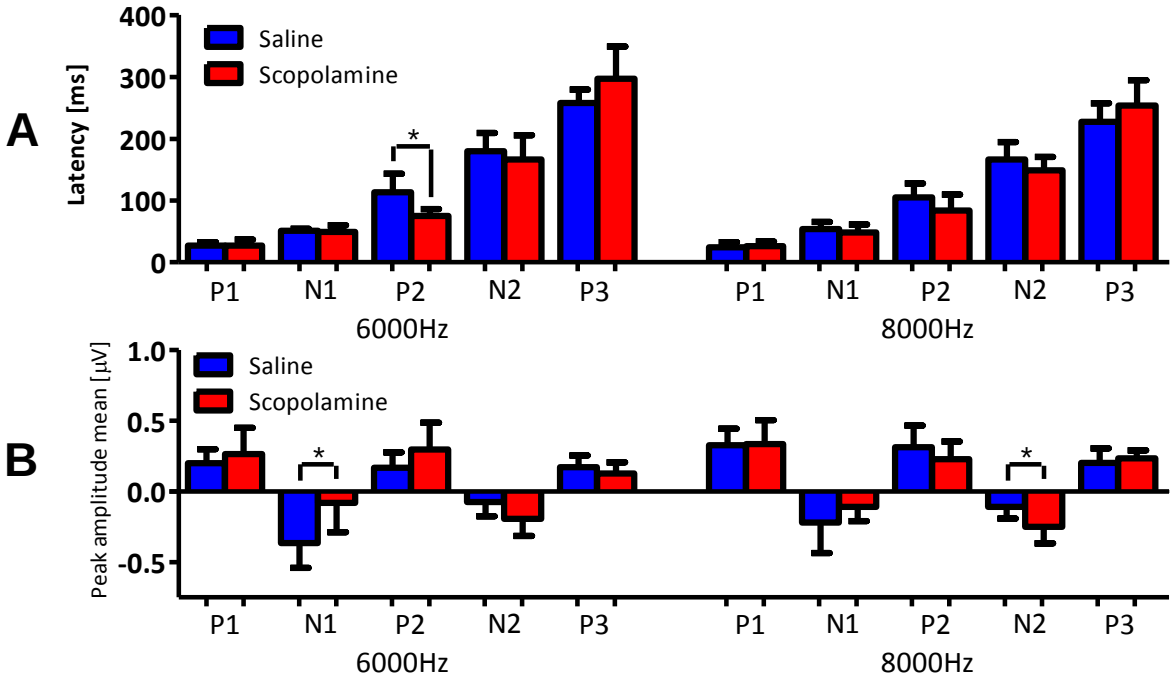


Figure 33: Latency (**A**) and amplitude (**B**) for parietal cortex in saline vs. scopolamine treatment. *=P<0.05.

4.2.4.4 Frontal cortex

The ERP for the frontal cortex consisted of a P1, N1, P2, N2 and P3 peak. The P3 was visible in both treatment groups, figure 34, A+B.

P1: A P1 peak at 25-30ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

N1: A N1 peak at 60-65ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

P2: A P3 peak at 258-268ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

N2: A N2 peak at 181-195ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

P3: A P3 peak at 258-268ms was observed for both tones in both treatment groups. No significant difference was observed for the latency and amplitude.

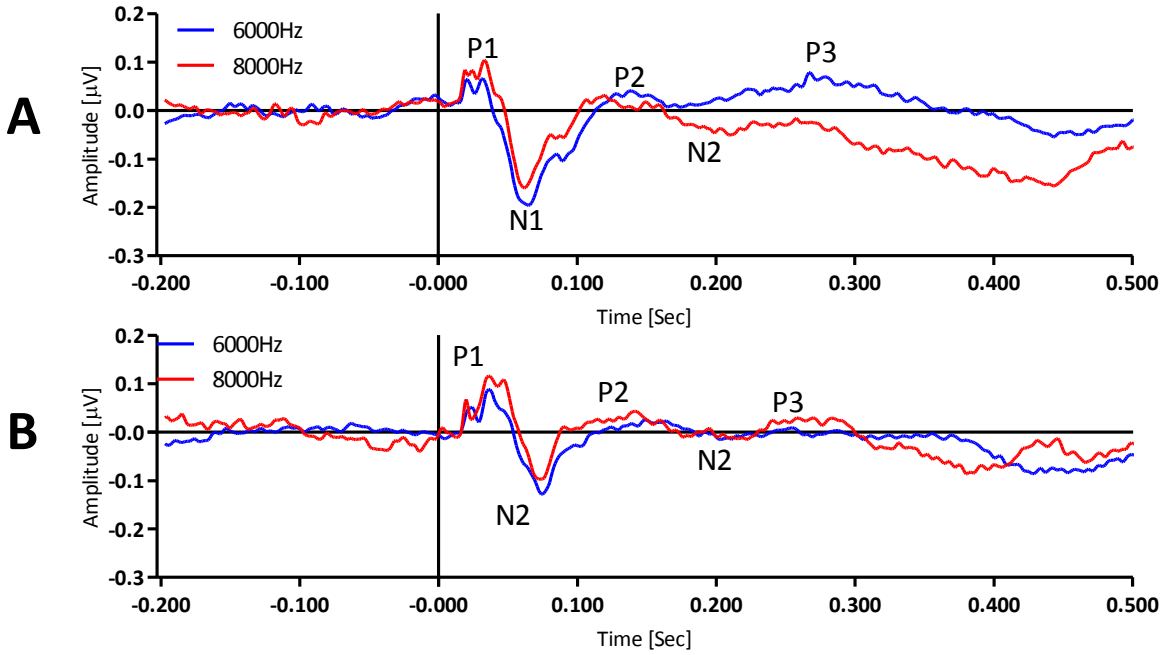


Figure 34: ERP for frontal cortex for all rats (N=9). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Saline treated group **B:** Scopolamine treated group.

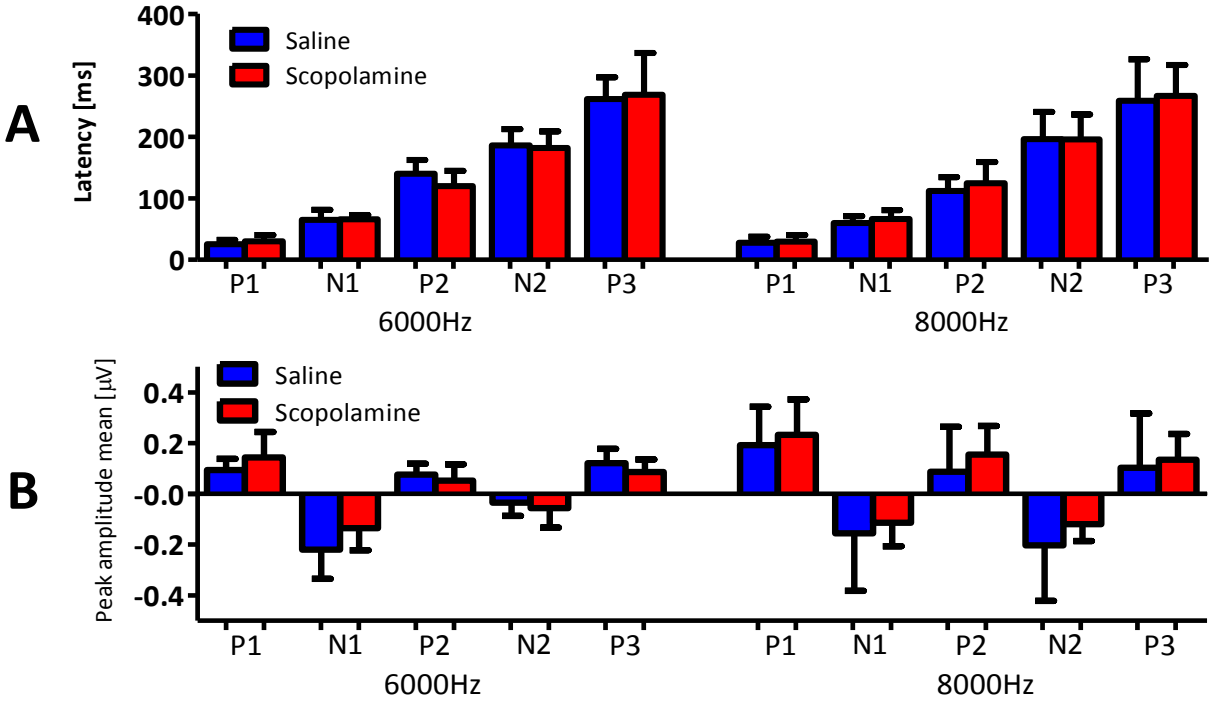


Figure 35: Latency (**A**) and amplitude (**B**) for frontal cortex in saline vs. scopolamine treatment.

4.3 Experiment 3

In experiment 3, ERP waveforms during the sensory paradigm and discrimination paradigm are shown for the hippocampal, primary auditory cortex, parietal association cortex, and prelimbic cortex electrodes.

The rats were screened prior to surgery and bad performers were excluded from further study. The good performing rats were better in the discrimination paradigm compared to the rats in experiment 2 and also learned to discriminate the tones faster, see figure 36.

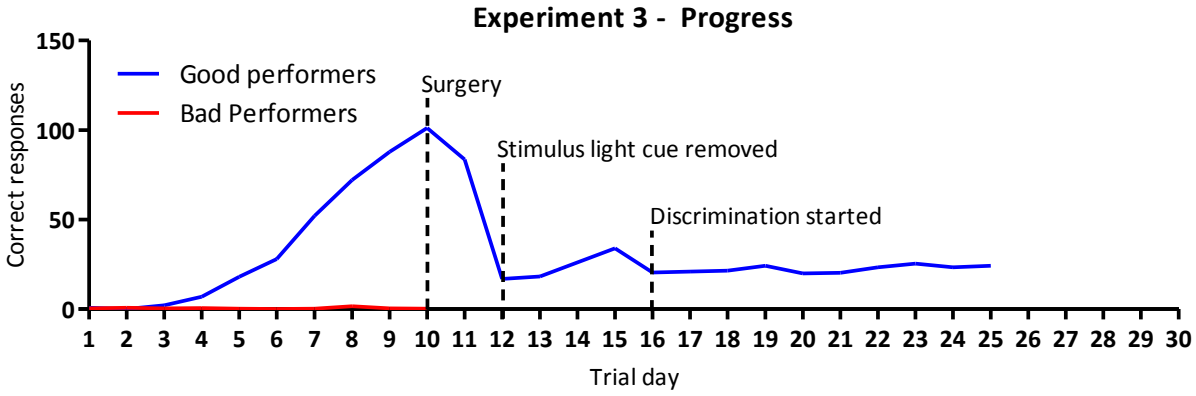


Figure 36: Progress of the rats. The amount of correct responses in 1 hour for each day is shown.

4.3.1 Sensory vs. discrimination paradigm

The data shown are for trial day 21

4.3.1.1 Hippocampus

The ERP for the hippocampus consisted of a N1, P2, N2 and P3 peak. The P3 were only visible during the discrimination paradigm, figure 37, A+B.

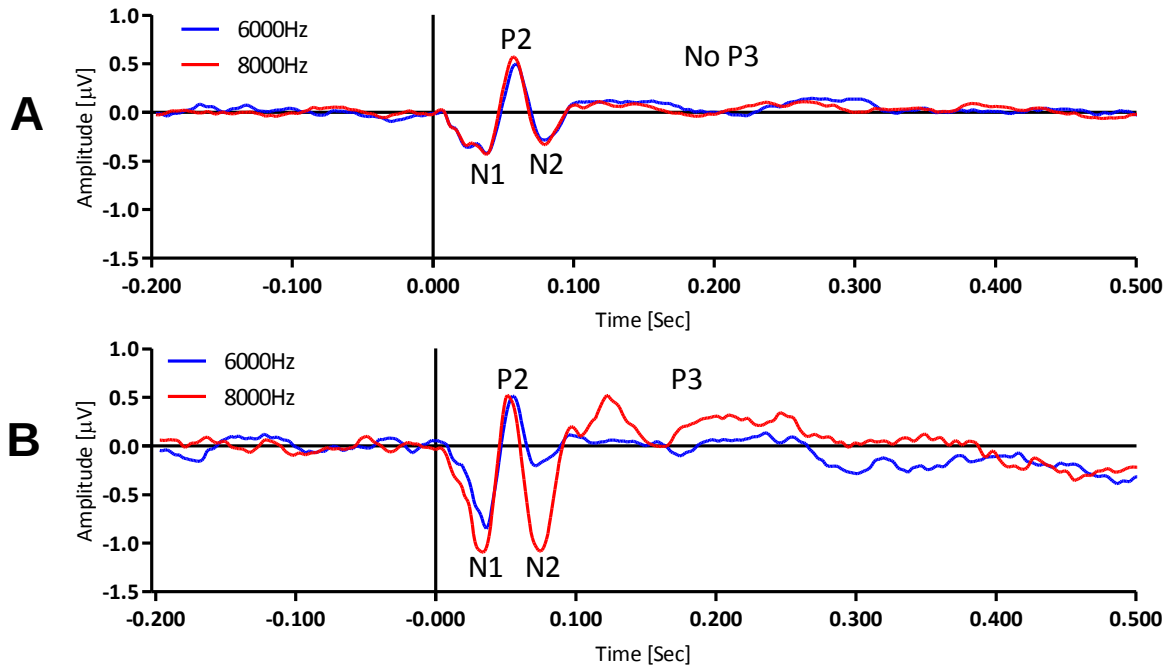


Figure 37: ERP for hippocampus for all rats (N=8). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

4.3.1.2 Primary auditory cortex

The ERP for the Primary auditory cortex consisted of a P1, N1, P2, N2 and P3 peak. The P3 was visible during both sensory and discrimination paradigm, but more clearly defined in the discrimination paradigm. The N2 and P3 appeared at a later latency for the 6000Hz tone compared to the 8000Hz tone, figure 38, A+B.

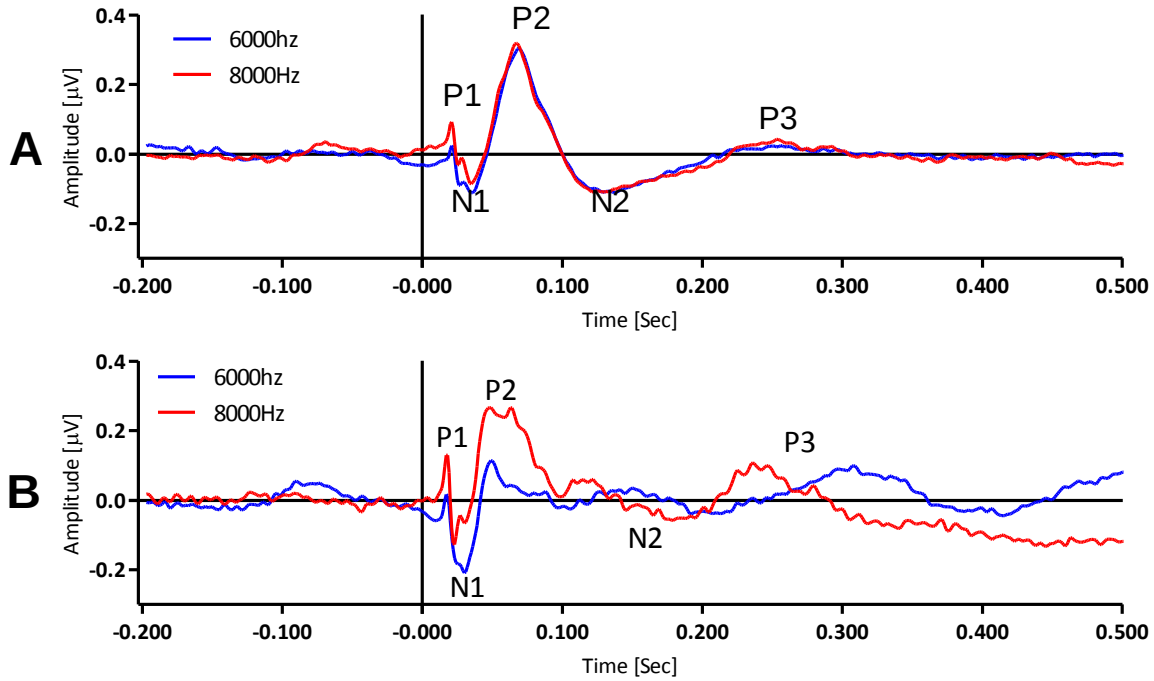


Figure 38: ERP for primary auditory cortex for all rats (N=8). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

4.3.1.3 Parietal cortex

The ERP for the Primary auditory cortex consisted of a P1, N1, P2, N2 and P3 peak. The P3 was only visible during the discrimination paradigm. The N2 and P3 appeared at a later latency for the 6000Hz tone compared to the 8000Hz tone, figure 39, A+B.

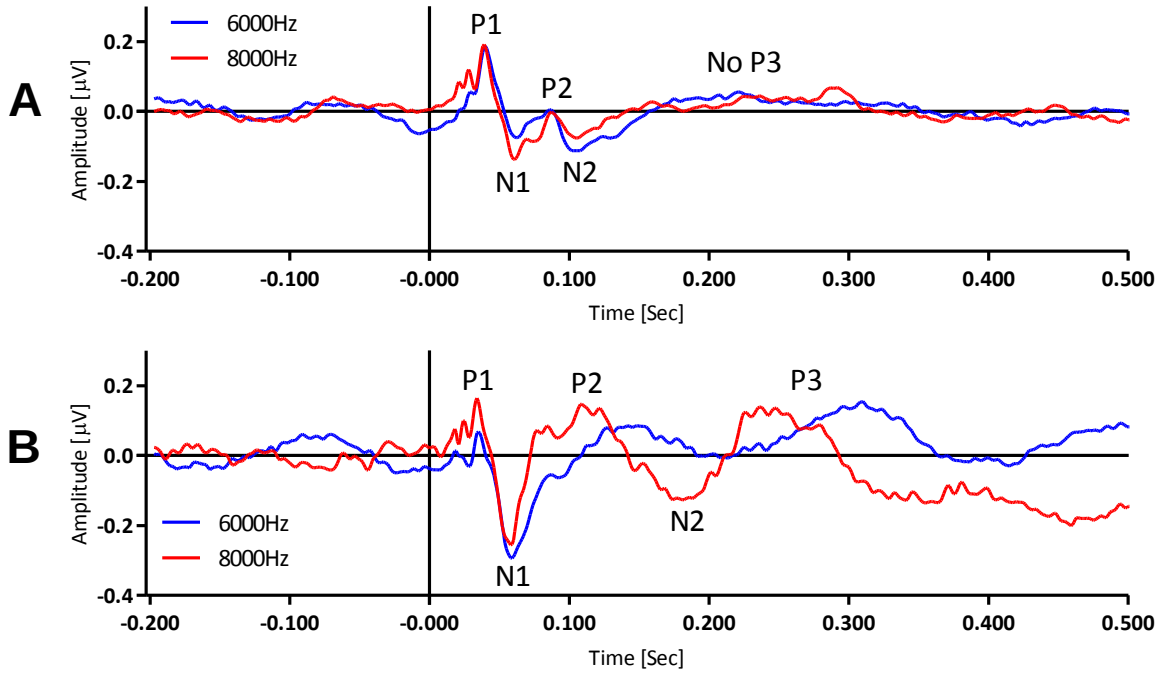


Figure 39: ERP for parietal cortex for all rats (N=8). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

4.3.1.4 Prelimbic cortex

The ERP for the prelimbic cortex consisted of a P1, N1, P2, N2 and P3 peak. The P3 was only visible during the discrimination paradigm, figure 40, A+B.

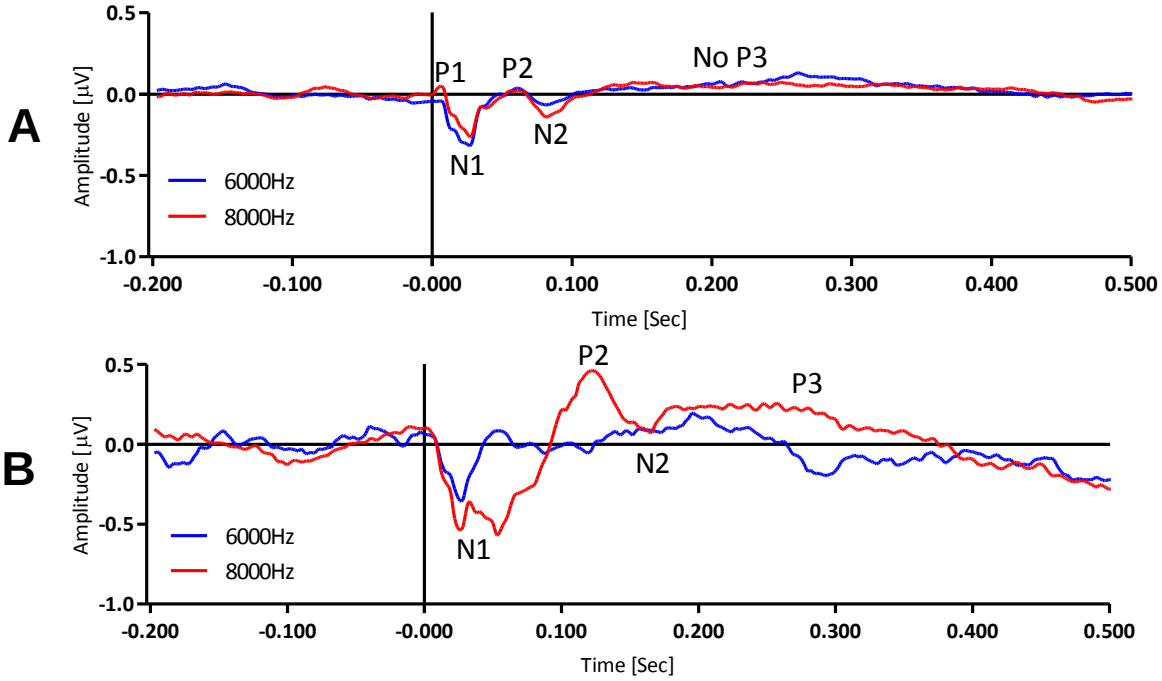


Figure 40: ERP for prelimbic cortex for all rats (N=8). Stimuli onset at time = 0 sec. **6000Hz** = Non-target tone. **8000Hz** = Target tone **A:** Auditory sensory paradigm **B:** Auditory discrimination paradigm.

5 Discussion

Overall, in the present thesis we have investigated several auditory discrimination paradigms and have identified a robust paradigm for eliciting a rat P3. In brief, we found a lack of clearly defined ERP signals for surface/cortical electrodes during experiment 1, but by improving the discrimination paradigm in experiment 2 and 3, a clearly defined ERPs could be identified for all electrodes and paradigms. In improved paradigms, a rat P3 ERP emerged in hippocampus and prelimbic cortex, which share similarities with the human P300 ERP.

5.1 Experiment 1

The paradigm for experiment 1 was problematic due to the fact that some rats apparently did not seem to use the target tone as cue for food reward. These rats seemed to have developed an alternative strategy by using the lever retraction that occurred 500ms after the target tone as cue for food reward. In general, this was problematic, since a P3 signal would not be locked to the occurrence of the auditory stimuli. This was particularly evident in surface electrodes placed on secondary auditory cortex, parietal cortex, and the frontal cortex where very variable or no sensory ERPs (P1, N1, and P2) could be observed during the discrimination paradigm.

One explanation for the large reduction in peak amplitude of sensory ERP components in cortical areas could relate to the observation that rats were very engaged in lever pressing at the time where the target tones were presented. High task engagement or high level of focused attention has been shown to produce reduced peak amplitudes of sensory ERP components[70].

However, in the hippocampus, a detectable sensory ERP was observed. A P3 peak appeared in hippocampus with a latency of 110-130ms. The fact that this P3 peak occurred at a latency of 110-130ms, and only during the discrimination paradigm may indicate that the P3 observed was similar to the P300 signal seen in humans as it has been hypothesized that the P300 in rats should be evoked faster in rats than humans[49, 63].

However, the fact that there were no P3 amplitude difference between target and non-target tone, as reported in humans[32], suggests that the rats did not distinguish the tones in regard to the task. Scopolamine have previously shown to be able to increase latency and decrease amplitude of the P300 processing in humans[71] and rats[63]. However, when dosing a pharmacologically active dose of scopolamine[68], no detectable change on the P3 peak amplitude or latency in hippocampus was observed.

However, scopolamine did evoke a reduction in the P2 amplitude which correlates with studies conducted by Sambeth et al.[72], which further supported that the scopolamine dose used was in the pharmacologically active range.

Even though the discrimination paradigm in the experiment has previously shown to be able to evoke a P3 signal in rats[64], it did not seem to be able to properly function in this experiment. In general, the paradigm was complex and many factors could affect the ERP signal and rats develop alternative strategies to solve the task. Thus, major improvements were required to ensure optimal conditions for evoking a P3 signal. This led to the development of a new discrimination paradigm implemented in experiment 2.

In summary, the P3 observed in experiment 1 was most likely not similar to the P300 in humans, due to the lack of difference between target and non-target tone ERPs and the fact that scopolamine did not have any effect on either latency or amplitude[63, 71].

5.2 Experiment 2 and 3

In experiment 2, clearly defined sensory ERP signals could be identified for all electrode placements and a P3 peak appeared in all electrodes.

On a technical level, the paradigm changes made from experiment 1 to experiment 2 were put in place due to the lack of a clearly defined ERPs in experiment 1. In experiment 2, the lever was always present inside the test chamber and the rat only had to press once for releasing a reward, which lead to a reduction of EEG noise.

These changes in the discrimination paradigm in experiment 2 were designed to reduce task engagement during presentation of the tones as well as direct rats towards using the target tone as cue for lever pressing and subsequent food reward. This strategy seemed to be effective by improving overall signal variability and ERP amplitudes. Some minor modifications were also put in place going from experiment 2 to 3. Even though all rats learned to discriminate the target tone from the non-target tone in experiment 2, some of the rats were not performing that well. To improve the performance of the rats, the training session in experiment 3 was performed before surgery and only good performers where then progressed for surgical implantation and further testing. The strategy of only selecting good performers also reduced the training time in experiment 3. Also removing the stimulus light cue in the last stages of the training phase improved the performance of the rats. In experiment 2, the stimulus light cue was removed in the later stages of discrimination training, as a consequence rats had bad performance on subsequent training days. Thus, requiring multiple training days before being able to distinguish the tones without light cue. This was most likely a result of rats using the visual cue rather than the target tone for food reward during early training when light cue was used. As a consequence, the light cue was removed during the training sessions in experiment 3. By removing the light cue prior to the discrimination task in experiment 3, the rats did not have to spend extra days relearning the discrimination.

One important factor for the variability of ERPs identified in the current study was that the frequency of the tones used, which have large impact on peak amplitude of the sensory component. At lower frequencies of 2000-4000Hz as used in experiment 1 clear sensory ERP could not always be detected in all rats. This observation correlates with the observations in another study that tried to identify the auditory components in rats. They observed that as frequency increased from 2000Hz to 8000Hz, the amplitude of the sensory components also increased when presented at identical intensities, but reached a plateau at 8-20kHz[73]. Due to the variable data in experiment 1 a more detailed investigation of sensory components were performed prior to the discrimination paradigm in experiment 2. In agreement to this, it was observed that the optimal frequencies were at 6-8000Hz compared to lower frequencies of 2-4000Hz. Even though 100dB provided the most consistent peaks, an intensity of 90dB was selected in experiment 2 and 3 as a startle reflex was observed at occasions at 100dB. Frequencies higher than 8000Hz was not tested as we did not want to deviate too much from the frequencies used in human studies which are in the 1000Hz region[38, 74]. Also, the use of tones that cannot be reliably detected by a human experimenter (>10kHz) is problematic, since experimental issues during the experiment e.g. stimulation software errors are less reliably detected. Most studies have a higher frequency for the target tone than the non-target tone. This could be problematic if tones have not been validated to produce identical ERP amplitudes across frequencies, since the difference in frequency *per se* could influence amplitude[75].

A puzzling finding, was in the secondary auditory cortex in experiment 2, where the latency of P1, N1, P2, and N2 were all shorter in the discrimination paradigm vs. the sensory paradigm on both tones. A possible answer for this could be that during the discrimination paradigm, the rats had been trained to distinguish the tones and therefore were more focused on the tones, leading to a faster processing of the sensory signal. However, one conflicting argument against this theory relates to the observation that this decreased latency only was observed in the auditory cortex and not at other electrode sites.

A very interesting finding was a large P3 peak in the hippocampus appeared at a latency of 170-180ms to the target tone as opposed to the non-target tone as the rats progressed and learned to distinguish the tones using the novel paradigm in experiment 2 and 3. This is interesting, since it indicates that the P3 peak observed in the hippocampus was part of the cognitive processing, which was needed to solve the task. To this end, a clear amplitude difference was observed on the P3 signal between target and non-target tone arguing that this hippocampal P3 observed is related to detection and processing of target tone response. This could indicate that the P3 was related to the human P300 signal. However, a small P3 signal was observed as a response to the non-target tone during the discrimination compared to the auditory sensory paradigm. The occurrence of a small P3 peak to the non-target tone could be due to the fact that ignoring a tone also requires some inclusion of the cognitive decision making, as the rat still have to decide whether to ignore or react to the tone, which may lead to a low amplitude P3.

Another interesting finding related to changes in the hippocampal N2 peak, which had significantly larger amplitude in response to the target tone than during the non-target tone, this may relate to the observation that N2 is believed to be involved in stimulus categorization and have also been shown to have a larger amplitude in response on rare tones (target tones) than frequent tones (non-target tones) in humans[34, 76, 77].

The findings of an increased N2 and P3 amplitude in the hippocampus in response to attended target tone stimulations was successfully reproduced in experiment 3. This suggests that the auditory discrimination paradigm generates a robust and reproducible hippocampal N2 and P3.

In the treatment trial in experiment 2, an equivalent dose of scopolamine, as used in experiment 1, produced a significantly increase of P3 latency and a tendency to a decrease P3 amplitude to the target tone. This effect on P3 latency and amplitude is similar to the effect of scopolamine on P300 in human trials[71].

Taken together, the P3 ERP observed in hippocampus during experiment 2 and 3 share several characteristics of human P300 as:

1: Rat P3 amplitude was larger on the target tone than the non-target tone.
2: Rat P3 appeared in the expected latency range.
3: Scopolamine induced similar effect as seen on P300 in humans.

This may suggest that rodent hippocampal P3 is similar to human P300.

During the auditory sensory paradigm a P3 appeared at about 200ms, mainly in auditory cortex. A large P3 would not be expected to occur as a response to non-conditioned tones. Nevertheless, this observation may be related to the fact that the rats were not habituated to any tones prior to initiating the sensory paradigm study. Thus, it could be speculated that the observed P3 during auditory sensory paradigm could

be related to novelty detection processes, which have been reported to produce a novelty P3a, which is evoked when a subject is exposed to an oddball or new tone[32].

In cortical electrodes, a P3 at 250ms could be detected during discrimination paradigm. This was a puzzling finding since P3 ERP latency was 170-180ms in the hippocampus. The P3 during the discrimination task was most likely not a novelty P3a as the rats by then had been habituated to the tones. In addition, it could also be speculated that the P3 peak observed in cortical electrodes during discrimination was properly not equivalent to the human P300 ERP, as no P3 amplitude difference was found between target and non-target tone, although this was not the case in frontal cortex in experiment 2. Moreover, scopolamine did not produce any significant effect on either latency or amplitude, which also challenges the hypothesis that the P3 ERP detected in surface placed electrodes resembles the human P300.

Most of the previous P300 studies in rats found a P300 in the 250-500ms region and it is possible that the P300 identified in these studies are similar to the “longer latency” cortical P3 ERPs that was identified in the three surface electrodes in the current study. But such a comparison is difficult as there are large experimental differences in how the experiments were conducted and the location of the electrodes.

One explanation for the occurrence of the P3 peak at about 250ms in cortical electrodes after target tones could be that rats need to perform a motoric response selectively to target tones. It is not unlikely, that motor cortex can be activated with a fast latency of 250ms, since well-trained rats in very simple reaction time task can respond with motor response with as low as 200ms latency to an external stimulus[78]. However, it was not possible to calculate the reaction time in the current experiments due to video recordings or electromyography recordings being necessary, as reaction time in rats are the time between onset of stimuli to first paw lifted from the floor[79]. The time between onset of target tone to lever press was measured for the best performing rat to 1.22sec (fastest response = 0.63sec, slowest response 3.24sec, data now shown in results) indicating that the rats could be quite fast in reacting to the target tone. However, it should be noted that as rats could move freely in the current study they had different distances from the lever when target tones were presented. Thus, in theory this means that different degree (and time) of motor activity would be needed from first motor cortex activation and lever press. However, in discrimination tasks, the reaction time is expected to occur slower, as the discrimination, e.g. P300 processing, must occur before the decision to react or not can be made. As a time difference of about 80ms were observed between the “short-latency” P3 in hippocampus to the “late-latency” P3 in cortical electrodes, it is possible that the “short-latency” P3 is part of the discrimination of the tones, and the “late-latency” P3 are the motor response to the stimulus. In favor of this hypothesis is the fact that this late cortical P3 was most pronounced in the frontal cortex, the cortically placed electrode that was located closest to the motor cortex. It cannot be excluded that an activation of motor cortex would be detected by electrodes at other cortical sites due to simple passive diffusion of electric charge over the surface of the cortex, known as volume conduction. In theory, a signal that are detected via volume conduction, should be larger closer to the source of the signal (here motor cortex source) and decrease in intensity as the distance from the source increases.

To confirm the hypothesis that the late cortical P3 was related to activation of motor cortex a depth electrode could be placed into the motor cortex to see if similar peak latencies are evoked during the discrimination paradigm or reaction time could be measured to see of a correlation to the “late latency” P3 could be made.

Nevertheless, in experiment 3 a depth electrode was placed in the rat prefrontal cortex (prelimbic cortex). The results from this experiment suggested that the late P3 observed using cortically placed screw electrodes in experiment 2 do not seem to resemble the P3 observed in prelimbic cortex. In prelimbic cortex, the P3 signal was very similar to that observed in hippocampus.

In humans, EEG cannot practically be recorded from the hippocampal region, due to its physical location in the brain. However, it is known that some P300 processing occur in the more accessible frontal brain region[32] Thus, it is interesting that a "short latency" P3, which was hypothesized to resemble the human P300 was observed by depth recordings from prelimbic cortex in experiment 3. The fact that a "short latency" P3 could also be detected in a brain area that have been shown to be involved in P300 generation by EEG in humans further validates this concept[32]. It is not surprising that a similar P3 can be observed both in ventral hippocampus and prelimbic cortex, since these interesting brain structures have monosynaptic connections[80] and have been reported to be involved in cognitive processing[81]. In addition, depth electrodes in other known generators e.g. temporal/parietal of the brain would also be very interesting to investigate as the generators for the P300 signal is believed to originate in these areas[32].

Although there are several arguments suggesting that the observed P3 in hippocampus and prelimbic cortex could be a model for the human P300, there are large differences in how the P3 paradigm is conducted in rats and humans. In human trials, the participants require no prior training whereas the rats require several days of training. In human trials no reward is required for motivating the participant to perform correctly but the rats require some sort of reward to be motivated to perform. This reward oriented motivation could possibly influence the ERP signal. It is also important to consider the anatomical brain differences which could influence how signals are processed. The study by Sambeth et al., in 2003 tried to compare the ERP signals between rats and humans in an auditory active oddball paradigm. They found that the sensory component correlate with the 40-50 % reduction in latency in rats compared to humans, but that the P300 latency recording by Sambeth et al., in the rats (P3 = 380ms) were within the expected latency range in humans[49]. They concluded that the relation between human and rat P300 peaks was not linear or that the P300 identified in rats does not correlate with the P300 found in humans[49].

As they used a surface electrode positioned at the parietal cortex, it is possible that the P300 observed in their study is similar to the "longer latency" P3 observed in the parietal cortex in this study. They concluded that if a rat P300 would follow the same reduction in latency seen on the sensory components, it should be in the 200ms region. It is possible that they have been an inappropriate region in their experiment as we observed a "short latency" P3 in hippocampus at exactly this latency in experiment 2 and 3.

In the present experiments we used scopolamine to show that it induced the same effect on the P300 signal in rats as it did in humans, e.g. decrease in amplitude and increase in latency[71]. Using such pharmacological tools to show similar effect of a drug on the signal is a valuable indicator that the signals observed are similar and also provide knowledge on the predictive validity of the rodent P3 assay. Further pharmacological testing could involve other compounds that are known to affect the human P300. Such pharmacological mechanisms could be nicotine, that have been reported to decrease P300 latency in humans[82], benzodiazepines that have been reported to increase P300 amplitude in schizophrenia patients[83], and d-amphetamine that very recently was shown to decrease P300 latency in healthy illicit stimulant users[84].

6 Conclusion

In the present study, we could successfully establish an auditory discrimination paradigm to evoke a P300-like ERP in hippocampus and possibly the prelimbic cortex, similar to the P300 seen in humans. Scopolamine showed a trend to reduce amplitude and significantly increased the latency of the hippocampal P3 further increasing the validity and translatability of the rat P3 to the human P300. Further testing to explain the P3 observed in the cortical electrodes, and also further validation of the rat P3 observed in hippocampus is warranted to increase the validity of the translatability between human and rat P300.

7 Future perspectives

Modifying the system to also be compatible with mice would enable the possibility to test various transgenic models of AD. This would enable the study of how Aβ and NFT impact the cognitive processing and would be highly interesting.

Further improving the system could be changing the lever with a nose poke mechanism where the rats instead of having to press the lever have to insert their nose into a hole which is then registered with a sensor, which could eventually reduce the mechanical artifacts even more. A nose poke mechanism could potentially also improve the training and response rate of the rats, due to some rats having difficulty learning how to press the lever. In addition, a nose poke system would be easier for a rat to learn as it only has to insert its nose into a hole and not correctly press down on a lever.

8 Appendix:

8.1 Protocols

8.1.1 Protocol for electrode operation in rats:

Materials:

This is a list of materials required for surgery on one rat.

All reusable surgery equipment must be cleaned between surgeries of multiple rats.

Item	Amount	Manufacturer	ID nr	Note
Forceps: - Curved, sharp end - Specimen - Kelly	 2 1 1			
Scissor	1			
Spatula	1			
Clamps	4			
Screwdriver	1			
Scalpel	1	Paragon®	P510	Nr 23
CMA 150 Temperature controller	1	Polygen	8315000	Heating pad
Stereotaxic alignment system	1	Kopf		Must be placed on ventilation table
Buster Op-Cover	1	Buster	141765	Sterile surgical cover
Magnifying glass	1			
Drilling machine Micro motor control Handpiece	 1 1	 Foredom Foredom	 FM3545	
Drill head	1	Hagar and Meisinger GmbH	310204001001014	
Electric razor	1			
Pro-Ophta sticks	As many as needed	Lohman & Rauscher	16515	
Q-sticks	As many as needed			
Sugi® Absorbant swabs	As many as needed	Kettenbach,	30601	
Depth electrode	1	Plastics1	E363/1	Intracraniel electrode W/Socket
Surface electrode	5	Plastics1	E363/20	Electrode W/Mounting Screw & Socket

Multi-channel electrode pedestal	1	Plastics1	MS363	
Prolene* surgery needle	1	Ethicon*	8682H	
Stop watch	1			
Felt-tip pen	1			
RelyX™ Unicem Self-Adhesive Resin Cement	As many as needed	3M ESPE	56818	
GC Fuji Plus Resin Reinforced Multipurpose Cement	As many as needed	GC America INC.	A3	
Syringes 1mL 3mL	As many as needed	 BD Plastipak™ BD Plastipak™	 300013 300910	
Canula 25G 5/8”	As many as needed	BD Microlance™	300600	

Chemicals/drugs:

Drugs:				
Name	**Concentration [mg/mL]**	**Manufacturer**	**Function**	**ID nr**
Rimadyl - Carprofen	 2.5	Orion Pharma Animal Health	Systemic analgesic	462986
Baytril - Enrofloxacin	5.0	Bayer	Antibiotic	508689
Marcain - Bupivacaine	 2.5	AstraZeneca	Local analgesic	169912
NaCl solution - Potassium Chloride	 9	Fresenius Kabi	Saline	
Hypnorm - Fentanyl - Fluanisone	 0.2 10	H. Lundbeck	Anesthetic	
Dormicum - Midazolam	 5	Roche	Anesthetic	081745
Iodine	1.6 % Solution	Novartis Agri A/S	Disinfectant	
Eyedrops Neutral “Ophtha” - Paraffin wax - Petroleum jelly	 200 800	Ophtha A/S	Eyedrops	539668
Sevorane - Sevoflurane	 100% solution	Abbott	Anesthetic	007462

Procedure:

Pre-surgery:

1) Weigh the rat
2) Calculate dose of drugs below and prepare them in syringes: See appendix for dose calculations.

   a. Hyp/dorm
   b. Baytril
   c. Rimadyl
3) Prepare a syringe with 1mL Marcain.
4) Inject Hyp/dorm (2.5mL/mL S.C.) to the rat (start the stopwatch. Every 30 min new injections of Hyp/Dorm are required)
5) While the anesthetics begin to work, prepare for the operation. The following materials must be present at operation table:
   a. Stereotaxic alignment system (Place on ventilation)
   b. Heating pad 37,5^{o}C (Remember to turn it on!)
   c. Sterile mat x1
   d. forceps x4, Scissor x1, scalpel x1-2, spatula x1, screwdriver x1, clamps x 4 (Place on the sterile mat)
   e. Magnifying glass x1
   f. Pro-Optha sticks and Q-tips
   g. Drill machine, incl. drill head (Drill head should be stored in 70% ethanol until it's needed)
   h. Depth electrodes x1, cut to 20mm length (Place on sterile mat)
   i. Surface electrode x 5 (Place on sterile mat)
   j. Electrode pedestal x 1 (Place on sterile mat)
   k. Eye drops
   l. 5mL syringe with NaCl solution
6) When the rat is anesthetized (check for reflexes), inject Baytril (4mL/kg, S.C.) and Rimadyl (1mL/kg, S.C.).
7) Shave the head of the rat with the shaving machine (located in the flow bench).
8) Disinfect the shaved area with iodine
9) Position the rat in the stereotaxic alignment system
   a. Remember to ensure the tongue is pushed to the side
10) Inject the Marcain (≈0.7mL S.C.) under the skin of the head and on the outside of the skin.
11) The rat is now ready for surgery.

Surgery:

1) Check for reflex every 5 min. Remember to keep the cut moist by injecting NaCl solution at different time slots (about every 5min should do).
2) Cut open the head from between the eyes down to between the ears. (about 2-3 cm)
3) Remove the membrane on top of skull by scraping them with the spatula
4) Place the clamps in each of the 4 corners of the cut.
5) Ensure that all membranes are removed and clean the skull with Pro-Optha sticks
6) Locate Bregma and Lambda on the skull.
7) Position a depth electrode on the stereotaxic alignment system and position it on top of Bregma (make a mark with a black felt pen). Measure the DV coordinate.
8) Reposition the depth electrode at Lambda and check of the DV coordinate correspond to the one at Bregma (+/- 2mm)
9) Reposition the depth electrode at Bregma and measure the AP and ML coordinates.
10) Calculate the coordinates for all surface electrodes
11) Reposition the depth electrode to each of these locations and mark the location with a black dot with a felt pen.
12) Drill holes at each electrode location. See illustration 1)
13) Screw in the 5 surface electrodes.

14) Reposition a depth electrode at Bregma. Measure the AP and ML coordinates (make sure the electrode is strait!)
15) Calculate vHipp coordinates and position the electrode correctly.
16) Fixate the electrode with RelyX™ dental cement.
17) Change the "electrode arm" of the Stereotaxic alignment system to the "electrode pedestal arm" and position an electrode pedestal in this.
18) Place each electrode head in its correct position in the pedestal, see illustration 2.
19) Fixate everything with GC Fuji Plus dental cement.
    a. Make sure that the cement is not in contact with any muscle or skin.
    b. Make sure that the surface of the cement is smooth so that it will not irritate the rat
20) Suture the wound with surgical simple interrupted sutures. About 2 sutures in front and 2 sutures in the back is enough.
    a. Make sure that the wound is properly closed (but NOT too tight).

Post-surgery:

1) Two food pellets that have been moistened are positioned inside the cage.
2) A recovery period of at least 10 days is needed
3) The rat is given ad libitum food during the recovery period
4) The rats are given analgesia, Rimadyl (1mL/kg S.C.), and antibiotics, Baytril (4mL/kg S.C.) every day in 5 days after surgery.
5) After at least 5 days sutures were removed.
    a. If a suture could not be removed due to the rat being uncooperative, it was anesthetized with Isoflurane Inhalation: Isoflurane induction at setting 5 for 5-10 minutes in induction chamber with an airflow of 0,5 O2 and 0,2 N2O. Maintenance at setting 1-2.

Comments:

Dosage calculations:

Volume to inject = weight of rat multiplied by dose of drug
Volume[mL] = dose[mL/kg] x m_{rat}[kg]

Anesthesia - Hypnorm/dormicum (Hyp/Dorm):

Initiation dose:	2.5mL/kg S.C.
Maintenance dose:	0.8mL/kg S.C.

Example:	m_{rat} = 250g
Initiation dose	2.5mL/kg x 0.250kg = 0,625mL
Maintenance dose:	0.8mL/kg x 0.250kg = 0,200mL

Analgesia – Rimadyl:

Dose:	1mL/kg S.C.

Example:	m_{rat} = 250g
Dose:	1mL/kg x 0.250g = 0,250mL

Antibiotics – Baytril:

Dose:	4mL/kg S.C.

Example:	m_{rat} = 250g
Dose:	4mL/kg x 0.250g = 1,00mL

Experiment 1 – Registration schema:

Date __ __ ____ (DD MM YYYY) Present at surgery _____

Rat # _____ Weight _____g

Hyp/Dorm initiation dose: _____mL

Hyp/Dorm maintenance dose: _____mL

Rimadyl: _____mL

Baytril: _____mL

Position of electrodes

	Level check Bregma/Lambda		Positioning of drill holes	Positioning of vHipp electrode
AP	X			
ML	X			
DV			X	X

[1] Frontal Association Cortex

	Target	Position of drill hole
AP	+4.7	
ML	-0.5	

[2] Parietal Association Cortex

	Target	Position of drill hole
AP	-3.8	
ML	-3.0	

[3] Reference (Front)

	Target	Position of drill hole
AP	>+8	
ML	-1.0	

[4] Temporal Cortex (secondary auditory Cortex)

	Target	Position of drill hole
AP	-4.8	
ML	-6.4	

[5] Hippocampus (Ventral CA1)

	Target	Position of drill hole
AP	-5,3	
ML	-4,6	
DV	-2,6	

[6] Ground (Mid temporal)

	Target	Position of drill hole
AP	-4,5	
ML	+4,0	

Illustration 1 – Electrode placement in experiment 1

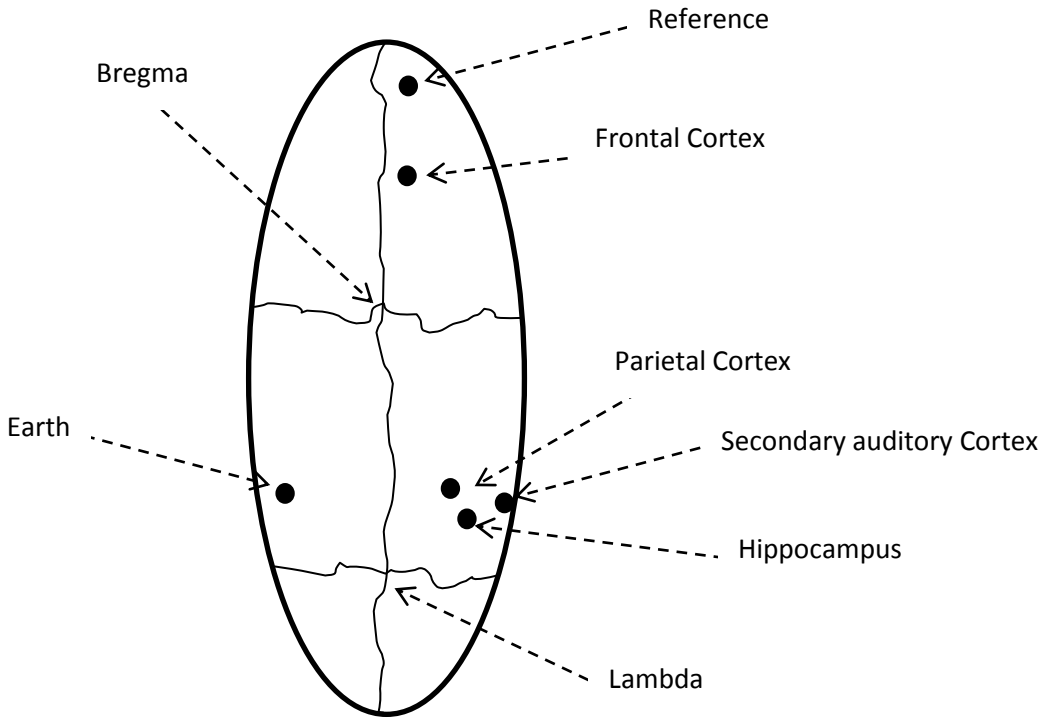
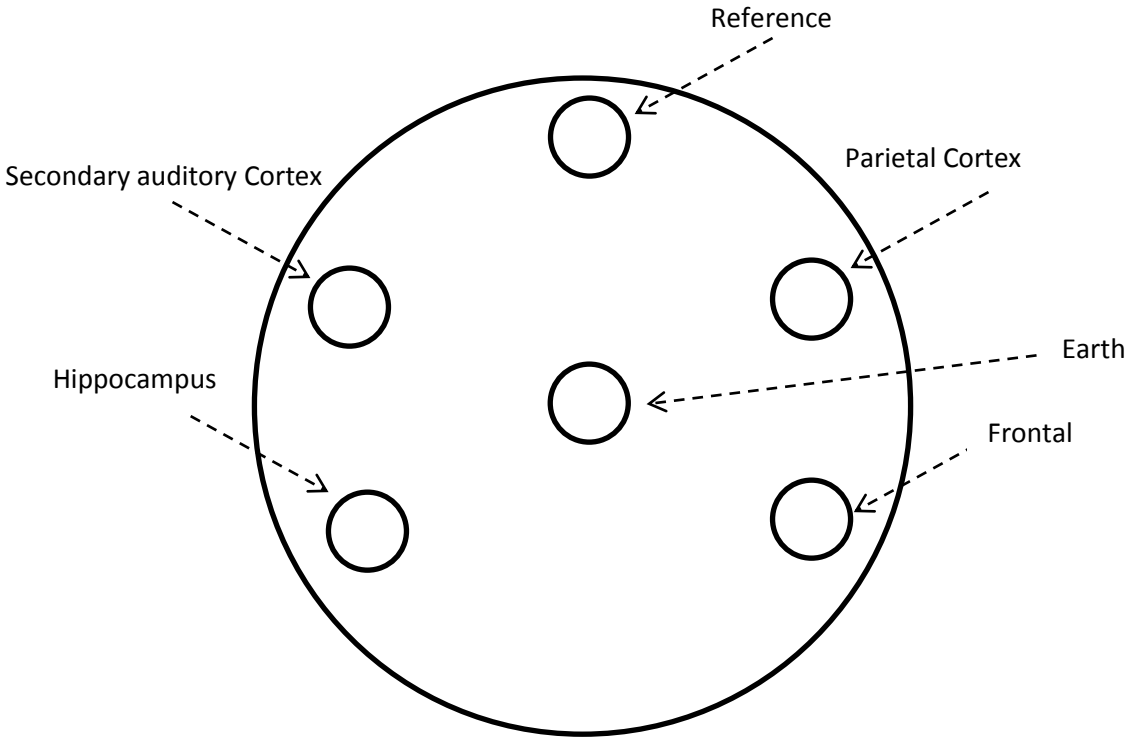


Illustration 2 – Position of electrode heads in pedestal for experiment 1



Experiment 2 – Registration schema:

Date __ __ ____ (DD MM YYYY) Present at surgery _____

Rat # _____ Weight _____g

Hyp/Dorm initiation dose: _____mL

Hyp/Dorm maintenance dose: _____mL

Rimadyl: _____mL

Baytril: _____mL

Position of electrodes

	Level check Bregma/Lambda		Positioning of drill holes	Positioning of vHipp electrode
AP	X			
ML	X			
DV			X	X

[1] Frontal Association Cortex

	Target	Position of drill hole
AP	+4.7	
ML	-0.5	

[2] Parietal Cortex

	Target	Position of drill hole
AP	-3.8	
ML	-3.0	

[3] Hippocampus (Ventral CA1)

	Target	Position of drill hole
AP	-5,3	
ML	-4,6	
DV	-2,6	

[4] Temporal Cortex (Secondary auditory cortex)

	Target	Position of drill hole
AP	-4.8	
ML	+6.4	

[5] Reference (Front)

	Target	Position of drill hole
AP	>+8	
ML	-1.0	

[6] Ground

	Target	Position of drill hole
AP	+2.3	
ML	+2.7	

Illustration 3 – Electrode placement in experiment 2

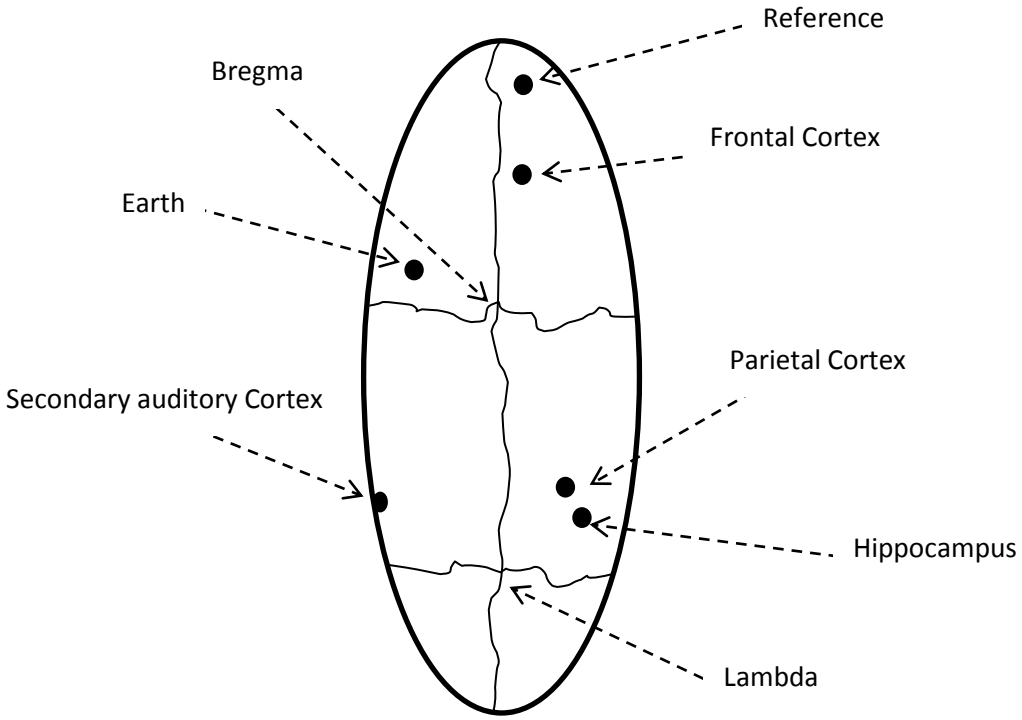
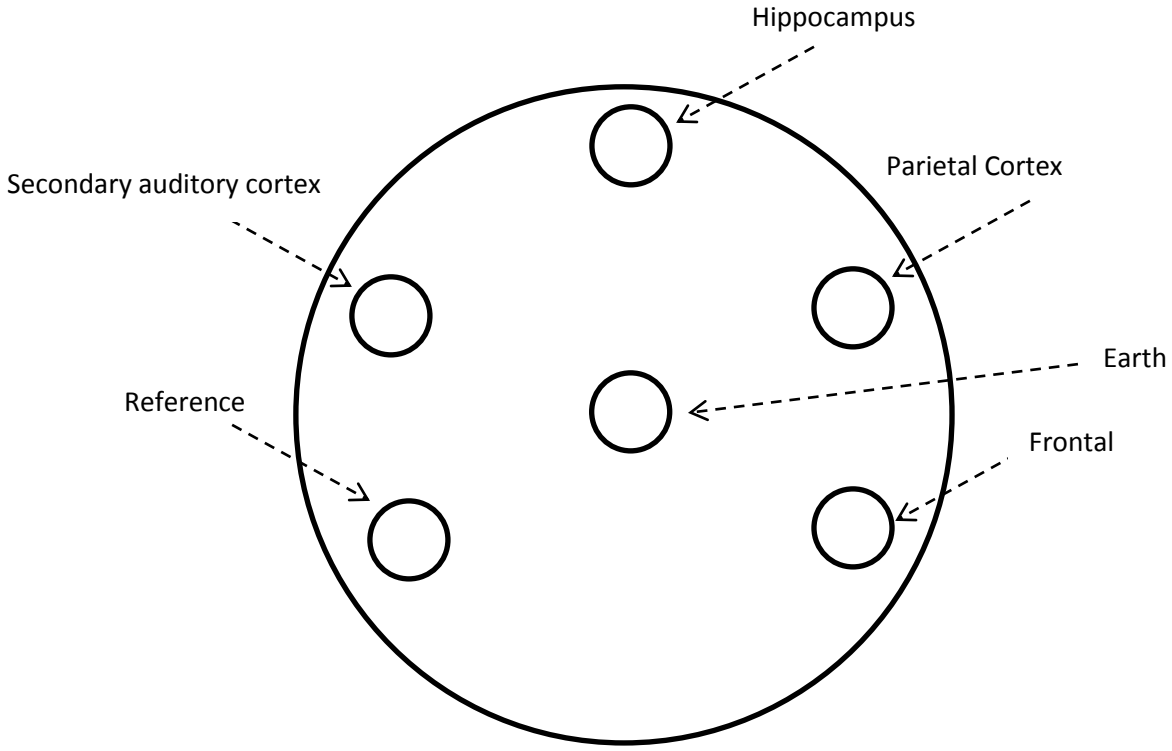


Illustration 4 – Position of electrode heads in pedestal for experiment 2



8.1.2 Rat weight control protocol

A normal rat eats about 5g food pr. 100g weight. To keep a rat on 80 % of normal weight it should be fed 4g of food pr. 100g weight. Feeding occur right after it have finished an experiment session, or 24 hour prior to conducting an experiment.

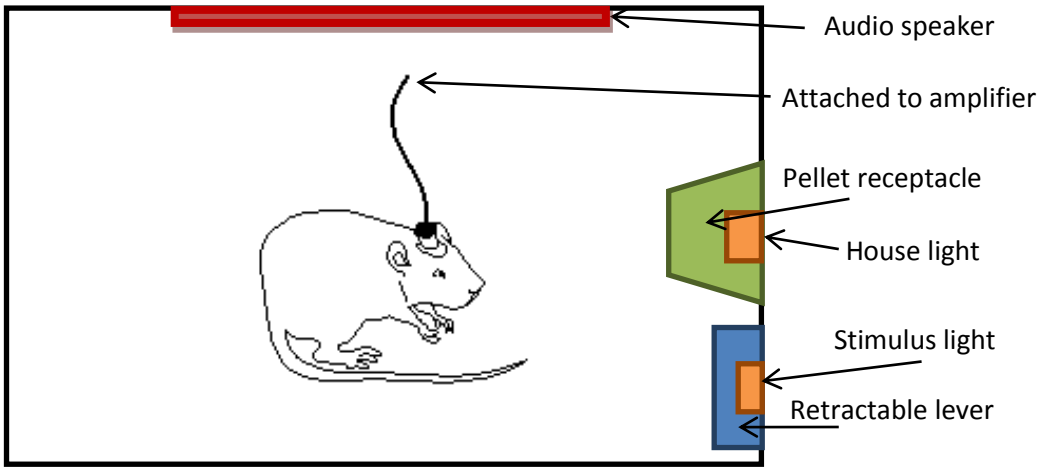
To ensure that the rats are not dropping below 80 % of normal weight, a control group (n=2-4) of same age received ad libitum food. If an experiment rat drops below 80% of the control weight, their food amount is increased until they are at 80 % again. If they are above 80 % their food amount can be decreased.

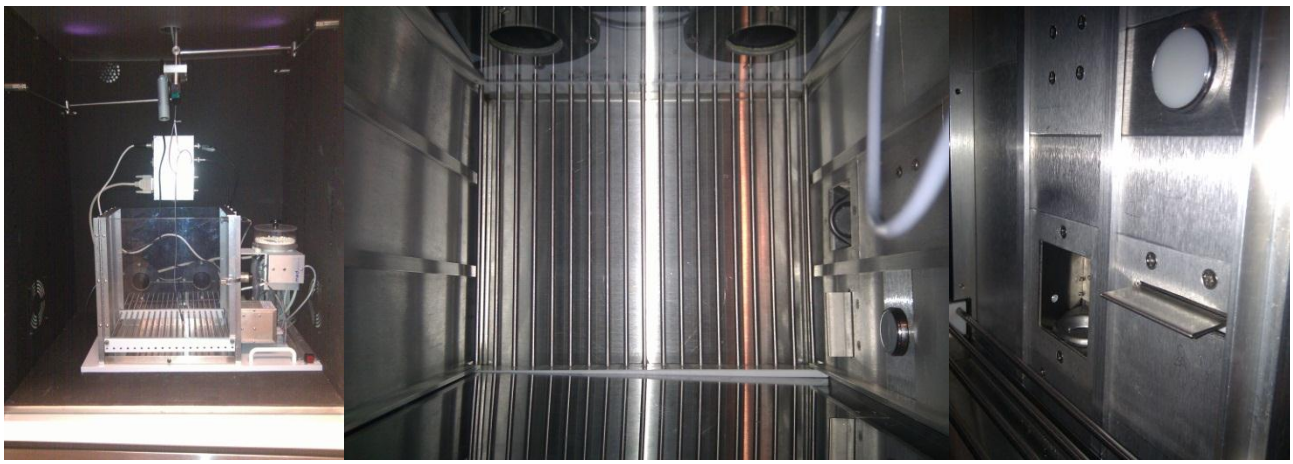
Procedure:

1. Weigh rotten, **Note the weight in a schema!**
2. Weight proper amount of food corresponding to the rat weight.
    - Food amount = 0.04 x m_{rat}
    - Example: Rat = 320g, 0.04 x 320g = 12.8g food

8.1.3 Experiment 1 – Setup

The experiment include two boxes in which the experiment can be conducted. A box consists of a soundproof metal box with a test chamber located inside. The test chamber consist of an audio speaker attached to the back wall, a pellet receptacle on the right side in which food pellets released from a pellet dispenser attached to the outside of the test chamber drops into. A retractable lever is positioned to the right of the pellet receptacle. Lighting of the box consists of a house light positioned above the pellet receptacle and a stimulus light above the retractable lever. An illustration of the test chamber can be seen below. Pictures of the test chamber can be seen below





Below, a table of the equipment used for the experiment 1 can be seen:

Item	Amount	ID number	Comment
Interface system			
Cabinet	1	Med Associates, SG-6080D	
SmartCtrl™	2	Med Associates, DIG-716	
Decode card	1	Med Associates, DIG-700G	
Audio Generator	2	Med Associates, ANL-926	
PCI Interface Card	1	Med Associates, DIG-704PCI-2	Installed in PC
Interface ribbon cable	1	Med Associates, DIG-700C	Connects Interface system with PC
1x Test chamber			
Test chamber	1	Med Associates, ENV-008	
Stainless steel grid floor	1	Med Associates, ENV-005	
Retractable lever	1	Med Associates, ENV-112CM	
Pellet receptacle	1	Med Associates, ENV-200R3M	
Pellet dispenser	1	Med Associates, ENV-203	
House light	1	Med Associates, ENV-215M	
Stimulus Light	1	Med Associates, ENV-229M	
Audio speaker	2	Monacor®, DT-254	Custom attachment to the back side of the test chamber
Connection Panel	1	Med Associates, SG-716B	
Camera system			
Camera system	2	Monacor®, TVCCD-160SCOL	
Recording unit	1		
EEG system			
Amplifier	2	Brownlee Precision Model 440 Amplifier	
Data acquisition interface	1	Cambridge Electronic Design, Power1401 mk 2	
Various			
Soundproof box	2		
Faraday cage	2		
Smart-UPS	1	APC, SMT750RMI2U	Backup power supply
Computer	1		Operating system: Windows XP

Software			
EEGO-Lab		Ellegaard Systems A/S, V. 1.0.1.6	Interface control software
Spike2		Cambridge Electronic Design, V. 6.09.	EEG recording software

8.1.4 Experiment 1 – Protocols

All Rats are kept on 80 % of normal free feeding weight throughout the whole experiment. A rat completes a protocol when it takes less than 30min to complete the tasks. A rat must complete a protocol at least 2 times (ea. 2 days) to continue to the proceeding protocol. A rat is exposed to the same test chamber every time. An experiment session is completed after 60 rewards or 1 hour. Rewards are 45mg Noyes feeding pellets.

Protocol 1A:

Tones used: Non-target tone: 2000Hz, 90dB, 20ms duration
Target tone: 4000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. The lever that the rat is required to press for release of food reward is present all the time. 1 correct press on the lever releases 1 food reward. In the start the reward can be released manually if the rat tries to press the lever but don't do it correctly. The non-target tone (2000Hz) are played randomly every 2, 3, 4 or 5sec (mean = 3.5sec). The target tone (4000Hz) are played when the rat correctly press's the lever, or if a food reward is given manually. A 0.500sec delay from target tone onset to release of food reward was inserted.

Protocol 1B:

Tones used: Non-target tone: 2000Hz, 90dB, 20ms duration
Target tone: 4000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. The lever that the rat is required to press for release of food reward is present all the time. The rat must press 1, 2, or 3 times (mean = 2 press's) on the lever to releases a food reward. The non-target tone (2000Hz) are played randomly every 6, 7, 8, 9 or 10sec (mean = 8sec). The target tone (4000Hz) are played when the rat correctly press's the lever, or if a food reward is given manually. A 0.500sec delay from target tone onset to release of food reward was inserted.

Protocol 1C:

Tones used: Non-target tone: 2000Hz, 90dB, 20ms duration
Target tone: 4000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. The lever that the rat is required to press for release of food reward is present all the time. The rat must press 1, 2, 3, 4, 5, or 6 times (mean = 3.5 press's) on the lever to releases a food reward. The non-target tone (2000Hz) are played randomly every 6, 7, 8, 9 or 10sec (mean = 8sec). The target (4000Hz) tone are played when the rat correctly press's the lever, or if a food reward is given manually. A 0.500sec delay from target tone onset to release of food reward was inserted.

Protocol 1D:

Tones used: Non-target tone: 2000Hz, 90dB, 20ms duration
Target tone: 4000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. The lever that the rat is required to press for release of food reward is present all the time. The rat must press 2, 4, 6, 8, or 10 times (mean = 6 press's) on the lever to releases a food reward. The non-target tone (2000Hz) are played randomly every 6, 7, 8, 9 or 10sec (mean =

8sec). The target tone (4000Hz) are played when the rat correctly press's the lever, or if a food reward is given manually. A 0.500sec delay from target tone onset to release of food reward was inserted.

Protocol 2A:

Tones used:	Non-target tone:	2000Hz, 90dB, 20ms duration
	Target tone:	4000Hz, 90dB, 20ms duration
	Lever tone:	3000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. The lever that the rat is required to press for release of food reward is retracted and a lever tone (3000Hz) is played when the lever is introduced to the test chamber. The lever is introduced every 10, 15 or 20 sec (mean = 15 sec). The rat must press 1, 2 or 3 times (mean = 2 press's) on the lever to releases a food reward. The non-target tone (2000Hz) are played randomly every 6, 7, 8, 9 or 10sec (mean = 8sec). The target tone (4000Hz) are played when the rat correctly press's the lever, or if a food reward is given manually. A 0.500sec delay from target tone onset to release of food reward was inserted. The rat has 5sec to begin pressing the lever, and 10 sec from the first press to finish the required amount. If the rat fails to press the required amount, the lever will retract, and all tones and lighting in the test chamber will turn off for 10sec.

Protocol 2B:

Tones used:	Non-target tone:	2000Hz, 90dB, 20ms duration
	Target tone:	4000Hz, 90dB, 20ms duration
	Lever tone:	3000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. The lever that the rat is required to press for release of food reward is retracted and a lever tone (3000Hz) is played when the lever is introduced to the test chamber. The lever is introduced every 10, 15 or 20 sec (mean = 15 sec). The rat must press 1, 2, 3, 4, 5 or 6 times (mean = 3.5 press's) on the lever to releases a food reward. The non-target tone (2000Hz) are played randomly every 6, 7, 8, 9 or 10sec (mean = 8sec). The target tone (4000Hz) are played when the rat correctly press's the lever, or if a food reward is given manually. A 0.500sec delay from target tone onset to release of food reward was inserted. The rat has 5sec to begin pressing the lever, and 10 sec from the first press to finish the required amount. If the rat fails to press the required amount, the lever will retract, and all tones and lighting in the test chamber will turn off for 10sec.

Protocol 2C:

Tones used:	Non-target tone:	2000Hz, 90dB, 20ms duration
	Target tone:	4000Hz, 90dB, 20ms duration
	Lever tone:	3000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. The lever that the rat is required to press for release of food reward is retracted and a lever tone (3000Hz) is played when the lever is introduced to the test chamber. The lever is introduced every 10, 15 or 20 sec (mean = 15 sec). The rat must press 2, 4, 6, 8, or 10 times (mean = 6 press's) on the lever to releases a food reward. The non-target tone (2000Hz) are played randomly every 6, 7, 8, 9 or 10sec (mean = 8sec). The target tone (4000Hz) are played when the rat correctly press's the lever, or if a food reward is given manually. A 0.500sec delay from target tone onset to release of food reward was inserted. The rat has 5sec to begin pressing the lever, and 10 sec from the first press to finish the required amount. If the rat fails to press the required amount, the lever will retract, and all tones and lighting in the test chamber will turn off for 10sec.

Protocol 2D:

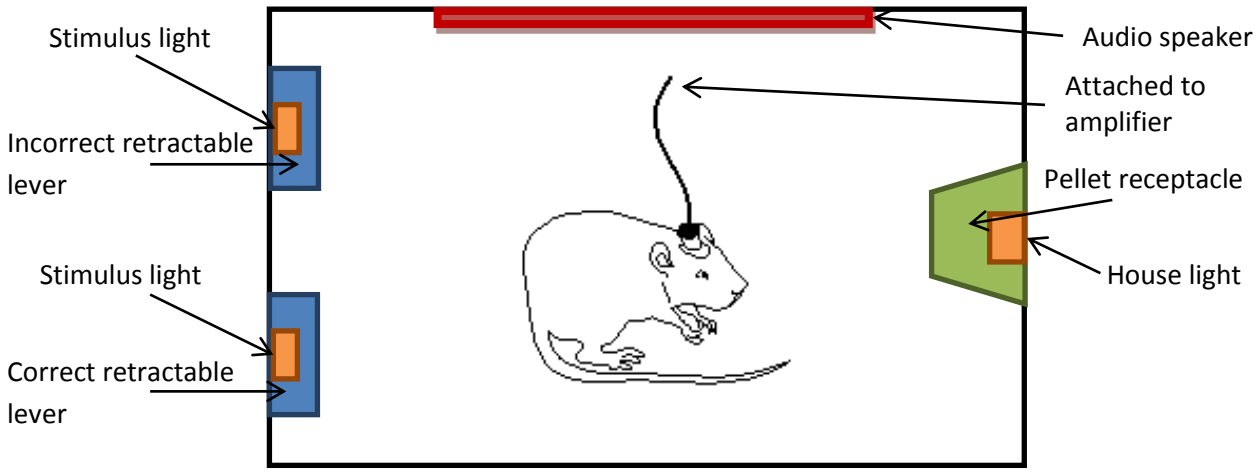
Tones used:	Non-target tone:	2000Hz, 90dB, 20ms duration
	Target tone:	4000Hz, 90dB, 20ms duration
	Lever tone:	3000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. The lever that the rat is required to press for release of food reward is retracted and a lever tone (3000Hz) is played when the lever is introduced to the test chamber.

The lever is introduced every 10, 15, 20, 25, 30, 35, 40, 45 sec (mean = 27.5 sec). The rat must press 2, 4, 6, 8, or 10 times (mean = 6 press's) on the lever to releases a food reward. The non-target tone (2000Hz) are played randomly every 6, 7, 8, 9 or 10sec (mean = 8sec). The target tone (4000Hz) are played when the rat correctly press's the lever, or if a food reward is given manually. A 0.500sec delay from target tone onset to release of food reward was inserted. The rat has 5sec to begin pressing the lever, and 10 sec from the first press to finish the required amount. If the rat fails to press the required amount, the lever will retract, and all tones and lighting in the test chamber will turn off for 10sec.

8.1.5 Experiment 2 – Setup

The experiment include two boxes in which the experiment can be conducted. A box consists of a soundproof metal box with a test chamber located inside. The test chamber consist of an audio speaker attached to the back wall, a pellet receptacle on the right side in which food pellets released from a pellet dispenser attached to the outside of the test chamber drops into. Two retractable levers are positioned to the left side of the test chamber. Lighting of the test chamber consists of a house light positioned above the pellet receptacle and two stimuli light above each retractable lever. An illustration of the test chamber can be seen below. Pictures of the test chamber can be seen below.



Below, a table of the equipment used for the experiment 2 can be seen:

Item	Amount	ID number	Comment
Interface system			
Cabinet	1	Med Associates, SG-6080D	
SmartCtrl™	2	Med Associates, DIG-716	
Decode card	1	Med Associates, DIG-700G	
Audio Generator	2	Med Associates, ANL-926	
PCI Interface Card	1	Med Associates, DIG-704PCI-2	Installed in PC
Interface ribbon cable	1	Med Associates, DIG-700C	Connects Interface system with PC
1x Test chamber			
Test chamber	1	Med Associates, ENV-008	

Stainless steel grid floor	1	Med Associates, ENV-005	
Retractable lever	2	Med Associates, ENV-112CM	
Pellet receptacle	1	Med Associates, ENV-200R2M	The access opening have been enlarged for easier access for the rat
Pellet dispenser	1	Med Associates, ENV-203	
House light	1	Med Associates, ENV-215M	
Stimulus Light	2	Med Associates, ENV-229M	
Audio speaker	2	Monacor®, DT-254	Custom attachment to the back side of the test chamber
Connection Panel	1	Med Associates, SG-716B	
Camera system			
Camera system	2	Monacor®, TVCCD-160SCOL	
Recording unit	1		
EEG system			
Amplifier	2	Brownlee Precision Model 440 Amplifier	
Data acquisition interface	1	Cambridge Electronic Design, Power1401 mk 2	
Various			
Soundproof box	2		
Faraday cage	2		
Smart-UPS	1	APC, SMT750RMI2U	Backup power supply
Computer	1		Operating system: Windows XP
Software			
EEGO-Lab		Ellegaard Systems A/S, V. 1.0.1.6	Interface control software
Spike2		Cambridge Electronic Design, V. 6.09.	EEG recording software

8.1.6 Experiment 2 – Protocols

All Rats are kept on 80 % of normal free feeding weight throughout the whole experiment.

A rat is exposed to the same test chamber every time. An experiment session is completed after 60 rewards or 1 hour. Rewards are 45mg Noyes feeding pellets.

Protocol 1 – Training:

Tones used: Training tone: 7000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. Both the correct and incorrect lever is present all the time. The training tone (7000Hz) is played every 8-16sec (mean = 12sec) from which the rat have 5sec to press on the correct lever to release a food reward. The stimulus light above the correct lever is turned on 0.500sec after the training tone has been played and turns off when the rat has pressed on the correct lever. If the rat press on the incorrect lever, press after 5sec of tone onset or do not press at all, a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off. When a rat is correct in >80% in 15min it was introduced to the next protocol, this takes about 1-2 weeks.

Protocol 2A – Day 1+2:

Tones used: Non-target tone: 6000Hz, 90dB, 20ms duration
Target tone: 8000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. Both the correct and incorrect lever is present all the time. Tones are played every 8-16 sec (mean = 12 sec). Target tone (8000Hz) is played 50% of the times and two target tones cannot precede each other. When a target tone (8000Hz) is played the rat have 5sec to press on the correct lever to release a food reward. The stimulus light above the correct lever is turned on 0.500sec after the training tone has been played and turns off when the rat has pressed on the correct lever. If the rat press on the incorrect lever, press after 5sec of tone onset or do not press at all, a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off. If a non-target tone (6000Hz) is played the rat must ignore it and do nothing. If the rat presses a lever after a non-target tone (6000Hz) a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off.

The rat is introduced to the next protocol after two days in this protocol

Protocol 2B – Day 3+4:

Tones used: Non-target tone: 6000Hz, 90dB, 20ms duration
Target tone: 8000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. Both the correct and incorrect lever is present all the time. Tones are played every 8-16 sec (mean = 12 sec). Target tone (8000Hz) is played 40% of the times and two target tones cannot precede each other. When a target tone (8000Hz) is played the rat have 5sec to press on the correct lever to release a food reward. The stimulus light above the correct lever is turned on 0.500sec after the training tone has been played and turns off when the rat has pressed on the correct lever. If the rat press on the incorrect lever, press after 5sec of tone onset or do not press at all, a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off. If a non-target tone (6000Hz) is played the rat must ignore it and do nothing. If the rat presses a lever after a non-target tone (6000Hz) a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off.

The rat is introduced to the next protocol after two days in this protocol

Protocol 2C – Day 5+6:

Tones used: Non-target tone: 6000Hz, 90dB, 20ms duration
Target tone: 8000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. Both the correct and incorrect lever is present all the time. Tones are played every 8-16 sec (mean = 12 sec). Target tone (8000Hz) is played 30% of the times and two target tones cannot precede each other. When a target tone (8000Hz) is played the rat have 5sec to press on the correct lever to release a food reward. The stimulus light above the correct lever is turned on 0.500sec after the training tone has been played and turns off when the rat has pressed on the correct lever. If the rat press on the incorrect lever, press after 5sec of tone onset or do not press at all, a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off. If a non-target tone (6000Hz) is played the rat must ignore it and do nothing. If the rat presses a lever after a non-target tone (6000Hz) a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off.

The rat is introduced to the next protocol after two days in this protocol

Protocol 2D – Day 7+:

Tones used:	Non-target tone:	6000Hz, 90dB, 20ms duration
	Target tone:	8000Hz, 90dB, 20ms duration

The rat is positioned in the test chamber. Both the correct and incorrect lever is present all the time. Tones are played every 8-16 sec (mean = 12 sec). Target tone (8000Hz) is played 30% of the times and two target tones cannot precede each other. When a target tone (8000Hz) is played the rat have 5sec to press on the correct lever to release a food reward. The stimulus light above the correct lever is NOT turned on. If the rat press on the incorrect lever, press after 5sec of tone onset or do not press at all, a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off. If a non-target tone (6000Hz) is played the rat must ignore it and do nothing. If the rat presses a lever after a non-target tone (6000Hz) a timeout period of 30sec is initiated in which no audio is played and all lighting is turned off.

8.2 Results

8.2.1 Experiment 1 – Auditory sensory paradigm

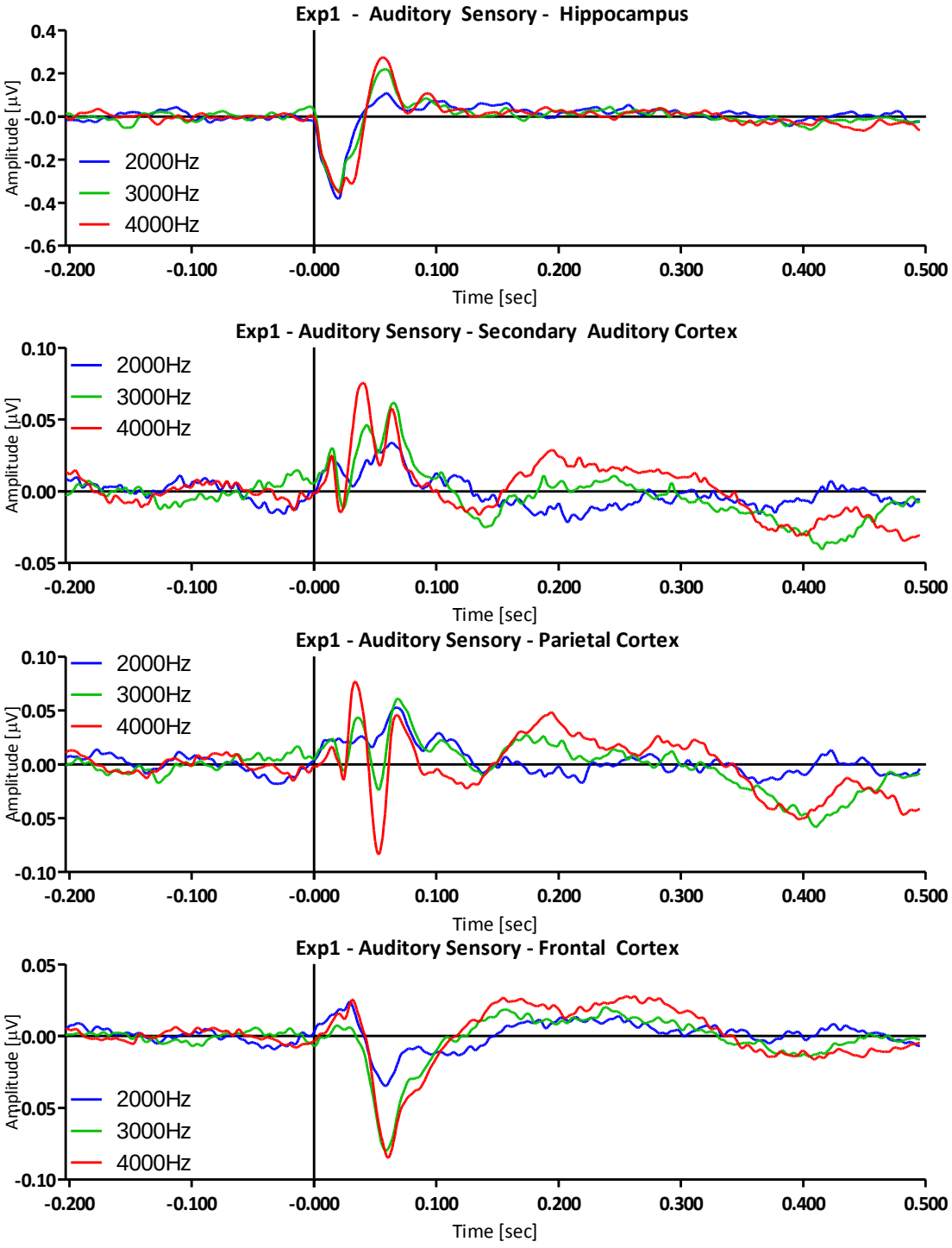


Figure 41: Audiotory sensory signals from hippocampus, secondary auditory cortex, parietal cortex, and frontal cortex during experiment 1.

8.2.2 Experiment 1 - Difficult peak localization on surface electrodes

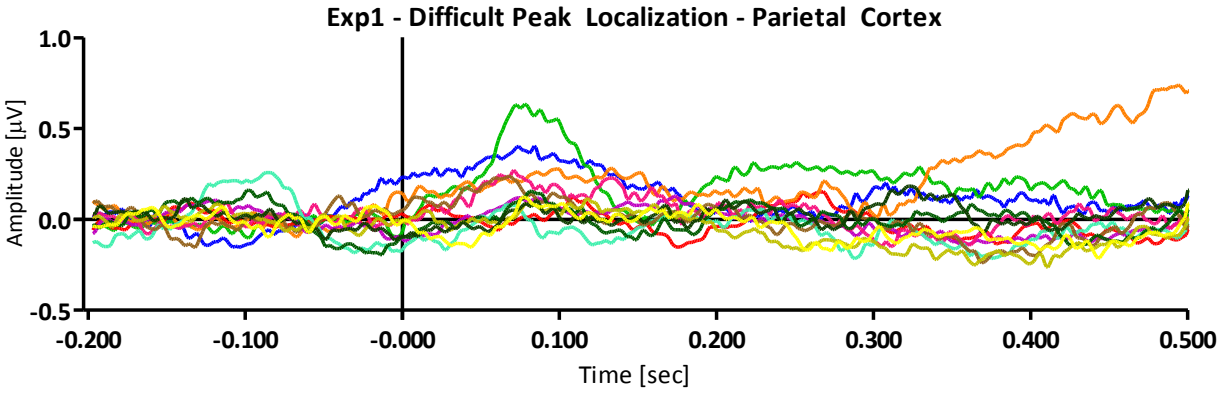


Figure 42: Each colored line indicates the ERP for a single rat. Notice that no peaks appear in unison. Similar results for the secondary auditory cortex and frontal cortex electrode. The secondary auditory cortex, parietal cortex, and frontal cortex electrodes were therefore excluded from further analysis in experiment 1.

8.2.3 Experiment 1 – Lever artifact

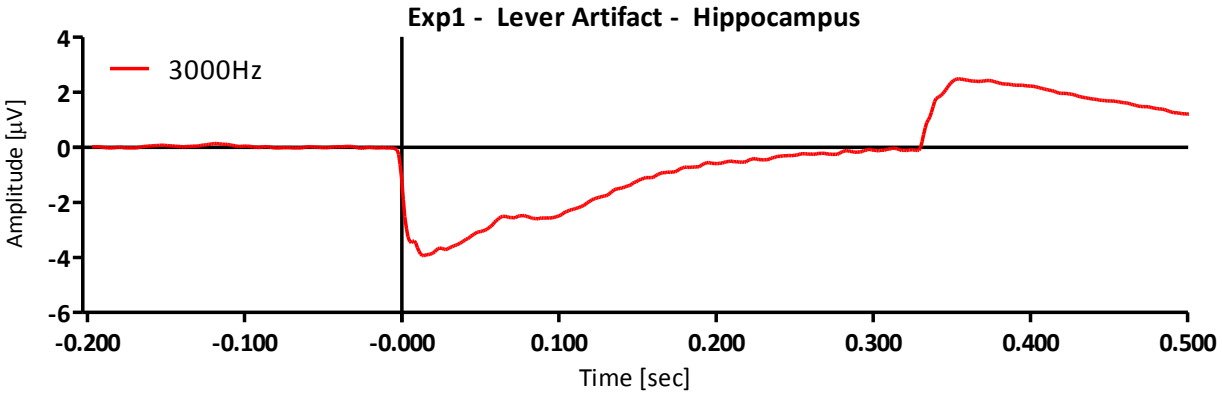


Figure 43: Artifact created by the introduction of the lever to the test chamber during the 3000Hz tone. This tone was therefore excluded from further analysis in experiment 1.

8.2.4 Experiment 2 – Auditory sensory paradigm – Tone validation

8.2.4.1 Hippocampus

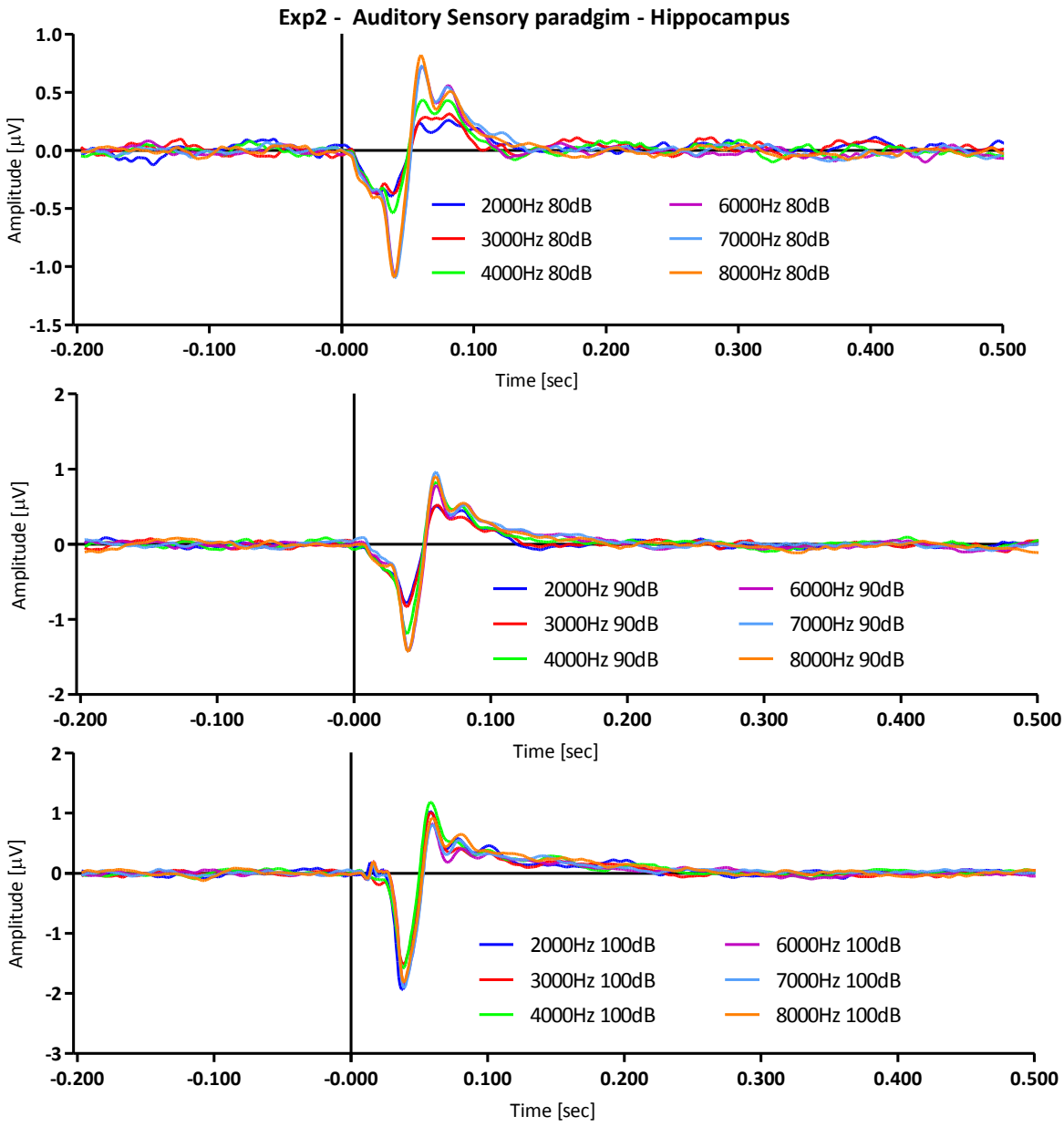


Figure 44: ERP components for hippocampus in sensory paradigm in experiment 2 for all frequencies and intensities tested.

8.2.4.2 Secondary auditory cortex

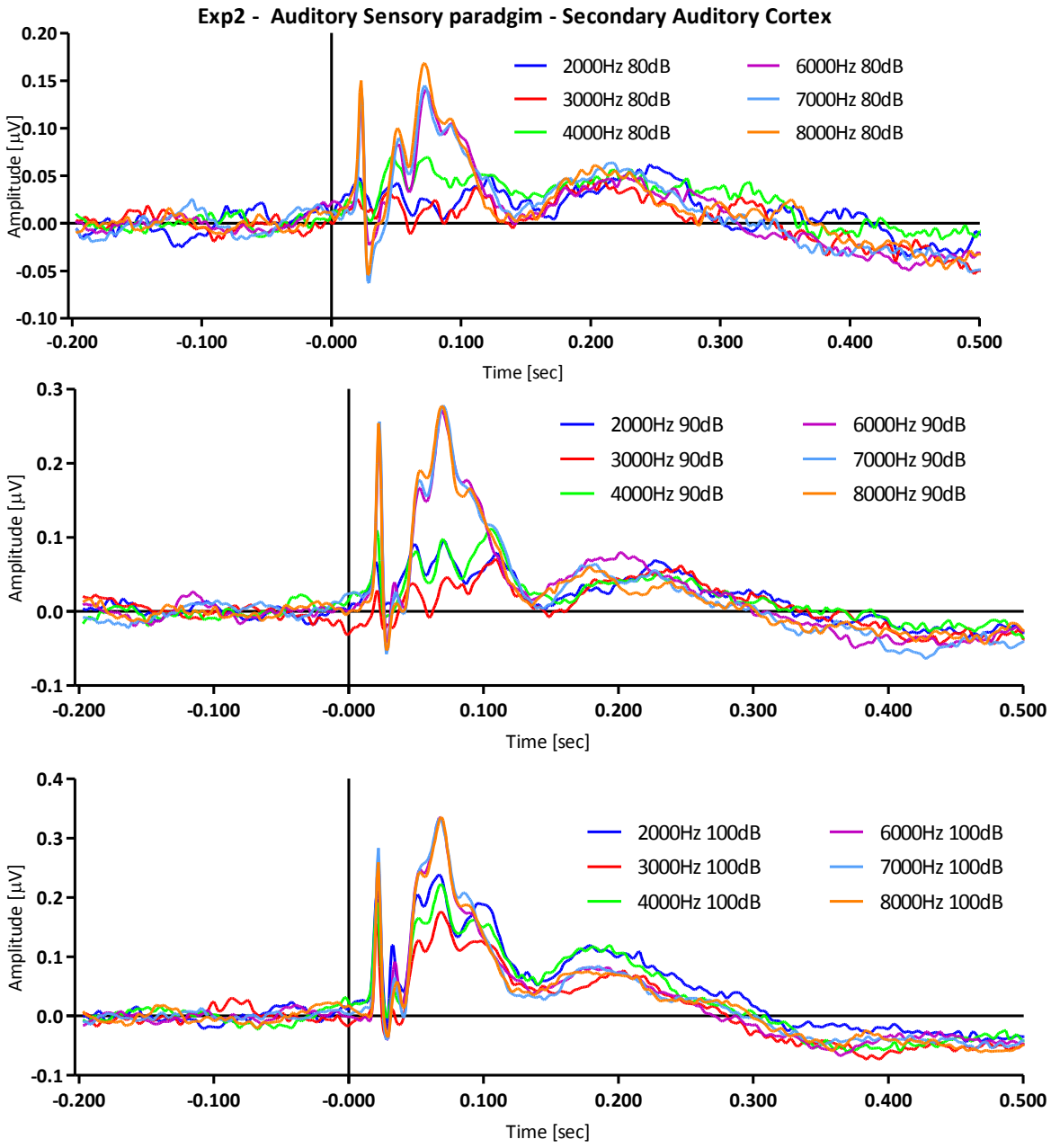


Figure 45: ERP components for secondary auditory cortex in sensory paradigm in experiment 2 for all frequencies and intensities tested.

8.2.4.3 Parietal Cortex

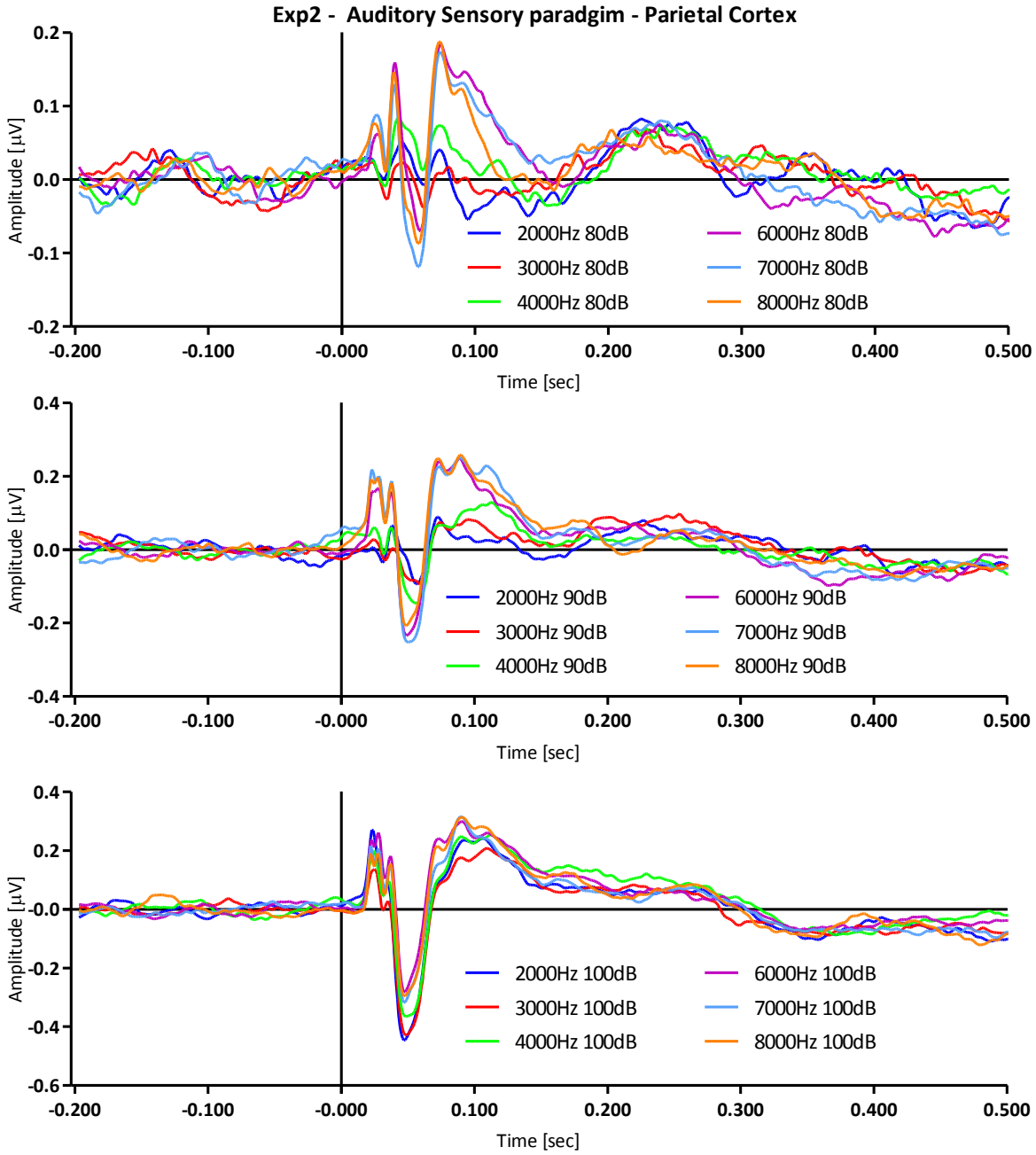


Figure 46: ERP components for parietal cortex in sensory paradigm in experiment 2 for all frequencies and intensities tested.

8.2.4.4 Frontal Cortex

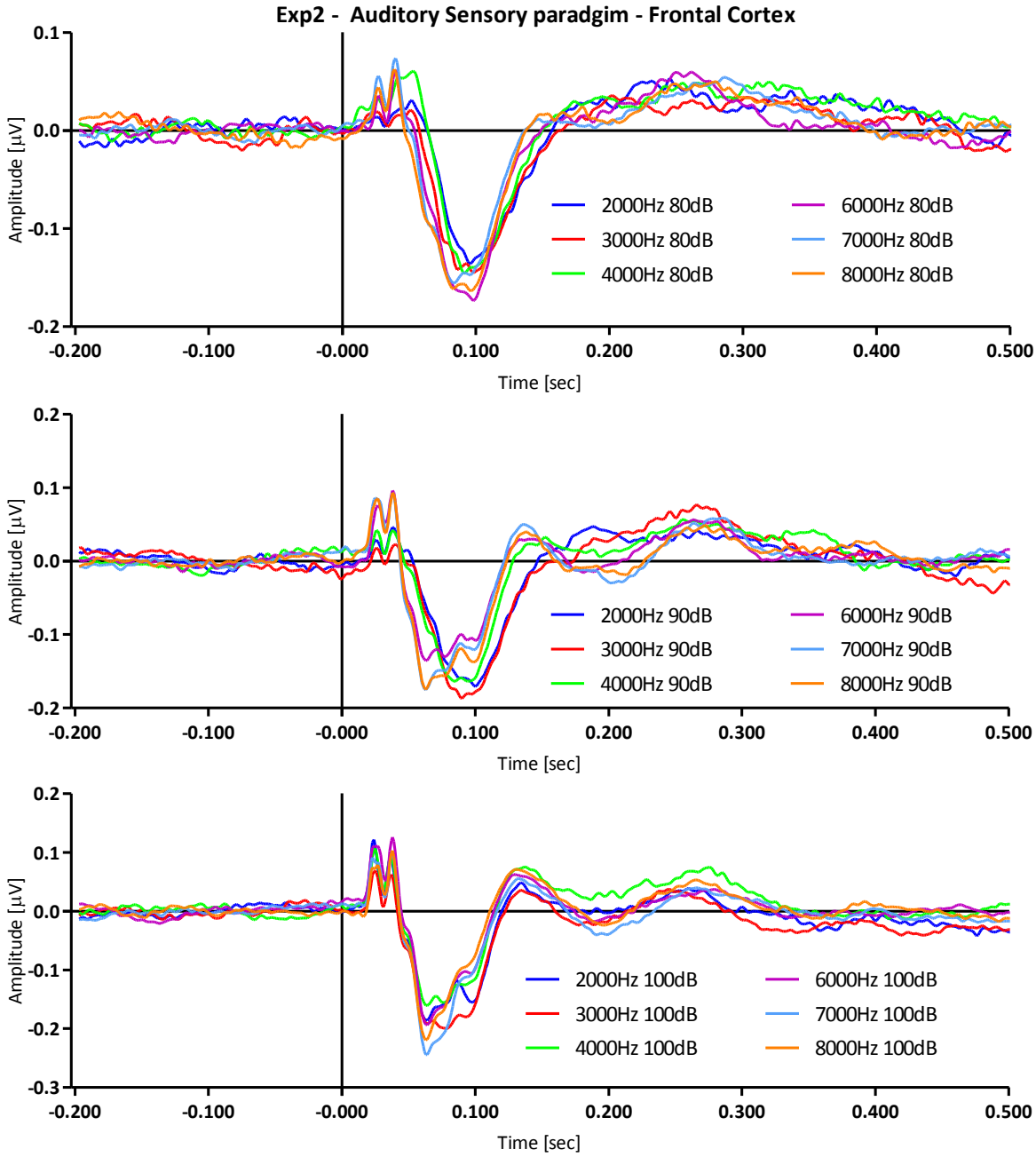


Figure 47: ERP components for frontal cortex in sensory paradigm in experiment 2 for all frequencies and intensities tested.

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