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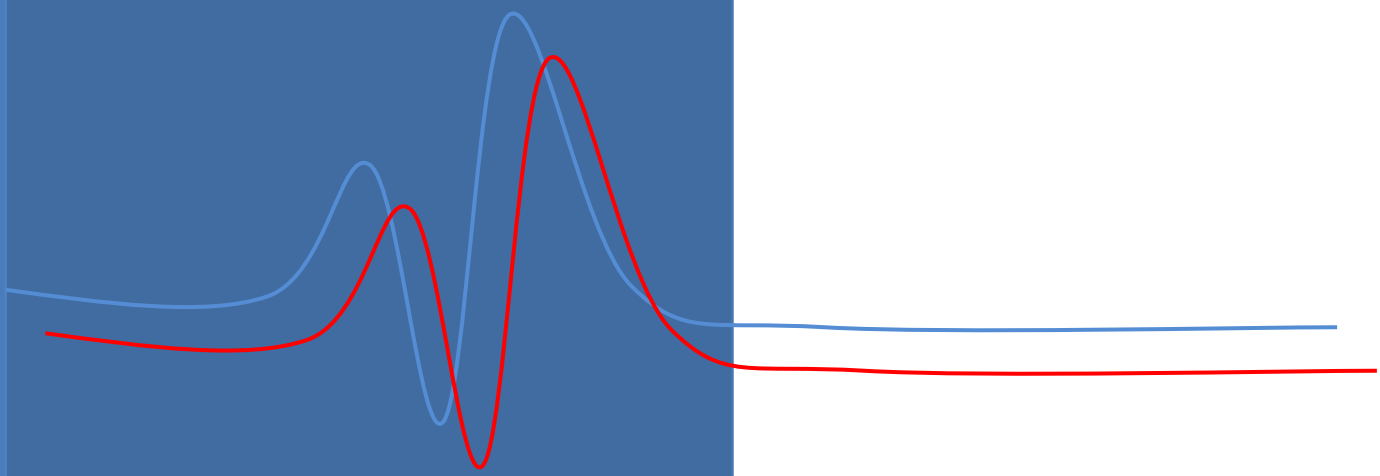
The Influence of Serotonergic Modulation on Auditory Evoked Potentials;

Mismatch Negativity a Potential Translational Assay in Schizophrenia



Master Thesis

Michelle Rosgaard Birknow

Medicine with Industrial Specialization

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Preface

This master thesis in Medicine with Industrial Specialization is based on experimental work performed at H. Lundbeck A/S, Valby in the period of August 2010 until June 2011.

Study results from the experimental work produce new knowledge and evidence in relation to mismatch negativity and the influence of the serotonergic system. Thus, it was decided to write an article manuscript, presenting these interesting findings. The article manuscript has been the major focus of this master thesis.

The master thesis consists of an introduction presenting schizophrenia and existing evidence related to mismatch negativity. The introduction is followed by a relatively short method and an initial validation section, presenting the choices that have been made in order to set up a valid assay for mismatch negativity in rats. Knowledge from the validation studies was used in the first escitalopram study. Subsequently, additional optimization was made prior to the second and primary escitalopram study, which is presented in an article manuscript "The Effect of Escitalopram on the Mismatch Negativity-like Response in Rats". The master thesis is completed with a general discussion of discussion points not raised in the article manuscript.

The master thesis presents only main results, in order to keep focus on results relevant to the study aims and the **article**. For a full overview of all test results see appendix E and F or attached disc.

Acknowledgement:

I would foremost like to thank my external supervisor at H. Lundbeck A/S, Jesper Frank Bastlund, Principal Scientist, Ph.D., Synaptic Transmission, for exceptional supervision and guidance throughout the entire project.

Moreover, a special thank to Kasper Larsen, Senior Technician at H. Lundbeck A/S, for instructions in surgical and experimental procedures.

I would like to thank people in the Research Department of Synaptic Transmission, office colleagues and fellow students at H. Lundbeck A/S for creating a pleasant, warm atmosphere.

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Michelle Rosgaard Birknow

Aalborg University, June 1st 2011

Abstract

Schizophrenia is a serious mental condition affecting 0.7% of the world's population. Schizophrenia leads to great disability and distress and is characterized by the presence of positive and negative symptoms, as well as cognitive deficits, which all affects the global functioning of the schizophrenic patient. The heterogeneity of the symptoms in schizophrenia and the complex pathophysiology hampers development of valid animal models and assays that address all the symptom clusters seen in schizophrenia.

Recent translational research has focused on the advantages of using mismatch negativity (MMN), a translational neurophysiological endophenotype, in relation to schizophrenia. MMN may represent a unique tool in pharmacological testing since it is an objective parameter, more closely linked to neurobiological foundations of disease processes rather than symptomatology. MMN deficits have been reported to be relatively selective for schizophrenia compared to other neuropsychiatric disorders. Furthermore, in chronically ill schizophrenic patients the severity of MMN deficits correlates with the severity of negative symptoms. Deficits in MMN generation in schizophrenia persist following treatment with both typical and atypical antipsychotics. Interestingly, the selective serotonin reuptake inhibitor (SSRI) escitalopram, which is frequently used in schizophrenia to treat depressive, cognitive or negative symptoms, significantly increases the MMN generation in healthy volunteers.

The objective of the present thesis was to back-translate the interesting finding that increased serotonergic signaling mediated by escitalopram increases MMN amplitude in healthy volunteers. This was performed by investigating the effect of increased serotonergic activity by dosing escitalopram alone or in combination with 5-HT3 antagonist ondansetron in an auditory oddball paradigm in rats. Secondarily, a validation of loudness dependence of auditory evoked potentials (LDAEP), as marker of serotonergic activity in rats, was performed.

Auditory evoked potentials (AEP) were recorded in hippocampus and parietal cortex of freely moving rats, when deviant tones were presented in a homogenous series of standard tones, to elicit a MMN-like response.

Results showed that 3mg/kg escitalopram significantly increased the MMN-like response in rat hippocampus. However, no synergistic effect on MMN was obtained when dosing escitalopram in combination with ondansetron.

In order to validate LDAEP as a marker of serotonergic activity, AEPs were recorded from hippocampus in freely moving rats, when presented to auditory stimuli with increasing intensity.

Results indicated that it was not possible to use LDAEP as a valid marker of serotonergic activity in the hippocampus.

Resume

Skizofreni er en alvorlig psykisk lidelse, der berører 0.7% af verdens befolkning. Skizofreni fører til mentalt handicap og angst og er kendetegnet ved tilstedeværelsen af positive, negative samt kognitive symptomer, som alle påvirker den generelle tilstand hos den skizofrene patient. Forskelligheden i symptomernes fremtoning samt en kompleks patofysiologi vanskeliggør udviklingen af valide dyremodeller og metoder, der kan anvendes til at adressere samtlige symptomer ved skizofreni.

Nylig translationel forskning har fokuseret på fordelene ved anvendelsen af mismatch negativitet (MMN), en translationel neurofysiologisk endofenotype, i forbindelse med forskning indenfor skizofreni. MMN repræsenterer et potentielt unikt redskab i farmakologiske forsøg, da det er en objektiv parameter, tættere knyttet til de neurobiologiske fundamenter af sygdommens processer snarere end symptomatologi.

Studier har vist, at afvigelser i dannelsen af MMN er relativt selektiv for skizofreni i forhold til andre neuropsykiatriske sygdomme. Desuden, hænger sværhedsgraden i MMN sammen med graden af negative symptomer hos kronisk syge skizofrene patienter. Afvigelser i dannelsen af MMN hos skizofrene viser sig fortsat efter behandling med typiske og atypiske antipsykotika. Det har imidlertid vist sig, at selektive serotoningenoptagshæmmere (selective serotonin reuptake inhibitors, SSRI), som escitalopram, der ofte anvendes i skizofreni til behandling af depressive, kognitive eller negative symptomer, signifikant forøger dannelsen af MMN i raske frivillige.

Formålet med denne afhandling var at "back-translate" den meget interessante iagttagelse at øget serotonerg signalering, medieret af escitalopram, er i stand til at øge MMN amplituden hos raske frivillige. Dette blev udført ved at undersøge effekten af øget serotonerg aktivitet gennem dosering af escitalopram alene eller i kombination med 5-HT3 antagonisten ondansetron i et auditorisk oddball paradigme. Sekundært, blev LDAEP valideret som en indikator for serotonerg aktivitet i hippocampus hos rotter.

Auditorisk fremkaldte potentialer (auditory evoked potentials, AEP) blev optaget fra parietal cortex og hippocampus i fritgående rotter under stimulering af auditoriske lydparadigmer bestående af en homogen serie af standardtoner afbrudt af enkelte afvigende toner, med det formål at udløse et MMN respons.

Resultater viste, at en dosis på 3mg/kg escitalopram signifikant øgede dannelsen af det MMN-lignende respons i hippocampus. Det var derimod ikke muligt at påvise en synergieffekt på det MMN-lignende respons ved at dosere escitalopram i kombination med ondansetron.

I forbindelse med valideringen af LDAEP (loudness dependence of auditory evoked potentials) som indikator for serotonerg aktivitet, blev der registreret AEPs i hippocampus hos fritgående rotter under auditiv stimulering med stigende intensitet.

Resultatet indikerede, at LDAEP ikke kunne anvendes som en valid indikator for serotonerg aktivitet i hippocampus.

List of abbreviations

AEP	Auditory evoked potential
ANOVA	Analysis of variance
CNV	Copy number variations
COMT	A gene at 22q11
CT	Computed tomographic
D_1	Dopamine D_1 receptors
D_2	Dopamine D_2 receptors
DRN	Dorsal raphe nucleus
DSM-IV	Diagnostic and statistical manual of mental disorders, fourth edition
EEG	Electroencephalography
EPS	Extrapyramidal symptoms
ERP	Event related potential
GABA	Gamma-aminobutyric acid
GWAS	Genome-wide association studies
ICH-10	International classification of diseases, tenth edition
ISI	Inter-stimulus interval
LDAEP	Loudness dependence of auditory evoked potentials
MARTA	Multi-acting receptor targeted antipsychotics
MMN	Mismatch negativity
MRI	Magnetic resonance imaging
NMDA	N-methyl-D-aspartate
PCP	Phencyclidine
PRODH	A gene at 22q11
REM	Rapid eye movement
SD	Standard deviation
SEM	Standard error of mean
SNP	Single nucleotide polymorphism

SSA	Stimulus specific adaptation
SSRI	Selective serotonin reuptake inhibitor
TRD	Treatment resistant depression
Val158Met	A valine to methionine mutation at position 158
VTA	Ventral tegmental area
ZDHHC8	A gene at 22q11
5-HT	5-hydroxytryptamin (serotonin)
5-HT$_{1A}$	Serotonin 5-HT$_{1A}$ receptors
5-HT$_{2A}$	Serotonin 5-HT$_{2A}$ receptors
5-HT$_{2C}$	Serotonin 5-HT$_{2C}$ receptors
5-HT$_{3}$	Serotonin 5-HT$_{3}$ receptors

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Introduction

Schizophrenia

At the beginning of the 20th century Kraepelin defined schizophrenia as an intellectual deterioration called *dementia praecox* ("early dementia"). The term *schizophrenia* ("split mind") was later introduced by Eugene Bleuler to explain the "fragmenting of the mind" that he believed to be the core abnormality of the disorder (Kaplan, 2008).

Today schizophrenia is known as a chronic debilitating psychiatric disorder. The life time risk of getting schizophrenia is estimated to 0.7% with similar rates of incidence throughout the world, although some variation is seen depending on urbanicity, migrant status, and social-economic class (for review see (McGrath et al., 2008)) The incidence of schizophrenia is higher in the male population compared to females (male:female ratio of 1.4) (Aleman et al., 2003; McGrath et al., 2008; Abel et al., 2010). Schizophrenia occurs as a sporadic and as a heritable disease, typically presenting in adolescence or early adulthood (Karam et al., 2010), with a peak onset in males between 15 and 25 years and with a 3-5 years delayed onset in women (Pearlson, 2000). Wide variation occurs over the course of the illness. The time course of schizophrenia can be either continuous or episodic, with one or more episodes with complete or incomplete remission. Despite the wide variation in the individual course of the illness, following overall stages of schizophrenia has been proposed; starting with a *premorbid phase*, followed by a *prodromal phase*, which is defined as the phase before the emergence of psychotic symptoms. The prodromal phase may progress into the *first psychotic episode* and subsequently a long term *chronic phase* (Singh et al., 2005; Agius et al., 2010). The course of schizophrenia is often more severe in men than in women (for review see (Abel et al., 2010)).

Diagnosis and symptoms

The diagnosis of schizophrenia builds on a clinical evaluation according to the International Classification of Diseases version 10 (ICD-10, the World Health Organization, 1993) or the Diagnostic and Statistical Manual of Mental Disorders version IV (DSM-IV-TR, the American Psychiatric Association, 2000). Both systems address schizophrenic symptomatology, without considering the underlying etiology (Tandon and Maj, 2008). One major difference between the two systems is that the DSM-IV-TR requires symptom duration of at least six months, whereas only one month is required in the ICD-10 criterion (Peralta and Cuesta, 2003). Thus, it is important to note which classification system has been implemented in the diagnosis.

The clinical abnormalities in schizophrenia are generally classified into three core symptom clusters; positive, negative and cognitive symptoms. (Pearlson, 2000; Tandon et al., 2009) There is significant heterogeneity in the clinical manifestations, and the severity of the different symptom clusters varies across patients and through the course of the illness (Tandon et al., 2008b; Tandon et al., 2009).

- *Positive symptoms* refer to a disturbance of normal behavior appearing as a result of the disease process in schizophrenia. The clinical manifestations include delusions and hallucinations (both auditory and visual), which often result in abnormal bizarre behavior and a distortion of reality. (for review see (Tandon et al., 2009))
- *Negative symptoms* refer to a reduction or absence of normal behavior found within schizophrenic patients. Common negative symptoms of schizophrenia include flattening of emotional expression, abulia (loss of motivation), alogia (poverty of speech), anhedonia (inability to experience pleasure), avolition (lack of initiative), apathy (lack of interest) and social withdrawal. (for review see (Tandon et al., 2009))
- *Cognitive symptoms* are core symptoms in schizophrenia and in the past decade intensive research has been made within this field. Cognitive abnormalities in schizophrenia have been suggested to include deficits in attention (Orzack and Kornetsky, 1966), processing speed (Dickinson et al., 2007), verbal fluency (Henry and Crawford, 2005), executive functions and working memory (Reichenberg and Harvey, 2007; Lee and Park, 2005; Barch and Smith, 2008) for review see (Tandon et al., 2009))

In addition to these three core symptom clusters, schizophrenia is often associated with co-morbid depression and anxiety, which are also regarded as important therapeutic targets in schizophrenia (Tandon and Jibson, 2003; Tandon et al., 2009). The depressive symptoms in schizophrenia are common but heterogeneous with respect to etiology, presentation, course, and treatment. (Bartels and Drake, 1989) It is estimated that approximately 60% of the schizophrenic patients experience a major depressive episode during the course of their illness (Martin et al., 1985).

In attempt to better understand the basis of the heterogeneity in clinical symptoms presented by patients with schizophrenia much recent research has focused on endophenotypes. Endophenotypes are stable, objective, state-independent measurements more closely linked to neurobiological underpinnings of disease processes rather than symptomatology (Gottesman and Gould, 2003). This approach aims at identifying quantifiable markers of pathophysiological processes that more closely resemble the primary effects of susceptibility genes than the clinical symptoms (Gottesman and Gould, 2003) (see section: Neurophysiological endophenotypes).

Risk factors

Although no common cause of schizophrenia has been identified, current evidence suggests that both genetic variations and environmental factors play a role in the development of schizophrenia, but neither acts alone in the development of the disease.

Genetic risk factors

Numerous studies in families, twins and adopted children have shown that genetic factors play a major role in the development of schizophrenia (Karam et al., 2010).

The incidence of schizophrenia is ~2% in third degree relatives (e.g. 1st. cousin) of an individual with schizophrenia; 2-6% in second degree relatives (e.g. grandparent) and 6-17% in first degree relatives (e.g. parent). Among twins, the incidence of schizophrenia is estimated to ~17% in dizygotic twins of affected individuals and ~50% in monozygotic twins. (Lewis and Lieberman, 2000; Tandon et al., 2008a)

Despite intensive research no single gene variations have consistently been associated with schizophrenia, which has led to the suggestion of schizophrenia as a polygenetic disorder. Further, recent Genome-Wide Association Studies (GWAS) have proposed that certain genetic copy number variations (CNVs) confer higher risk of schizophrenia. CNVs consist of genomic rearrangements such as deletions, duplications, inversions, or translocations, which either can be inherited or caused by *de novo* mutations. In relation to schizophrenia chromosome 22 has been extensively investigated. (Karam et al., 2010; Sebat et al., 2009) Approximately 30% of all individuals with 22q11.2 microdeletions develop symptoms of schizophrenia (Karayiorgou and Gogos, 2004). Following genes located in this region have been implicated in the development of schizophrenia; PRODH, ZDHHC8 and COMT. (Karam et al., 2010; Sebat et al., 2009) Furthermore, single nucleotide polymorphisms (SNPs) in the COMT gene (e.g. Val158Met) have been associated with schizophrenia like manifestations (Ohnishi et al., 2006; Costas et al., 2011).

Environmental risk factors

Although genetic risk factors clearly play a role in the etiology of schizophrenia, a variety of specific environmental exposures have also been implicated in the etiology of schizophrenia (for review see (Tandon et al., 2008a))

These environmental risk factors may include both biological and psychosocial risk factors during the perinatal period, early and late childhood, adolescence and early adulthood (Maki et al., 2005).

Especially risk factors in the perinatal period have received a lot of attention. Maternal influenza (Mednick et al., 1988) or infections (Brown et al., 2001; Brown et al., 2005; Brown,

2006) in these periods have been associated with increased liability of developing schizophrenia. Furthermore, severe nutritional deficiency (St Clair et al., 2005; Susser et al., 1996; van Os, 1997) and severe adverse life events (Khashan et al., 2008) experienced by the mother during the first trimester of pregnancy have been linked to increased risk of developing schizophrenia.

Risk factors in childhood may include trauma (Read et al., 2005), head injury (David and Prince, 2005) and parental separation or death (Morgan et al., 2007).

During adolescence, especially drug abuse e.g. cannabis use has been linked to an increased risk of developing schizophrenia (Semple et al., 2005). In early adulthood, social adversity and stressful life events have been associated with the risk of developing schizophrenia. (Norman and Malla, 1993; Allardyce and Boydell, 2006)

None of the environmental risk factors appear sufficient or necessary to cause schizophrenia alone.

Pathophysiology

The specific underlying pathophysiology of schizophrenia is still not entirely known. However, schizophrenia appears to involve brain structural, functional and neurochemical alterations. (Keshavan et al., 2011)

Morphological findings in schizophrenia

Structural brain abnormalities have been extensively documented in individuals with schizophrenia, assessed primarily with magnetic resonance imaging (MRI) (Harrison and Roberts, 2000). MRI studies show enhanced ventricle volume, especially enlargements of the lateral ventricles and the third ventricle in schizophrenia (Andreasen et al., 1990; Andreasen et al., 1994; Schwarzkopf et al., 1991). A meta-analysis of available post-mortem, computed tomographic (CT) and MRI studies showed that patients with schizophrenia have a highly significant reduction in brain size and to a lesser extent intracranial size (Ward et al., 1996). These findings have been further substantiated by a systematic review of MRI studies which concluded that there is a 3% reduction of brain volume and a ~40% enlargement of the lateral ventricles in schizophrenia (Lawrie and Abukmeil, 1998). The hippocampal formation has been a region of extensive clinical investigation in schizophrenia and also the most commonly studied region in post-mortem research (Harrison and Roberts, 2000). Structural neuroimaging studies have identified selective volume deficits in the amygdalohippocampal region and parahippocampal gyrus in schizophrenia (Nelson et al., 1998).

Neurochemical alterations in schizophrenia

Much evidence suggests that alterations in several neurotransmitter systems are involved in the pathophysiological processes leading to the development of schizophrenia. Among these, the dopamine and glutamate systems have received most attention, although other systems such as serotonergic, GABAergic, and cholinergic systems also have been implicated. (Laruelle et al., 2003)

The dopamine hypothesis

Dopamine plays an important role in the brain and is transmitted via three main pathways, Figure 1.

- *The nigrostriatal pathway* consists of cell bodies in the substantia nigra with axons terminating in the striatum. This pathway is mainly responsible for motor control.
- *The mesocorticolimbic pathway* is subdivided into two pathways; the mesocortial and the mesolimbic pathway. The mesocortical pathway connects the ventral tegmental area (VTA) to the prefrontal cortex. Whereas the mesolimbic pathway runs from the VTA to the nucleus accumbens. These pathways are essential to cognitive functions and are involved in motivational and emotional responses.
- *The tuberohypophyseal pathway* connects cell bodies in the hypothalamus to the pituitary gland. This pathway is associated with the endocrine control of the hypothalamic-pituitary system.

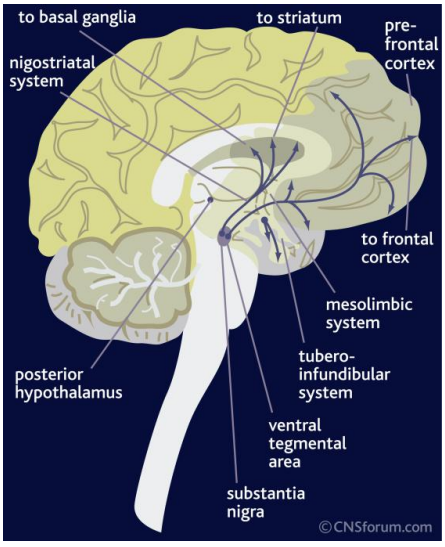

Figure 1. Dopamine pathways in the brain. The nigrostriatal pathway: Extends from substantia nigra and ends in the striatum. The mesolimbic/mesocortical pathway: Extends form ventral tegmental area (VTA) and ends in 1) prefrontal cortex 2) nucleus accumbens. The tuberohypophyseal pathway: Extends from hypothalamus and ends in pituitary gland. (Taken from www.cnsforum.com)

The dopaminergic synaptic transmission underlies at least some aspects of the pathogenesis of schizophrenia and receives its primary support from pharmacological studies (Laruelle et al., 2003).

In 1963 Carlsson discovered that antipsychotic drugs increased the metabolism of dopamine when administered to animals. The discovery by Carlsson formed the basis of the dopamine hypothesis of schizophrenia, which proposed that a hyperactivity of dopamine neurotransmission was responsible for positive symptoms (Seeman, 1987). Later it became evident that the reduction of positive symptoms was mediated by the ability of antipsychotic drugs to potently block the D_2 receptor. This was supported by pharmacological studies, which reported a correlation between increased psychotic symptoms and dopamine release following treatment with amphetamine (an inhibitor of the monoamine transporters including the dopamine active transporter (DAT)) in patients with schizophrenia (Laruelle et al., 1996; Abi-Dargham et al., 1998). Additionally, it was noted that drugs that increase the level of dopamine such as cocaine and amphetamine could induce psychotic episodes resembling those of schizophrenia in healthy humans, and that antipsychotic drugs were able to reverse these cocaine- and amphetamine-induced psychoses (Lieberman et al., 1987; Johnson and Milner, 1966).

To this end, an elevated density of dopamine D_2 receptors in post-mortem striatal tissue of schizophrenic patients has been reported (Seeman and Niznik, 1990). Moreover, schizophrenic patients have been shown to have increased occupancy of D_2 receptors by dopamine (Abi-Dargham et al., 2000). Taken together; there is an overwhelming evidence for a hyperactive dopamine system in schizophrenia.

In 1991, Davis et al. published a landmark article describing what they called "a modified dopamine hypothesis of schizophrenia". This article challenged the original dopamine hypothesis in the light of studies reporting reduced dopamine metabolites in some parts of the brain while elevated in other brain regions (Davis et al., 1991; Howes and Kapur, 2009). Several studies confirmed these findings by showing that D_1 receptors are decreased in the prefrontal cortex of schizophrenic patients (Okubo et al., 1997), contributing to a reduced dopamine activation of the frontal cortex (hypofrontality). Thus, according to the newer and more refined version of the dopamine hypothesis, schizophrenia may be caused by a hyperactive mesolimbic dopamine pathway, responsible for the positive symptoms and a hypoactive mesocortical dopamine pathway, which may be responsible for negative and cognitive symptoms. (Howes and Kapur, 2009)

The glutamate theory

Glutamate is the primary excitatory neurotransmitter in the brain and is widely distributed in the brain, Figure 2

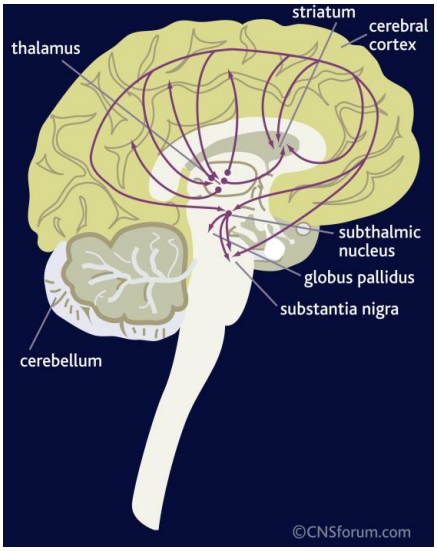

Figure 2. Glutamatergic pathways in the brain. Glutamate is widely distributed in the brain. (Taken from www.cnsforum.com)

The idea of glutamatergic abnormalities in schizophrenia was first proposed by Kim and Kornhuber and colleagues in 1980 based on their findings of reduced glutamate in the cerebrospinal fluid of patients with schizophrenia (Kim et al., 1980)

Later, it was published that the N-methyl D-aspartate (NMDA) antagonists phencyclidine (PCP) induce psychotic symptoms in healthy volunteers almost indistinguishable from those seen in schizophrenic patients (Javitt and Zukin, 1991) Further, studies report that ketamine, another NMDA antagonists, exacerbates the psychotic symptoms in schizophrenic patients (Lahti et al., 1995).

Post-mortem studies of schizophrenic patients have reported reduced expression of glutamatergic NMDA receptors in a variety of brain regions, notably the prefrontal cortex and the hippocampus (Harrison et al., 2003). In addition, positive modulators of the glutamate signaling, namely glycine and glycine transport inhibitors have beneficial effects in schizophrenia (Buchanan et al., 2007; Tsai et al., 2004; Lane et al., 2008). Thus, according to the glutamate hypothesis, the pathogenesis of schizophrenia involves NMDA receptor hypofunction.

Treatment

The broad objectives of the treatment of schizophrenia are to reduce the mortality and morbidity of the disorder by decreasing the frequency and severity of psychotic episodes and improving the quality of lives of the individuals afflicted with the illness. (Tandon et al., 2010)

Antipsychotic medication is the generally recommended treatment for schizophrenia. Antipsychotics are divided into typical (1.generation) and atypical (2.generation) antipsychotic agents. (Tandon et al., 2010)

Typical antipsychotics have been available since the 1950's and are characterized by their high affinity for the dopamine D_2 receptor, where they act as antagonists, decreasing the dopamine neurotransmission in the brain. In general, typical antipsychotics, such as haloperidol, are efficacious in attenuating the positive symptoms during acute psychotic episodes and preventing psychotic relapse. However, typical agents have limited effects on negative and cognitive symptoms in schizophrenic patients. Furthermore, it is recognized that typical antipsychotics induce acute extrapyramidal symptoms (EPS) such as akathisia, parkinsonism, dystonia and dyskinesia, as a result of inhibition of the dopaminergic motor control pathway in the nigro-striatal area of the brain. It is estimated that approximately 50-75% of patients taking typical antipsychotics experience these unwanted and unpleasant symptoms (Lublin et al., 2005).

In the 1970's the second generation of antipsychotic medication was developed. The atypical antipsychotics are a heterogeneous group of agents that act at multiple receptor sites, including dopaminergic, serotonergic, muscarinic, histaminergic and adrenergic receptors. (Horacek et al., 2006)

Atypical antipsychotics can be categorized according to the pharmacological properties, which reflect their affinities for specific receptors. Serotonin-dopamine antagonists (SDA) have high affinity for serotonin 5-HT_{2A} receptors and dopamine D_2 receptors (and also adrenergic α_1 receptors). Multi-acting receptor targeted antipsychotics (MARTA) show affinity for 5-HT_{2A}, D_2 and receptors of other neurotransmitter systems (cholinergic, histaminergic, 5-HT_{1A}, 5-HT_{2C} and others). (Horacek et al., 2006)

A final class of atypical antipsychotics is the partial dopamine receptor agonists, which are also known as the third generation of antipsychotics. (Horacek et al., 2006) The multi target approach in atypical agents has proved to be efficacious in treating the positive symptoms in schizophrenia and to less extent some negative and cognitive symptoms. Concerning adverse effects, atypical antipsychotic drugs are associated with a significantly lower risk of EPS than typical agents, which may be reflected in the greater affinity for other neurotransmitter receptors. However, atypical antipsychotics give rise to a range of other unwanted side-

effects, such as weight gain, diabetes mellitus, sedation and sexual dysfunction. (Lublin et al., 2005; Horacek et al., 2006)

The management of schizophrenia is continuous and often lifelong treatment with antipsychotic drugs to minimize relapse and provide clinical benefit to the patient (Keith and Kane, 2003). Regardless of the antipsychotic applied approximately 30% of schizophrenic patients remain refractory to the first line treatment, 40% respond only partially and 20-30% relapse within two years (Ban, 2004). Furthermore, non-compliance (up to 80%) is one of the primary barriers for successful treatment of schizophrenia (Keith and Kane, 2003). The high treatment resistance among patients and the fact that the currently available drugs mainly treat positive symptoms, while having little or no effect on negative and cognitive symptoms emphasizes the need of finding novel pharmacological treatment options.

One approach in optimizing the treatment of negative symptoms has been the use of combination treatment (Rummel et al., 2005). Several studies indicate that antipsychotics in combination with antidepressant (selective serotonin reuptake inhibitors, SSRI) may be more efficacious in treating negative symptoms than antipsychotic drugs alone (Spina et al., 1994; Jockers-Scherubl et al., 2005; Silver and Nassar, 1992). Although, SSRIs are often used in combination with antipsychotics in the management of schizophrenia, surprisingly little neurochemical evidence exists for this combination.

Drug discovery of new and innovative antipsychotics have been surprisingly slow. The reason for the slow progress in identifying effective drug treatments of schizophrenia is partly due to the high genetic and phenotypic heterogeneity of the disease. As well as the use of inadequate animal models, that mainly focuses on mimicking the phenotypic manifestations of the disease. Recently, investigators have focused on the advantages of using neurophysiological endophenotypes in translational drug discovery, which might contribute to more valid animal assays. (Thaker, 2007)

Neurophysiological endophenotypes

According to the classical definition, neurophysiological endophenotypes are stable, objective, state-independent measurements more closely linked to neurobiological underpinnings of disease processes rather than symptomatology. Neurophysiological endophenotypes are heritable and therefore present in first-degree relatives at higher rates than in the general population. (Gottesman and Gould, 2003) This means that neurophysiological endophenotypes have a distinct advantage in the development of new translational and valid animal models and assays. Neurophysiological endophenotypes provide a tool to examine different etiological factors and associated pathophysiology related to schizophrenia and are an ideal platform for development of novel treatments (Thaker,

2007). Moreover, it is possible to use the endophenotypes in diagnosis and classification of psychiatric diseases (Gottesman and Gould, 2003).

In the past decade, investigators have identified several neurophysiological deficits in schizophrenia that meet the criteria of a neurophysiological endophenotype; these include deficits in P50, P300 and mismatch negativity (MMN) (Thaker, 2007; Javitt et al., 2008). Neurophysiological endophenotypes have been widely studied both in humans and in animals, since they represent pre-attentive neuronal processing in the brain. Neurophysiological endophenotypes are all electrophysiological measures that can be obtained via electroencephalography.

Electroencephalography

Electroencephalography (EEG) is a noninvasive technique for recording brain electrical activity. Neurons of the brain generally process information by electrical signals. These electrical signals are generated when neurotransmitters activate specific postsynaptic receptors, and excitatory or inhibitory postsynaptic potentials are generated by the flow of ions across the membrane, ultimately leading to the generation of an action potential. The postsynaptic potentials cause electric fields in the surrounding extracellular environment by means of volume conduction. The volume conduction of the brain acts as a spatial low-pass filter and smears the electric potentials over rather large brain areas. With volume conduction there is only a microscopic delay between the brain activity and its reflection in the electrode-recorded signal. (Kenemans & Kähkönen, 2011) Thus, the measurement of EEG is the sum of electric fields formed from many postsynaptic potentials generated in the same time, transported through the brain via volume conduction until reaching the recording electrode.

In humans, EEG is recorded from many electrodes, placed in different areas on the scalp. In rodent studies, it is furthermore possible to routinely record EEG from intracranial electrodes in deep brain areas.

The arrangement/orientation of the neurons in the brain plays an important role in EEG measurement. The scalp-recorded EEG relies on the postsynaptic excitatory and inhibitory potentials generated predominantly in the dendrites of pyramidal cells. The reason for this is that the pyramidal neurons possess a well-developed dendritic system of equally oriented fibers. In the case of synchronous firing activity, the co-oriented dendritic fibers of pyramidal cells produce fairly large electrical potentials, which can be measured on the surface of the head. (Nunez and Srinivasan, 2006)

Event-related potentials

EEG can also be used to record event-related potentials (ERPs). ERPs are measured during external stimulation (e.g. visual or auditory). Due to a time-lock of brain response to external stimuli, ERPs can be recorded by averaged time-locked EEG signals. The advantage of making an average of many recordings is to maximize signal-to-noise ratio, due to the random nature of noise. (Javitt et al., 2008)

Auditory evoked potentials

Auditory evoked potentials (AEP) are ERPs elicited by auditory stimulation. The AEPs are named based on the latency from stimulus and the polarity of the waveform, such that P50 in humans is a positive deflection 50msec after stimuli. An AEP consists of a positive deflection (P50 in humans, also termed P1) followed by a negative deflection (N100 in humans, also termed N1) and ends with a large positive deflection (P200 in human, also termed P2). (Siegel et al., 2003), see Figure 3.

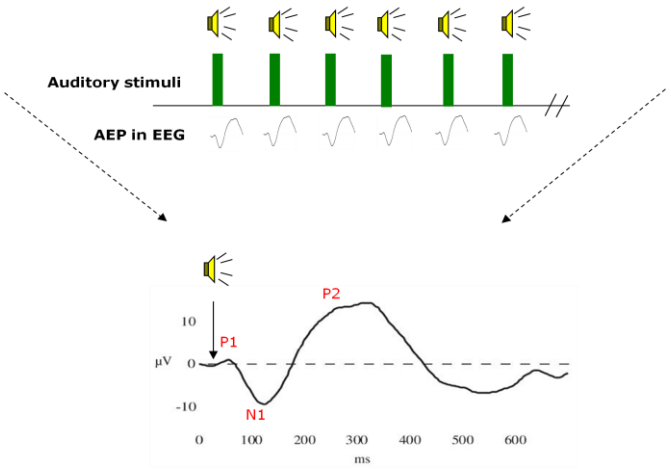

Figure 3. Average auditory evoked potential. Auditory evoked potentials (AEPs) are time-locked to the external stimuli. An average AEP is generated by averaging many individual AEPs recordings. P1: First positive deflection, at 50msec. N1: First negative deflection, at 100msec. P2: Second positive deflection, at 200msec. Modified from (Siegel et al., 2003)

Mismatch negativity

One of the most recently discovered neurophysiological endophenotypes in schizophrenia is mismatch negativity (MMN). MMN was first described by Näätänen and co-workers in 1978 (Näätänen et al., 1978). MMN represents the ability to detect changes in sensory information e.g. the auditory domain (Näätänen, 1995)

MMN is elicited when a sequence of repeated stimuli (standards) is interrupted by tones, which deviate in sensory characteristics such as e.g. frequency or duration (oddballs), see Figure 4 A. MMN is the negative component of the difference waveform generated by

subtraction of the standard AEP from the oddball AEP. (Näätänen, 1995), see Figure 4 B. The MMN (the negative component of the difference waveform) response in humans, peaks at about 100-200msec. after stimulus onset, but its latency vary according to the specific paradigm. (Näätänen, 1995)

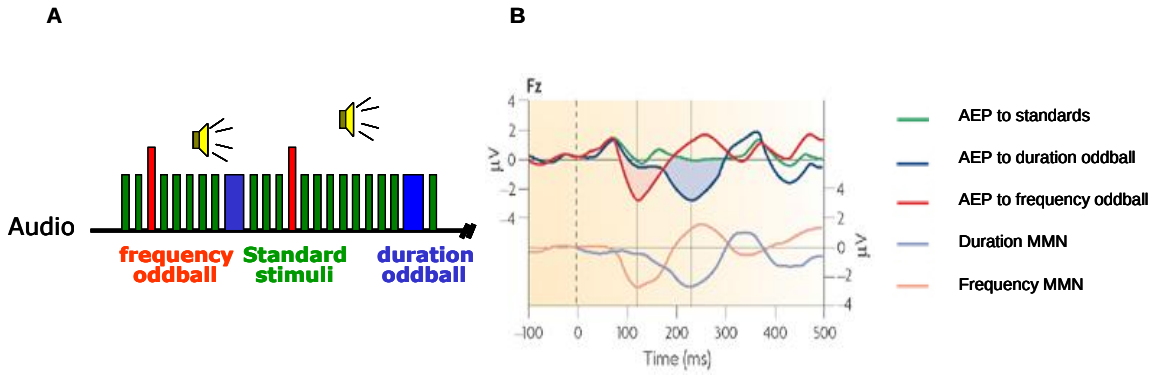

Figure 4. Generation of mismatch negativity. A) Auditory stimulation paradigm (oddball paradigm). Green bars represent standard stimuli, red bars represent frequency oddballs and blue bars represent duration oddballs. B) Average auditory evoked potentials generation in response to the auditory stimulation paradigm. Modified from (Javitt et al., 2008)

It is commonly accepted that MMN automatically arises if there is mismatch between the physical features of a deviant stimulus and a neural sensory-memory trace produced by repetitive standard stimuli. Thus, in order to elicit the MMN, a memory trace with the characteristics of the standard stimulus must be present. (Näätänen, 1995) For this reason a deviant stimulus only generates MMN if at least two standards have been presented (Umbricht et al., 2005).

Auditory MMN is a pre-attentional phenomenon (Näätänen et al., 1978), which means that it can be detected even when the subject is not paying attention. MMN can be measured without any task requirements and elicited even when the subject performs a task that is not related to the stimulus (Näätänen, 1995). The best condition to observe MMN has been suggested to be when the subject's attention is directed away from the stimulus (Näätänen, 1995). The fact that MMN can be detected in the absence of attention, makes it particularly suitable for testing different clinical populations, newborns and animals (Garrido et al., 2009). Since MMN is best observed in the absence of attention, it is believed to reflect an automatic orienting reflex based on memory and comparisons processes (Wienberg et al., 2009).

Although the MMN has been studied extensively, the neurophysiological mechanisms underlying the MMN are not well understood (Garrido et al., 2009). However, MMN is believed to represents a higher level of sensory information processing (Näätänen, 1995).

Many stimulus parameters influence the MMN. One parameter that influences the MMN is the length of the inter-stimulus interval (ISI). It has been shown that the MMN amplitude in

humans decreases with an increasing ISI (Pekkonen et al., 1993). Normally, an ISI between 300 and 1000msec. is used when MMN is measured in humans. (Umbricht et al., 2002; Umbricht et al., 2006; Sato et al., 2002)

Another parameter that influences MMN is the oddball probability. Several studies have shown that the MMN decreases with an increasing oddball probability (Sato et al., 2002; Umbricht et al., 2005). An increased occurrence of standard stimuli, is believed to reflect the strengthening of the memory trace of the standard stimuli, which is a determining factor of the MMN generation (Umbricht et al., 2005).

Brain structures involved in mismatch negativity

In 1979 Näätänen and Michie proposed two intracranial generators for the MMN, one temporal generator in auditory cortex and one in the frontal cortex (Näätänen and Michie, 1979; Näätänen, 1995). The temporal generator has been interpreted as reflecting direct activity in sensory memory and the automatic mismatch process (Sato et al., 2002). The role of the frontal cortex in the generation of MMN has been associated with the involuntary switching of attention to stimulus deviance (Sato et al., 2002). Furthermore, a slight time delay has been observed in the frontal activation relative to the auditory cortex activation, supporting the assumption that the change in auditory cortex triggers the frontal mechanisms of attention switch (Näätänen, 2000).

Pharmacology of mismatch negativity in healthy volunteers

Pharmacologically induced changes in the MMN have been investigated in numerous studies, using a variety of drugs affecting different neurotransmitter systems (for review see (Kenemans and Kahkonen, 2011))

Interestingly, several studies have found strong reductions of the MMN amplitude under the administration of the NMDA antagonist ketamine in healthy volunteers (Heekeren et al., 2008; Umbricht et al., 2000; Umbricht et al., 2002). Although some studies have failed to reproduce this (Oranje et al., 2000).

The effects of dopamine signaling have also been studied, but studies are inconsistent. Pekkonen et al. investigated the effect of haloperidol (a dopamine D_2 receptor antagonist) on MMN and showed that haloperidol shortened MMN latencies to frequency change, with no effect on MMN amplitudes or latencies to duration change (Pekkone et al., 2002). Moreover a study using dopamine D_2 receptor agonists (bromocriptine) and dopamine D_1/D_2 receptor agonist (pergolide) found no significant effect on MMN generation (Leung et al., 2007). Further, Leung et al. demonstrated in 2010 that tyrosine/phenylalanine depletion did not affect the MMN latencies or amplitude (Leung et al., 2010).

Data on serotonin modulation of MMN are also inconsistent. Kähkönen et al. used acute tryptophan depletion in healthy volunteers to reduce serotonin synthesis in the brain and found significantly increased depressed mood, increased MMN amplitude and a shortened latency (Kähkönen et al., 2005). EEG studies in healthy volunteers, using 5-HT2A receptor agonists psilocybin and dimithyltryptamine, found no evidence of MMN modulation (Umbricht et al., 2003b; Heekeren et al., 2008). However, Oranje et al. discovered in 2008 that a low oral dose of escitalopram (10mg) significantly increased the serotonergic activity and the MMN amplitude in healthy volunteers (Oranje et al., 2008). These findings were supported by Wienberg et al., in 2009 who demonstrated that a higher oral dose of escitalopram (15mg) also significantly increased the MMN response in healthy volunteers compared to placebo (Wienberg et al., 2009).

One proposed way to assess serotonergic activity after dosage of escitalopram is loudness dependence of auditory evoked potentials (LDAEP). LDAEP has been identified as a potential marker for central serotonergic activity and indicates the increase or decrease in amplitude of the N1/P2 component in response to an increase in auditory stimulus intensity (Wutzler et al., 2008; Hegerl et al., 2001). A weak LDAEP reflects a high serotonergic activity and a strong LDAEP reflects a low serotonergic activity (Wutzler et al., 2008; Hegerl et al., 2001). The advantages of this method, in estimation of serotonergic activity, are that LDAEP constitute a non-invasive procedure, which can be determined in an auditory paradigm in continuation of MMN oddball paradigms.

Mismatch negativity in schizophrenia

Schizophrenic patients show impaired MMN, as first reported by Shelly et al. in 1991. Subsequently, numerous studies have published results on MMN deficits in schizophrenia, both for frequency and duration oddballs (Umbricht and Krljes, 2005).

Reduced MMN has been reported to be relatively selective for schizophrenia over other neuropsychiatric disorders (Umbricht et al., 2003a) in that e.g. treatment resistant depression (TRD) show increased MMN (He et al., 2010). A study investigating MMN in different stages of the disease found that patients with recent onset and chronic schizophrenia have reduced MMN amplitude compared with healthy volunteers (Umbricht et al., 2006). The reduced MMN observed in schizophrenic patients indicate that these patients have deficient ability to distinguish changes in incoming auditory stimuli, which may have a devastating impact on their ability to interact with surroundings. To this end, MMN deficits have been reported to correlate with the occurrence and severity of negative symptoms (Catts et al., 1995; Javitt et al., 2008), cognitive dysfunction (Baldeweg et al., 2004) and even everyday functioning in schizophrenic patients (Light and Braff, 2005).

Interestingly, MMN deficits in chronically ill schizophrenic patients persist following treatment with atypical antipsychotics like clozapine (Umbricht et al., 1998) and risperidone (Umbricht et al., 1999). The insufficient efficacy of current antipsychotic drugs, against negative- and cognitive symptoms coupled with lack of effect on MMN deficits, suggest that drugs that improve MMN deficit may have beneficial effects in the management of schizophrenia.

Mismatch negativity like response in rodents

AEPs recorded in humans and rodents shows the same waveform, but the latencies in rodents AEP subcomponents are approximately 60% shorter than those observed in humans. The rodent correlate of human P50 is P20, human N100 is rodent N40 and human P200 correlates to the rodent P80 (Siegel et al., 2003), see Figure 5.

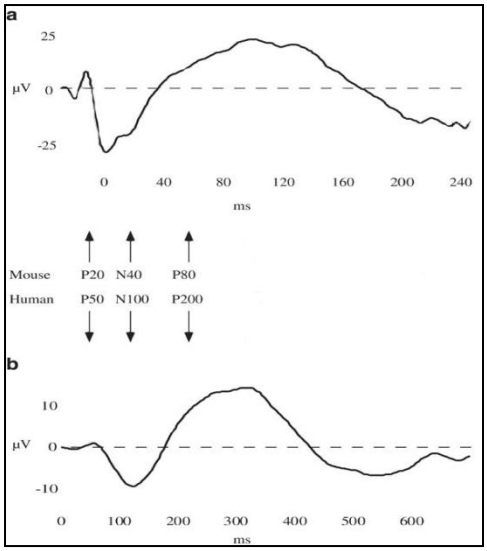

Figure 5. Auditory evoked potentials (AEPs) recorded in mouse (a) and human (b) elicited by the same stimuli. The AEP was elicited by a 3000 Hz sound stimulation with a length of 50msec. and an intensity of 75 dB. Modified from (Siegel et al., 2003).

MMN-like responses have also been studied in rodents (Umbricht et al., 2005; Ehrlichman et al., 2008; Farley et al., 2010). Although increased N40 response to an oddball tone has been reported by several authors, it is still debated if MMN-like response in rodents represent the neuronal construct of the MMN response observed in humans (Umbricht et al., 2005; Ehrlichman et al., 2008; Farley et al., 2010)

In line with human studies, pharmacologically induced changes of the MMN-like response in rodents have been investigated in several studies. Importantly the role of NMDA receptors in the generation of the MMN-like response has been studied in rodents. Ehrlichman et al.

reported that the NMDA antagonist ketamine significantly reduced MMN-like responses, which displays characteristics similar to those seen in human MMN (Ehrlichman et al., 2008). Tikhonravov et al. presented the same year that MK-801, a NMDA receptor antagonist, reduce the MMN-like response in rats (Tikhonravov et al., 2008). Further, it was shown that memantine, a low-affinity NMDA receptor antagonist, blocked the generation of the auditory MMN-like response in rats (Tikhonravov et al., 2010).

Taken together, MMN may represent a translational endophenotype, which measures pre-attentive neuronal processing deficits important for the functioning of patients with schizophrenia. Importantly, the current literature also suggests that this basic processing deficit is not normalized with current treatment. Thus, ultimately the use of MMN-like response in rodents may facilitate the development of potential novel drugs for the treatment of schizophrenia.

Aims of study

The aim of the present master thesis is to back-translate the interesting finding that increased serotonergic signaling mediated by escitalopram increases MMN amplitude in healthy volunteers. This will be performed by investigating the effect of increased serotonergic activity by dosing escitalopram alone or in combination with 5-HT3 antagonist ondansetron in an auditory oddball paradigm in rats. Secondarily, validate loudness dependence of auditory evoked potentials (LDAEP) as a marker of serotonergic activity in rats.

Methods

Animals

Male Wistar HanTac rats (Taconic MB A/S, Denmark) were used in all experiments. Rats were obtained, when the body weight were between 225-250g. The study was performed at Lundbeck A/S, Denmark and carried out in accordance with Danish legislation, granted by the animal welfare committee, appointed by the Danish Ministry of Justice.

Housing

Rats were group-housed (two per cage) before surgery under controlled conditions (12h of light starting at 06:00; temperature of 21±2°C; 55±5% humidity) in Macrolon (type III) cages with standard sawdust bedding and environmental enrichment (plastic house and wooden chew blocks). Food (Altromin 1323 pills, Brogaarden, Denmark) and tap water were available ad libitum. Twice a week rats were enriched with a rabbit mixture food (Chudley´s Rabbit Royal, Brogaarden, Denmark). Rats were allowed to acclimatize for minimum a week after arrival, before surgery took place.

Surgical procedures

Surgical procedures were performed on a test-batch (n=14), used for initial validation and later on a primary batch (n=24), which was used in the first and second escitalopram study.

Rats were treated with prophylactic antibiotic and peripheral acting analgesia prior to surgery by injection of 5 mg/kg Baytril vet® (SC, 50mg/ml enrofloxacin, Bayer, Germany) and 1.5mg/kg Rimadyl vet® (SC, 50mg/ml carprofen, Pfizer, USA) respectively. Rats were anesthetized with a mixture of, one part Hypnorm® (0.315mg/ml fentanyl and 10mg/ml fluanisone, Janssen-Cilag Inc., USA) and one part Dormicum® (5mg/ml midazolam, F. Hoffman-LaRoche AG, Switzerland) in two parts of sterilized isotonic water. After ensuring deep animal anesthesia level the hair was clipped from neck to nose, swabbed with iodine and the rat immobilized in a stereotaxic frame (Kopf, David Kopf Instruments, Germany). Body temperature was maintained at 37°C by means of an isothermal heating pad (CMA/150 temperature controller, CMA Microdialysis AB, Sweden). Before incision over the skull local analgesia, Marcain® (5mg/ml bupivacain, AstraZeneca A/S, Denmark) was injected under the skin. Then eye gel (Neutral Ophtha, Ophtha A/S, Denmark) was applied on the eyes. Rats were covered with a sterile operation cover, leaving only the incision area form neck to nose exposed. Surgical instruments were sterilized prior to use and placed on a separate sterile operation cover. The top of the skull and both lateral ridges were exposed by means of a

scalpel and the loom was released from the skull by a blunt spatula. Bregma was identified and holes were drilled in the skull according to coordinates found in the rat brain atlas (Paxinos and Watson, 1998)

Target area for the epidural stainless steel screw electrodes (Plastic One, Virginia, USA) were parietal cortex (-4.0mm posterior and +2.0mm lateral relative to bregma) and reference (+8.0mm anterior and -1.0mm lateral relative to bregma). In the test-batch a teflon-coated stainless steel depth electrode (0.125mm, Plastic One, Virginia, USA) was placed in either dorsal hippocampus (n=7) (-3.6mm posterior and -3.4mm lateral relative to bregma and -3.1mm deep relative to dura mater) or ventral hippocampus (n=7) (-5.2mm posterior and -5.0mm lateral relative to bregma and -4.8mm deep relative to dura mater). In the primary batch the depth electrode was placed in the ventral hippocampus (n=24) according to the following coordinates (-5.2mm posterior and -5.0mm lateral relative to bregma and -4.8mm deep relative to dura mater). Additionally, a ground electrode (0.125mm, Plastic One, Virginia, USA) was inserted in the subcutaneous area lateral to bregma. An anchor stainless steel screw (Plastic One, Virginia, USA) was placed in the skull to secure that the depth electrode stayed in place. The depth electrode was secured by means of dental cement (GC Fuji PLUS Capsule, GC Corporation, Japan), see appendix A and B.

Electrodes were connected to a 6-channel pedestal (Plastic One, Virginia, USA), which was fixated to the skull using a two component dental cement (Kemdent simplex rapid ™ powder mixed with liquid, Kemdent® Associated Dental Products Ltd., UK). The incision area was closed with a 5/0 resorbable suture (Vicryl, Ethicon', Belgium). For immediate pain relief rats were given 0.1mg/kg Temgesic® (SC, 0.3mg/ml buprenorphin, Schering-Plough, USA), see appendix A and B.

After surgery rats were housed individually in clean cages and water soaked food pellets were given. Rats were closely observed and treated once daily for five days post surgery with 5mg/kg Baytril vet® (SC, 50mg/ml enrofloxacin, Bayer, Germany) and 1.5mg/kg Rimadyl vet® (SC, 50mg/ml carprofen, Pfizer, USA). Rats were allowed a minimum of two weeks post surgical recovery. After recovery from surgery rats were habituated to auditory stimulation and the test environment.

EEG recordings

EEG was recorded in a test box between 8am - 6pm (light phase) consisting of a plastic chamber (L: 40cm, W: 40cm, H: 50cm) shielded with a copper net to avoid interference of external electrical installations (50 Hz noise), see appendix C. EEG was recorded by connecting a 6-channel cable attached to a 6 channel commutator (Plastic One, Virginia, USA) allowing the rat to move freely during the experiment.

The EEG signals were filtered (filter setting: low pass = 100Hz; high pass = 1.0Hz; notch filter was "on" to remove 50Hz noise) and amplified (gain = 5000) using a Brownlee Precision Model 440 amplifier. After filtration and amplification, the EEG signals were digitized with a Power 1401 (Cambridge Electronic Design, Cambridge, UK) and stored using Spike 2 version 6.09 software package (Cambridge Electronic Design, Cambridge, UK), see Figure 6. The raw EEG was recorded continuously through the test session, and stored on a computer hard disk along with time-locked digital stimulus tags.

Auditory stimuli were generated and controlled by Spike 2 software version 6.09 (Cambridge Electronic Design, Cambridge, UK) and digitized via Power 1401 (Cambridge Electronic Design, Cambridge, UK). The stimulus intensity was controlled by a custom made attenuator (Ellegaard Systems, Denmark) and an amplifier (Tony Lee DJ201, JCLEON International Electronic, China) and finally transmitted to the four loudspeakers in the test box (two placed in the ceiling and two in the rear wall of the box), see Figure 6.

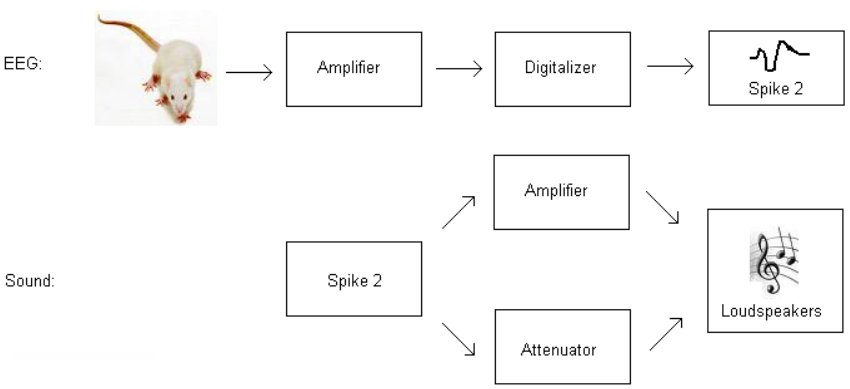

Figure 6. Schematic overview of the EEG recorded and the sound played. The EEG was recorded from the rat brain, subsequently it was amplified and digitalized and then stored in Spike 2. The sound waveforms were generated in Spike 2, then amplified and attenuated simultaneous and finally played through the loudspeakers.

Data analysis

Data analysis of raw EEGs was performed off-line in Spike 2 version 6.09 (Cambridge Electronic Design, Cambridge, UK). Following manual and visual artifact rejection of large amplitude artifacts (>2µV), e.g. due to movement, epochs were averaged off-line for each animal separately. Average auditory evoked potential (AEP) waveforms were generated for the electrodes in hippocampus and parietal cortex (settings for average: width = 0.5sec; offset = 0.1sec).

Following parameters were assessed during the different studies:

- P1/N1 amplitude, the distance from P1 peak to N1 peak, see Figure 7 A
- N1/P2 amplitude, the distance from N1 peak to P2 peak, see Figure 7 A
- Baseline/N1 amplitude, the distance from baseline to N1 peak, see Figure 7 B
- Baseline/P2 amplitude, the distance from baseline to P2 peak, see Figure 7 B

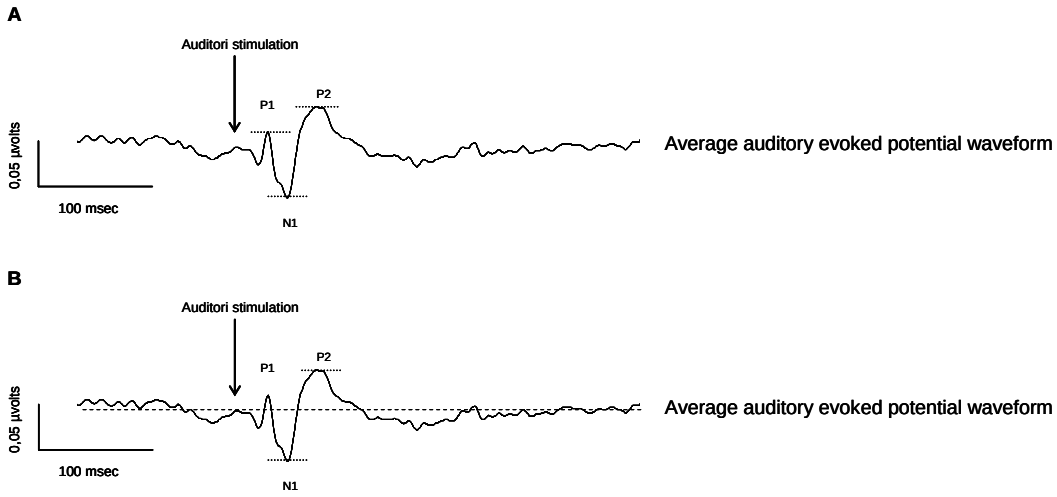

Figure 7. Average auditory evoked potential (AEP) waveform. Illustrating the localization of P1, N1 and P2 and how the different parameters were measured in this study. (A) Determination of P1/N1 and N1/P2, from peak to peak; (B) Determination of Baseline/N1 and Baseline/P2, from baseline to peak.

It should be noted that not all results and parameters found during the studies are presented in the following paragraphs. For a full overview of all the result see appendix E and F or attached disc.

Validation of stimulus parameters

Initial validation was carried out in order to determine the best settings for the primary escitalopram studies.

Validation of the oddball paradigm, which was used in the generation of MMN-like responses, has previously been carried out in rats at Lundbeck A/S (Hansen, 2010). The previous validation included; validation of tone duration, tone intensity, oddball probability, inter-stimulus interval (ISI) and a validation of the P1/N1 amplitude of all individual single tones (6000Hz, 7000Hz, 8000Hz) (Hansen, 2010).

The present study is based on this previous validation set. However, at the initiation of the present study uncertainties were raised in relation to the previously used ISI (700msec) and consequently further validation was needed. Furthermore, a validation of the loudness dependence of auditory evoked potentials (LDAEPs) was necessary, in order to validate the use of this parameter as a marker for central serotonergic neurotransmission in the escitalopram studies.

Validation of the inter-stimulus interval (ISI)

Validation of the ISI was carried out on the test-batch (n= 14) using auditory oddball paradigms with different ISI. The auditory oddball paradigm used to generate MMN-like responses was composed of standard tones with a frequency of 7000Hz and a probability of 94%, and deviant tones (oddballs) with a frequency of either 6000Hz or 8000Hz and a total probability of 6%. The ISI was (A) 300msec (randomized between 100 and 500msec) (B) 500msec (randomized between 300 and 700msec) and (C) 700msec (randomized between 500 and 900msec). All stimuli had an intensity of 80dB and a duration of 20msec (3msec rise and fall), see Figure 8

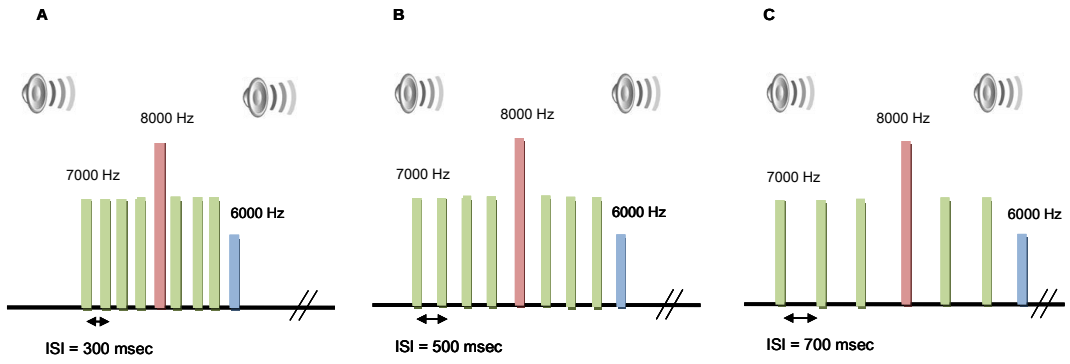

Figure 8. Auditory oddball paradigms with different inter-stimulus intervals (ISI). Standard tones =7000Hz (green bars). Frequency oddballs = 6000 and 8000Hz (blue and red bars respectively). Randomized oddballs with a probability of 6%. Duration of tones = 20msec (3msec rise and fall). Intensity of tones = 80dB. Inter-stimulus interval (A) 300msec (randomized between 100 and 500msec) (B) 500msec (randomized between 300 and 700msec) (C) 700msec (randomized between 500 and 900msec). Length of each paradigm = 30min

Rats with P1/N1 AEP amplitude smaller than the amplitude of noise at baseline were excluded from the results.

At first sight, electrode location in the dorsal hippocampus and an ISI of 300msec seemed to generate the best MMN-like response, see Figure 9 C. However, large variation occurred between the rats with electrode placement in the dorsal hippocampus, when presented to auditory stimuli with an ISI of 300msec. Moreover, over half of the rats tested were executed due to low signal to noise ratio (AEP amplitude < noise). In contrast, all rats with electrode placement in the ventral hippocampus, had good signal to noise ratio and the optimal ISI was judged to be 500msec, based on an acceptable mean MMN-like response, see Figure 9.

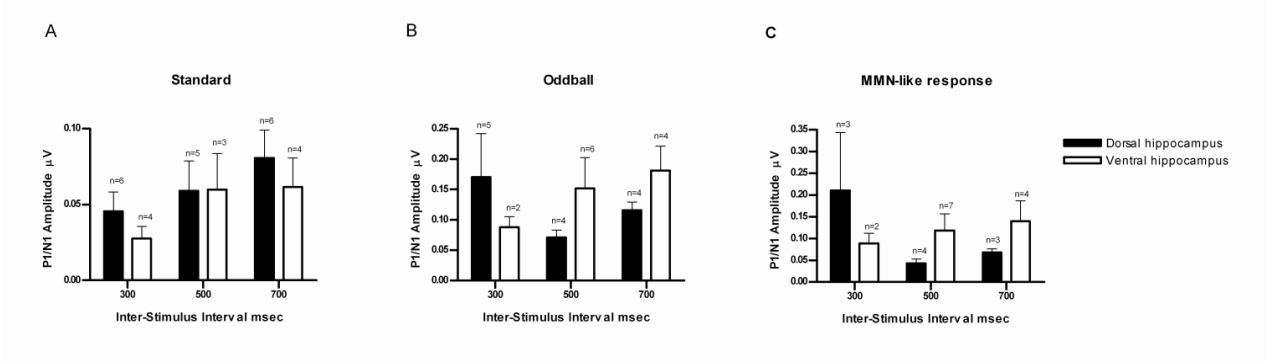

Figure 9. P1/N1 amplitudes of auditory evoked potentials in dorsal and ventral hippocampus, generated in response to different inter-stimulus intervals (ISI). (A) P1/N1 estimated in the standard AEP waveform; (B) P1/N1 estimated in the oddball AEP waveform and (C) P1/N1 in the difference AEP waveform (corresponding to the MMN-like response). Data are expressed as mean ± SEM.

To further investigate the relation between ISI and P1/N1 amplitude 7000Hz tones (tone intensity of 80dB and a tone duration of 20msec (3msec rise and fall)) were presented with different ISIs (250, 500, 750, 1000, 1500, 2000, 3000, 4000, 6000msec), see Figure 10

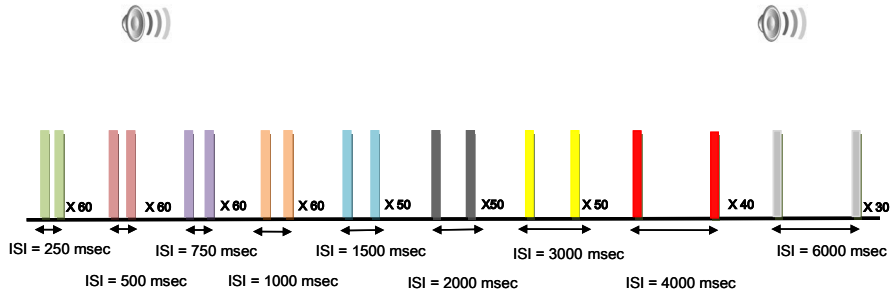

Figure 10. Auditory paradigm, varying inter-stimulus interval (ISI). Frequency of tones = 7000Hz. Duration of tones = 20msec (3msec rise and fall). Intensity of tones = 80dB. Inter-stimulus interval = 250msec (60 repeats), 500msec (60 repeats), 750msec (60 repeats), 1000msec (60 repeats), 1500msec (50 repeats), 2000msec (50 repeats), 3000msec (50 repeats), 4000msec (40 repeats) and 6000msec (30 repeats). Length of paradigm ~ 15min

The results demonstrated that the P1/N1 amplitude increased with increasing ISI and reached a plateau at an ISI of 3000msec, see Figure 11.

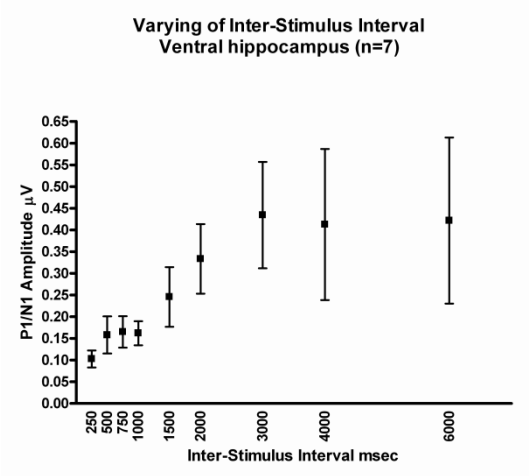

Figure 11. The effect of increasing inter-stimulus interval (ISI) on the P1/N1 amplitude in ventral hippocampus. Data are expressed as mean ± SEM.

In summary, the validation studies of the ISI, demonstrated that an ISI of 500msec was optimal in the generation of MMN-like responses in rats. Furthermore the optimal depth electrode location in relation to the MMN-like response was estimated to be in the ventral hippocampus. These settings were used in the following studies.

Validation of the loudness dependence of auditory evoked potentials (LDAEP)

Validation of the LDAEP was carried out on rats from the test-batch with depth electrode placement in the ventral hippocampus (n= 7). The auditory paradigm used for validation was composed of 7000Hz tones (tone duration of 20msec (3msec rise and fall)) presented with different tone intensities (60, 70, 80, 90 and 100dB) and with an ISI randomized between 1800 and 2200msec (mean 2000msec), see Figure 12.

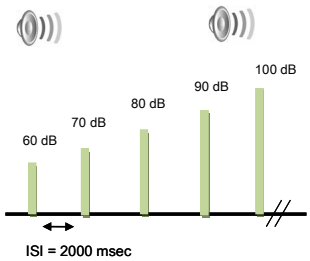

Figure 12. Auditory paradigm, loudness dependence of auditory evoked potentials (LDAEP). Frequency of tones = 7000Hz. Duration of tones = 20msec (3msec rise and fall). Intensity of tones = 60, 70, 80, 90, 100dB. Inter-stimulus interval 2000msec (randomized between 1800 and 2200msec). Length of paradigm = 15min

The LDAEP measure indicates changes in the auditory evoked N1/P2 component in response to an increase in stimulus intensity. The LDAEP was measured as the slope of the linear correlation between N1/P2 amplitude and tone intensity, see Figure 13.

The results showed a positive linear correlation between N1/P2 amplitude and tone intensity in the ventral hippocampus. However, variations in the data occurred; illustrated as a low squared correlation coefficient (R^2) of 0.5496, see Figure 13 B. A R^2 of 1.0 would indicate that the regression line perfectly fits data. The variation in data and the low squared correlation coefficient may be related to the small sample size in this study.

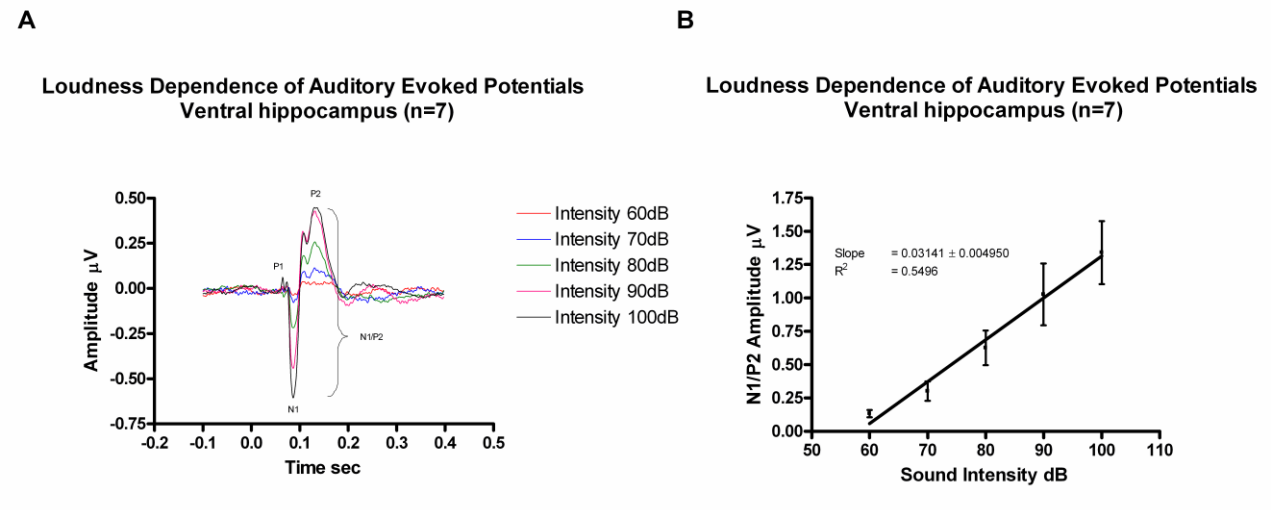

Figure 13. Estimation of loudness dependence of auditory evoked potentials (LDAEP) in hippocampus. (A) Illustrates the average auditory evoked potentials (AEP) in response to increasing intensity and how N1/P2 amplitudes were estimated. (B) Illustrates the correlation between N1/P2 amplitude and tone intensity. LDAEP was estimated as the slope of this linear correlation. Data are expressed as mean ± SEM.

In summary, the validation of the LDAEP demonstrates that LDAEP can be reliably measured in the hippocampus of rats.

Taken together, initial validation studies, demonstrated that the optimal depth electrode location in relation to the MMN-like response was in the ventral hippocampus. Further, the optimal oddball paradigm settings were determined to be 7000Hz standard tones with a probability of 94%, a 50:50 mix of 6000Hz and 8000Hz deviant tones (oddballs) with a probability of 6%. All tones were presented with a tone intensity of 80dB, a duration of 20msec (3msec rise and fall) and an inter-stimulus interval (ISI) of 500msec (randomized between 300msec and 700msec).

First escitalopram study

The first escitalopram study was based on results from the previous validation (see section: Validation of stimulus parameters) and executed on a new batch of rats (primary batch, n=24).

On test days, rats were allowed to habituate to the test environment for 45 minutes with auditory stimulation. The habituation session was followed by a LDAEP baseline session (10min) and a baseline oddball paradigm session (30min). Hereafter rats were injected subcutaneous (injection volume 5mg/kg) with escitalopram in different dosage (0.25mg/kg, 0.5mg/kg or 1.0mg/kg) or vehicle (0.9% isotonic saline), see appendix D. Twenty minutes after dosing, LDAEP was recorded for 10 minutes, followed by a 30 minutes oddball paradigm, see Figure 14. This study design was based on previous studies measuring the effect of escitalopram on serotonin in the hippocampus by microdialysis (Mørk et al., 2003).

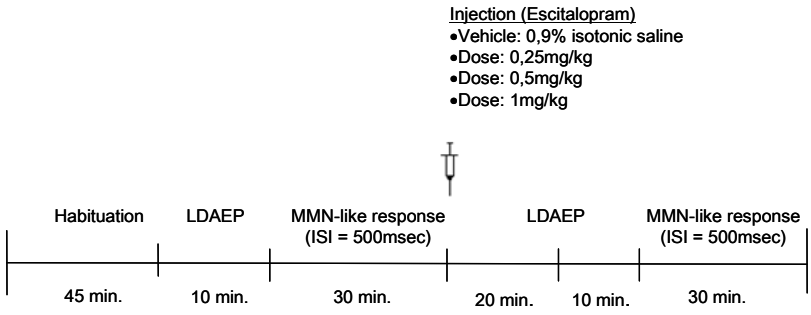

Figure 14. Overview of the test session. LDAEP = Loudness dependence of auditory evoked potentials. MMN-like response = Mismatch negativity-like response. ISI=Inter-stimulus interval

The auditory paradigm used to generate LDAEPs was determined in validation studies (see section: Validation of the loudness dependence of auditory evoked potentials (LDAEP)). The auditory paradigm was composed of 7000Hz tones with a tone duration of 20msec (3msec rise and fall) and an ISI randomized between 1800 and 2200msec (mean 2000msec). The tones were presented with varying intensity (60, 70, 80, 90 and 100dB) in a 10 minute long paradigm, see Figure 15.

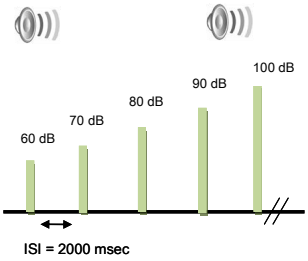

Figure 15. Auditory paradigm, loudness dependence of auditory evoked potentials (LDAEP). Frequency of tones = 7000Hz. Duration of tones = 20msec (3msec rise and fall). Intensity of tones = 60, 70, 80, 90, 100dB. Inter-stimulus interval of 2000msec (randomized between 1800 and 2200msec). Length of paradigm = 10min

The results demonstrated a positive linear correlation between N1/P2 amplitude and tone intensity. Figure 16 illustrates baseline LDAEP independent of treatment group. An almost perfect linear correlation was obtained as R^2 equals 0.9534, see Figure 16 B.

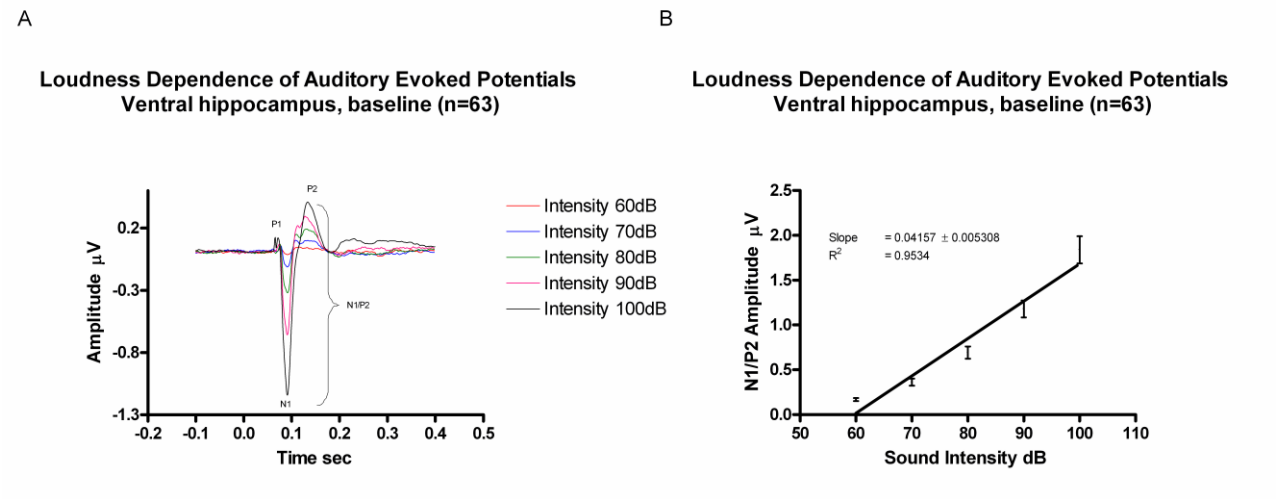

Figure 16. Estimation of loudness dependence of auditory evoked potentials (LDAEP) in hippocampus. (A) Illustrates baseline average auditory evoked potentials (AEP) in response to increasing intensity and how N1/P2 amplitudes were estimated. (B) Illustrates the correlation between N1/P2 amplitude and tone intensity. Baseline LDAEP was estimated as the slope of this linear correlation. Data are expressed as mean ± SEM.

A strong LDAEP has been reported to reflect low serotonergic activity and weak LDAEP a high serotonergic activity, when recorded in the auditory cortex. It was hypothesized that escitalopram would weaken the LDAEP, due to increased serotonergic activity. However, the results of the present study did not demonstrate a consistent reduction in LDAEP after dosing escitalopram. Statistical analysis, two-way ANOVA, revealed that the difference in the absolute LDAEP among the different levels of treatment were significantly greater than would be expected by chance after allowing for effects of differences in time (baseline/after dose) [F(3, 125) = 3.806, p=0.012]. Isolation of treatment groups and multiple comparison testing (bonferroni t-test, all pairwise multiple comparison procedures) revealed a statistically significant difference between 0.25mg/kg escitalopram and 0.5mg/kg escitalopram (t=3.044, p=0.017) as well as between 0.25mg/kg escitalopram and 1.0mg/kg escitalopram (t=2.719, p=0.045), see Figure 17 A. No significant differences were obtained in relative changes (from baseline measure) of the LDAEP between treatment groups, see Figure 17 B.

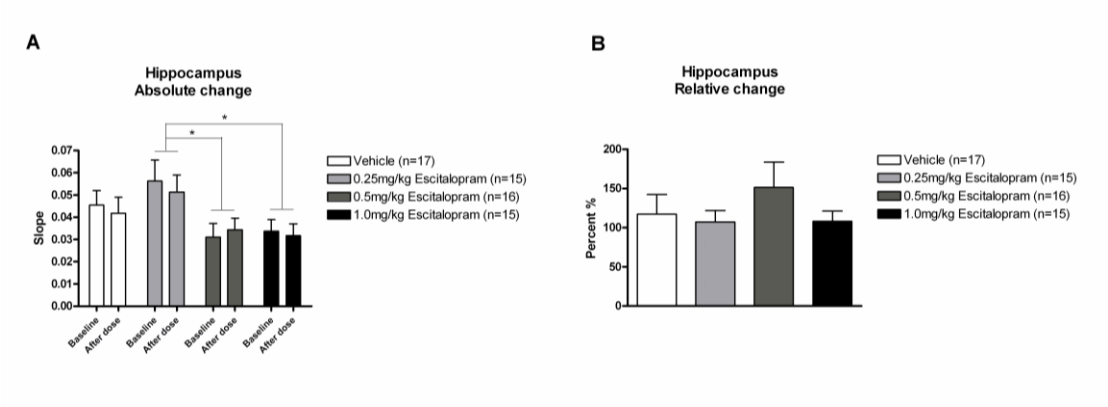

Figure 17. Mean absolute- and relative changes in the loudness dependence of auditory evoked potentials (LDAEP) in hippocampus. (A) Absolute changes in the LDAEP in hippocampus, measured as the slope of the linear correlation between N1/P2 amplitude and tone intensity; (B) Relative changes in the LDAEP in hippocampus. Data are expressed as mean ± SEM. *p<0.05 (Bonferroni t-test, all pairwise multiple comparison procedures).

The auditory oddball paradigm used to generate MMN-like responses was determined in initial validation studies (see section: Validation of stimulus parameters) and composed of standard tones presented with a frequency of 7000Hz and a probability of 94%, whereas deviant tones (oddballs) were presented with a frequency of either 6000Hz or 8000Hz and a total probability of 6%. All stimuli had a tone intensity of 80dB, a duration of 20msec (3msec rise and fall) and an inter-stimulus interval (ISI) randomized between 300msec and 700msec (mean 500msec), see Figure 18

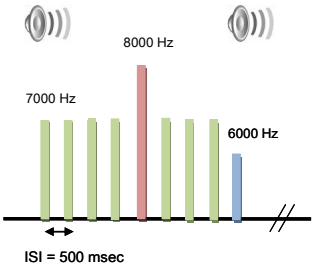

Figure 18. Auditory oddball paradigm. Standard tones =7000Hz (green bars). Frequency oddballs = 6000 and 8000Hz (blue and red bars respectively). Randomized oddballs with a probability of 6%. Duration of tones = 20msec (3msec rise and fall). Intensity of tones = 80dB. Inter-stimulus interval of 500msec (randomized between 300 and 700msec). Length of paradigm = 30min

In the analysis, rats with no observed MMN-like response at baseline recording and outliers (average ± 2xSD) were excluded from the statistical analysis. Statistical analysis, one-way ANOVA, showed a significant difference in the relative change of the MMN-like response (measured as the P1/N1 component in the difference waveform) in the hippocampus between treatment groups [$F(3, 46) = 5.255$, $p<0.004$]. Bonferroni t-test, all pairwise multiple comparison procedures demonstrated that a dose of 1.0mg/kg escitalopram significantly increased the relative change in the MMN-like response in hippocampus compared to vehicle

(t=3.588, *p*=0.005) and 0.5mg/kg escitalopram (t=3.318, *p*=0.011), see Figure 19 B. However no significant differences were found in absolute differences, see Figure 19 A.

When measuring baseline/N1, only a trend of escitalopram dose-response effect was observed, but this effect did not reach statistical significance in neither absolute- nor relative changes of the MMN-like response between treatment groups, see Figure 19 C and D.

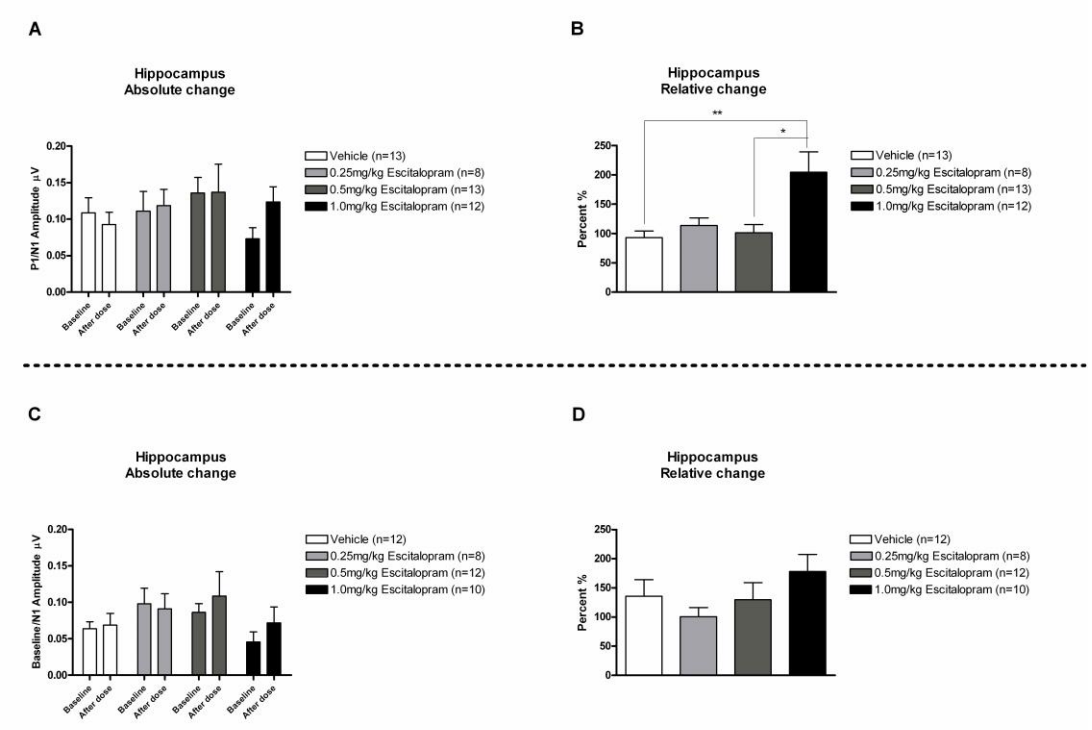

Figure 19. Mean absolute- and relative changes in the mismatch negativity (MMN)-like response in hippocampus. (A) Absolute changes in the MMN-like response in hippocampus, measured as P1/N1 in the difference waveform; (B) Relative changes in the MMN-like response in hippocampus, measured as P1/N1 in the difference waveform; (C) Absolute changes in the MMN-like response in hippocampus, measured as baseline/N1 in the difference waveform; (D) Relative changes in the MMN-like response in hippocampus, measured as baseline/N1 in the difference waveform. Data are expressed as mean ± SEM. *p<0.05; **p<0.01 (Bonferroni t-test, all pairwise multiple comparison procedures)

In summary, the first escitalopram study demonstrated a significant increase in the MMN-like response, measured as P1/N1, after administration of 1.0mg/kg escitalopram. Furthermore, the results indicated that the baseline/N1 measure may be a less sensitive readout in rats. Moreover, escitalopram did not produce consistent changes in LDAEP when measured in hippocampus.

Due to speculations as to whether the rats were sleeping during the experiments, the circadian rhythm of the rats was changed prior to the second escitalopram study to allow for testing during the dark phase. Thus, the second escitalopram study was performed during the dark phase, to minimize variation due to changes in vigilance state. The second escitalopram study aimed to further investigate the effect of increased serotonergic activity by dosing escitalopram alone or in combination with 5-HT3 antagonist ondansetron in an auditory oddball paradigm in rats, see following article.

The Effect of Escitalopram on the Mismatch Negativity-like Response in Rats

M.R. Birknow[1,2], J.F. Bastlund[1]

[1]*Department of Synaptic Transmission, H. Lundbeck A/S, Denmark;*

[2]*Faculty of Medicine, Department of Health Science and Technology, Aalborg University, Denmark*

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ABSTRACT

Mismatch negativity (MMN) is an event-related potential that measures pre-attentive novelty detection processes in the auditory domain. MMN deficits is a neurophysiological endophenotype of schizophrenia and deficits have been reported to correlate with the severity of negative symptoms. Deficits in MMN generation persist following treatment with both typical and atypical antipsychotics. Interestingly, the selective serotonin reuptake inhibitor (SSRI) escitalopram, which is frequently used in schizophrenia to treat depressive and negative symptoms, significantly increases the MMN generation in healthy volunteers. The main objective of the present study was to investigate the effect of escitalopram on the MMN-like response in rats. Further the effect of ondansetron (5-HT_3 receptor antagonist) on MMN was investigated as it was hypothesized that ondansetron would boost escitaloprams enhancing effect on MMN.

Auditory evoked potentials (AEPs) were recorded from parietal cortex and the hippocampus of freely moving rats. Auditory oddball paradigms using frequency oddballs were utilized to elicit MMN-like responses. Rats were treated with vehicle, 1mg/kg escitalopram, 3mg/kg escitalopram or 0.5mg/kg ondansetron alone or in combination with 1mg/kg escitalopram. AEPs and MMN-like responses were recorded before and after treatment for each rat.

The results showed that 3mg/kg escitalopram significantly increased the MMN-like response in rodent hippocampus. However, no synergistic effect on MMN was obtained when dosing escitalopram in combination with ondansetron.

INTRODUCTION

Schizophrenia is a serious mental condition affecting 0.7% of the world's population (McGrath et al., 2008). Schizophrenia leads to great disability and distress and is characterized by the presence of positive and negative symptoms, as well as cognitive deficits, which all affects the global functioning of the schizophrenic patient (Tandon et al., 2009; Karam et al., 2010; Javitt et al., 2008). The heterogeneity of the symptoms in

schizophrenia and the complex pathophysiology hampers development of valid animal models and assays that address all the symptom clusters seen in schizophrenia (Tandon et al., 2008).

Recent translational research has focused on the advantages of using translational neurophysiological endophenotypes in relation to schizophrenia (Thaker, 2007). The advantages of using neurophysiological endophenotypes are that they represent underlying neuronal processing deficits of the disease. Endophenotypes are primarily state-independent and are objective measurements more closely linked to neurobiological underpinnings of disease processes rather than symptomatology (Gottesman and Gould, 2003).

One such neurophysiologic endophenotype is mismatch negativity (MMN). MMN reflects a pre-attentive process and represent the ability to detect changes in sensory information e.g. the auditory domain (Shelley et al., 1991; Näätänen, 1995). Auditory MMN is elicited when frequent (standard) tones occasionally are replaced by infrequent deviant (oddball) tones. The difference in the N100 component of the auditory evoked potential (AEP) generated by subtraction of the standard AEP from the oddball AEP represents the MMN (Näätänen, 1995).

A number of studies in humans have demonstrated that patients with schizophrenia have a significant reduction of MMN amplitude (Umbricht and Krljes, 2005; Catts et al., 1995; Sato et al., 2003; Shelley et al., 1991; Javitt et al., 1993). Reduced MMN has been reported to be relatively selective for schizophrenia over other neuropsychiatric disorders (Umbricht et al., 2003a), in that e.g. treatment resistant depression (TRD) show increased MMN (He et al., 2010). The reduced MMN observed in schizophrenic patients indicates that these patients have deficient ability to distinguish changes in incoming auditory stimuli, which may have a devastating impact on their ability to interact with surroundings. To this end, MMN deficits have been reported to correlate with negative symptoms (Catts et al., 1995; Javitt et al., 2008), cognitive dysfunction (Baldeweg et al., 2004) and even everyday functioning in schizophrenic patients (Light and Braff, 2005).

Interestingly, MMN deficits in chronically ill schizophrenic patients persist following treatment with atypical antipsychotics like clozapine and risperidone (Umbricht et al., 1998; Umbricht et al., 1999). The insufficient efficacy of current antipsychotic drugs, against negative- and cognitive symptoms coupled with lack of effect on MMN deficits, suggest that drugs that improve MMN deficits may have beneficial effects in the management of schizophrenia.

Clinically, serotonin reuptake inhibitors (SSRIs) are frequently combined with antipsychotic medication in schizophrenic patients (Oranje et al., 2008; Rummel et al., 2005). Several studies indicate that antipsychotic drugs in combination with SSRIs may be more efficacious in treating negative symptoms of schizophrenia than antipsychotic drugs alone (Jockers-Scherubl et al., 2005; Silver and Nassar, 1992; Spina et al., 1994). However, no convincing

neurochemical theory does exist for this combination. Interestingly, the effect of the SSRI escitalopram has been demonstrated to increase the amplitude of MMN in healthy volunteers (Oranje et al., 2008; Wienberg et al., 2009).

MMN-like responses have also been studied in rodents (Umbricht et al., 2005; Ehrlichman et al., 2008; Farley et al., 2010). AEPs recorded in humans and rodents show similar waveform, but the latencies in rodents AEP subcomponents are approximately 60% shorter than those observed in humans, so that the rodent correlate of human N100 is N40 (negative component occurring 40msec after stimuli, termed N1 in this study) (Siegel et al., 2003). Although, increased N40 response to oddball tone has been reported by several authors, it is still debated if MMN-like response in rodents represents the MMN response observed in humans (Umbricht et al., 2005; Farley et al., 2010; Ehrlichman et al., 2008).

The aim of the present study is to back-translate the interesting finding that increased serotonergic signaling produced by escitalopram increases MMN amplitude in healthy volunteers. This will be performed by investigating the effect of increased serotonergic activity by dosing escitalopram alone or in combination with 5-HT3 antagonist ondansetron in an auditory odd ball paradigm in rats.

METHODS

Animals

Male Wistar HanTac rats (Taconic MB A/S, Denmark) were used in all experiments. Rats were obtained when the body weight were between 225-250g. The study was performed at Lundbeck A/S, Denmark and carried out in accordance with Danish legislation, granted by the animal welfare committee, appointed by the Danish Ministry of Justice.

Housing

Rats were group-housed (two per cage) before surgery under controlled conditions (12h of light starting at 06:00; temperature of 21±2°C; 55±5% humidity) in Macrolon (type III) cages with standard sawdust bedding and environmental enrichment (plastic house and wooden chew blocks). Food (Altromin 1323 pills, Brogaarden, Denmark) and tap water were available ad libitum. Twice a week rats were enriched with a rabbit mixture food (Chudley´s Rabbit Royal, Brogaarden, Denmark). Rats were allowed to acclimatize for minimum a week after arrival, before surgery took place.

Surgical procedures

Rats were treated with prophylactic antibiotic and peripheral acting analgesia prior to surgery by injection of 5 mg/kg Baytril vet® (SC, 50mg/ml enrofloxacin, Bayer, Germany) and 1.5mg/kg Rimadyl vet® (SC, 50mg/ml carprofen, Pfizer, USA) respectively. Rats were

anesthetized with a mixture of, one part Hypnorm® (0.315mg/ml fentanyl and 10mg/ml fluanisone, Janssen-Cilag Inc., USA) and one part Dormicum® (5mg/ml midazolam, F. Hoffman-LaRoche AG, Switzerland) in two parts of sterilized isotonic water. After ensuring deep animal anesthesia level the hair was clipped from neck to nose, swabbed with iodine and the rat immobilized in a stereotaxic frame (Kopf, David Kopf Instruments, Germany). Body temperature was maintained at 37°C by means of an isothermal heating pad (CMA/150 temperature controller, CMA Microdialysis AB, Sweden). Before incision over the skull local analgesia, Marcain® (5mg/ml bupivacain, AstraZeneca A/S, Denmark) was injected under the skin. Then eye gel (Neutral Ophtha, Ophtha A/S, Denmark) was applied on the eyes. Rats were covered with a sterile operation cover, leaving only the incision area from neck to nose exposed. Surgical instruments were sterilized prior to use and placed on a separate sterile operation cover. The top of the skull and both lateral ridges were exposed by means of a scalpel and the loom was released from the skull by a blunt spatula. Bregma was identified and holes were drilled in the skull according to coordinates found in the rat brain atlas (Paxinos and Watson, 1998).

Target area for the epidural stainless steel screw electrodes (Plastic One, Virginia, USA) were parietal cortex (-4.0mm posterior and +2.0mm lateral relative to bregma) and reference (+8.0mm anterior and -1.0mm lateral relative to bregma). A teflon-coated stainless steel depth electrode (0.125mm, Plastic One, Virginia, USA) was placed in the hippocampus according to the following coordinates (-5.2mm posterior and -5.0mm lateral relative to bregma and -4.8mm deep relative to dura mater). Additionally a ground electrode (0.125mm, Plastic One, Virginia, USA) was inserted in the subcutaneous area lateral to bregma. An anchor stainless steel screw (Plastic One, Virginia, USA) was placed in the skull to secure that the depth electrode stayed in place. The depth electrode was secured by means of dental cement (GC Fuji PLUS Capsule, GC Corporation, Japan).

Electrodes were connected to a 6-channel pedestal (Plastic One, Virginia, USA), which was fixated to the skull using a two component dental cement (Kemdent simplex rapid ™ powder mixed with liquid, Kemdent® Associated Dental Products Ltd., UK). The incision area was closed with a 5/0 resorbable suture (Vicryl, Ethicon', Belgium). For immediate pain relief rats were given 0.1mg/kg Temgesic® (SC, 0.3mg/ml buprenorphin, Schering-Plough, USA).

After surgery rats were housed individually in clean cages and water soaked food pellets were given. Rats were closely observed and treated once daily for five days post surgery with 5mg/kg Baytril vet® (SC, 50mg/ml enrofloxacin, Bayer, Germany) and 1.5mg/kg Rimadyl vet® (SC, 50mg/ml carprofen, Pfizer, USA). Rats were allowed a minimum of two weeks post surgerical recovery. Subsequently, rats were habituated to auditory stimulation and the test environment, and the circadian rhythm of the rats was reversed (12h of light starting at 18:00) to allow electrophysiological experiment to be performed during the dark phase in awake rats.

EEG recordings

EEG was recorded by connecting a 6-channel cable attached to a 6 channel commutator (Plastic One, Virginia, USA) allowing the rat to move freely during the experiment.

The EEG signals were filtered (filter setting: low pass = 100Hz; high pass = 1.0Hz; notch filter was "on" to remove 50Hz noise) and amplified (gain = 5000) using a Brownlee Precision Model 440 amplifier. After filtration and amplification, the EEG signals were digitized with a Power 1401 (Cambridge Electronic Design, Cambridge, UK) and stored using Spike 2 version 6.09 software package (Cambridge Electronic Design, Cambridge, UK). The raw EEG was recorded continuously through the test session, and stored on a computer hard disk along with time-locked digital stimulus tags.

Auditory stimuli were generated and controlled by Spike 2 software version 6.09 (Cambridge Electronic Design, Cambridge, UK) and digitized via Power 1401 (Cambridge Electronic Design, Cambridge, UK). The stimulus intensity was controlled by a custom made attenuator (Ellegaard Systems, Denmark) and an amplifier (Tony Lee DJ201, JCLEON International Electronic, China) and finally to the four loudspeakers in the test box (two placed in the ceiling and two in the rear wall of the box).

The study was designed as a pseudo latin square design, with animals receiving randomly assigned treatments. A "wash-out" period of one week was applied between the different treatments to limit "carry-over" effects.

On test days, animals were allowed to habituate in the test environment for 45 minutes with auditory stimulation. The MMN-like response was determined 0-30 minutes prior to and 30-60 minutes after drug administration. Following auditory oddball paradigm was used to generate MMN-like responses: Standard tones were presented with a frequency of 7000Hz and a probability of 94%, whereas deviant tones (oddballs) were presented with a frequency of either 6000Hz or 8000Hz and a total probability of 6%. All stimuli had an intensity of 80dB, a duration of 20msec. (3msec. rise and fall) and an inter-stimulus interval (ISI) randomized between 300msec and 700msec. All 3 tones were previously validated so they produced identical AEP amplitude (data not shown).

Drugs

The compounds used in the study were: Escitalopram oxalate, a SSRI (H. Lundbeck A/S, Denmark) and ondansetron, a 5-HT3 antagonist (Bosche Scientific LLC, New Jersey, USA). Both compounds were dissolved in 0.9% isotonic saline and had a pH >4 and <8. All compounds were injected subcutaneously, as dose free base, with an injection volume of 5ml/kg.

Data analysis

Data analysis of raw EEGs was performed off-line in Spike 2 version 6.09 (Cambridge Electronic Design, Cambridge, UK). Following manual and visual artifact rejection of large amplitude artifacts (>2µV), e.g. due to movement, epochs were averaged off-line for each animal separately. Average auditory evoked potential (AEP) waveforms were generated for the electrodes in hippocampus and parietal cortex (settings for average: width = 0.5sec; offset = 0.1sec) both for the standard and deviant tones. Subsequently, the difference wave was generated by subtracting the standard AEP from the deviant AEP. The individual average AEP waveforms in hippocampus were identified as the maximum amplitudes in the following time windows: P1: between 5 and 40msec; N1: between 20 and 60msec; P2: between 50 and 100msec. Whereas the individual average AEP waveforms for parietal cortex were identified as the maximum amplitudes in the following time windows: P1: between 10 and 45msec; N1: between 30 and 70msec; P2 between 50 and 100msec.

The N1 amplitude was assessed in standard, deviant and difference AEPs by measuring the distance from P1 to N1 in the individual average AEP waveforms. The MMN-like response was determined as the N1 amplitude in the difference waveform.

Statistics

All statistical calculations were carried out using the software package SigmaStat™ for Windows™ (Jandel, San Rafael, CA, USA).

Rats with no observed MMN-like response at baseline recording and outliers (average ± 2xSD) were excluded from the statistical analysis.

Two-way ANOVA wase applied on logarithmic transformed data to determine differences in absolute N1 amplitudes in relation to treatment before and after dose (data: absolute N1 amplitudes; factors: treatment and time). Logarithmic transformation was used to secure normal distribution and equal variance in the data set. If statistical significant differences occurred Bonferroni t-test post-hoc analysis with all Pairwise Multiple Comparison Procedures was performed.

The absolute baseline N1 amplitude was normalized to 100% and the relative changes from baseline to after compound administration was calculated. One-way ANOVA was applied on logarithmic transformed data to determine differences in relative changes for N1 amplitudes in relation to treatment (data: relative change in N1 amplitudes; factor: treatment). Logarithmic transformation was used to secure normal distribution and equal variance in the data set. If statistical significant differences occurred Bonferroni t-test post-hoc analysis with all Pairwise Multiple Comparison Procedures was performed.

To determine differences in absolute baseline latencies in hippocampus versus parietal cortex independent t-tests were performed on logarithmic transformed data. If no normal distribution or equal variance were obtained Mann-Whitney Rank Sum Test were performed instead.

All results are expressed as mean values ± SEM. A probability value of less than 5% ($p<0.05$) was pre-set to indicate a statistically significant difference.

RESULTS

The effects of increased serotonergic activity on the MMN-like response in hippocampus and parietal cortex were investigated.

Results demonstrated a significant change in the relative change in the MMN-like response between treatment groups in the hippocampus [$F(4, 48) = 4.908$, $p<0.002$]. A dose of 3mg/kg escitalopram significantly increased the relative change in the MMN-like response in hippocampus compared to vehicle ($t=4.013$, $p=0.002$) and 0.5mg/kg ondansetron ($t=2.963$, $p=0.049$), see Figure 20 (A). Further, a dose of 0.5mg/kg ondansetron in combination with 1mg/kg escitalopram was found to be significantly different from the vehicle ($t=3.018$, $p=0.042$), but not significantly different from 1mg/kg escitalopram dosed alone, see Figure 20(A)

The same tendency, in relation to the effect of 3mg/kg escitalopram on the MMN-like response, was observed in parietal cortex, but statistical significance was not reached, see Figure 20(B).

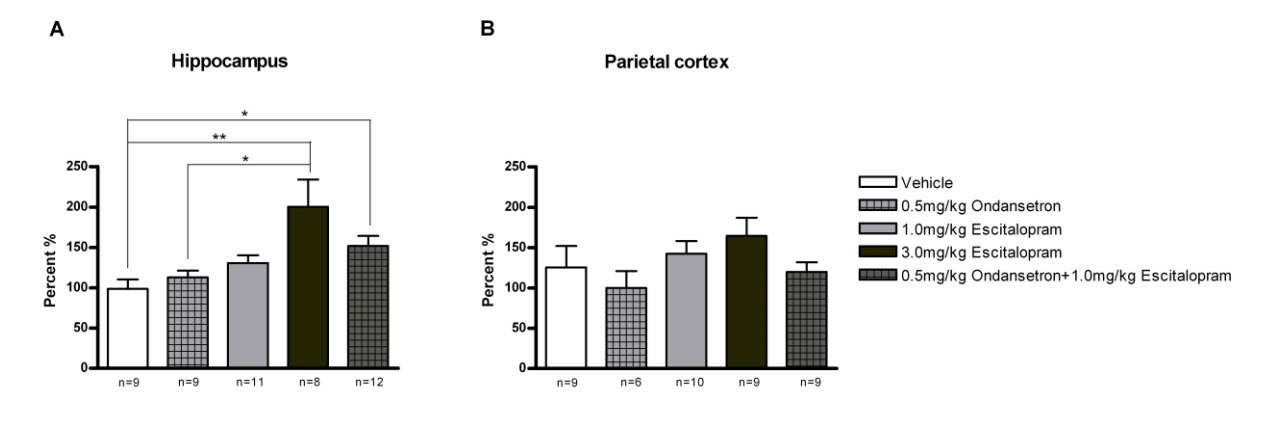

Figure 20. Mean relative changes in the mismatch negativity (MMN)-like response. (A) In hippocampus, (B) In parietal cortex. Vehicle (hippocampus n=9; parietal cortex n=9), 0.5mg/kg ondansetron (hippocampus n=9; parietal cortex n=6), 1.0mg/kg (hippocampus n=11; parietal cortex n=10), 3.0mg/kg (hippocampus n=8; parietal cortex n=9) and 0.5mg/kg ondansetron+1.0mg/kg escitalopram (hippocampus n=12; parietal cortex n=9). *$p<0.05$; **$p<0.01$

The difference in the absolute MMN-like response among the different levels of treatment was significantly greater than would be expected by chance after allowing for effects of differences in time (baseline/after dose) [F(4, 48) =2.725, p=0.034]. Isolation of treatment groups and multiple comparison testing revealed a statistically significant difference between vehicle and 3mg/kg escitalopram (t=3.171, p=0.021), see Table 1. This result further supports escitaloprams enhancing effect on the MMN-like response.

N1 amplitude	Standard		Oddball		Mismatch negativity like response	
Treatment	Hippocampus mean(±SEM)	Parietal cortex mean(±SEM)	Hippocampus mean(±SEM)	Parietal cortex mean(±SEM)	Hippocampus mean(±SEM)	Parietal cortex mean(±SEM)
Vehicle (Baseline)	0.058 (±0.016) (n=9)	0.132 (±0.021) (n=9)	0.103 (±0.015) (n=9)	0.198 (±0.027) (n=9)	0.074 (±0.014) (n=9)	0.110 (±0.020) (n=9)
Vehicle (After dose)	0.059 (±0.019) (n=9)	0.145 (±0.028) (n=9)	0.118 (±0.032) (n=9)	0.235 (±0.046) (n=9)	0.070 (±0.014) (n=9)	0.118 (±0.023) (n=9)
Escitalopram 1mg/kg (Baseline)	0.102 (±0.035) (n=11)	0.120 (±0.022) (n=10)	0.165 (±0.044) (n=11)	0.188 (±0.026) (n=10)	0.083 (±0.015) (n=11)	0.095 (±0.015) (n=10)
Escitalopram 1mg/kg (After dose)	0.094 (±0.018) (n=11)	0.142 (±0.022) (n=10)	0.149 (±0.022) (n=11)	0.175 (±0.026) (n=10)	0.101 (±0.017) (n=11)	0.122 (±0.011) (n=10)
Escitalopram 3mg/kg (Baseline)	0.070 (±0.020) (n=8)	0.130 (±0.021) (n=9)	0.135 (±0.027) (n=8)	0.205 (±0.042) (n=9)	0.088 (±0.022) (n=8)	0.114 (±0.031) (n=9)
Escitalopram 3mg/kg (After dose)	0.098 (±0.019) (n=8)	0.167 (±0.023) (n=9)	0.200 (±0.034) (n=8)	0.251 (±0.035) (n=9)	0.146 (±0.022) (n=8)	0.156 (±0.026) (n=9) *
Ondansetron 0,5mg/kg (Baseline)	0.082 (±0.025) (n=9)	0.119 (±0.025) (n=6)	0.124 (±0.028) (n=9)	0.180 (±0.022) (n=6)	0.075 (±0.009) (n=9)	0.096 (±0.012) (n=6)
Ondansetron 0,5mg/kg (After dose)	0.075 (±0.023) (n=9)	0.133 (±0.023) (n=6)	0.127 (±0.029) (n=9)	0.179 (±0.030) (n=6)	0.083 (±0.011) (n=9)	0.090 (±0.020) (n=6)
Ondansetron 0,5mg/kg + Escitalopram 1mg/kg (Baseline)	0.099 (±0.025) (n=12)	0.118 (±0.021) (n=9)	0.128 (±0.021) (n=12)	0.180 (±0.024) (n=9)	0.068 (±0.010) (n=12)	0.112 (±0.009) (n=9)
Ondansetron 0,5mg/kg + Escitalopram 1mg/kg (After dose)	0.097 (±0.013) (n=12)	0.156 (±0.017) (n=9)	0.146 (±0.014) (n=12)	0.213 (±0.029) (n=9)	0.097 (±0.012) (n=12)	0.121 (±0.011) (n=9)

Table 1. Mean absolute N1 amplitudes at baseline and after dose during auditory odd ball stimulation. In hippocampus; vehicle (n=9), escitalopram 1mg/kg (n=11), escitalopram 3mg/kg (n=8), ondansetron 0.5mg/kg (n=9) and escitalopram 1mg/kg in combination with 0.5mg/kg ondansetron. In parietal cortex; vehicle (n=9), escitalopram 1mg/kg (n=10), escitalopram 3mg/kg (n=9), ondansetron 0.5mg/kg (n=6) and escitalopram 1mg/kg in combination with 0.5mg/kg ondansetron (n=9). *p<0.05 between vehicle and escitalopram 3mg/kg.

Latencies of P1, N1 and P2 at baseline were also investigated and were significantly different between hippocampus and parietal cortex see Figure 21 (A). The latency of P1 in the standard waveform was significantly increased in parietal cortex compared to hippocampus (p<0.001), see Figure 21 (C). Whereas the latencies of both N1and P2 were significantly increased in parietal cortex compared to hippocampus in the standard, oddball and the difference waveform (standard, N1 p<0.001 P2 p=0.005)(oddball, N1 p<0.001, P2 p<0.001)(Difference, N1 p<0.001, P2 p<0.001), see Figure 21 (B, C, D)

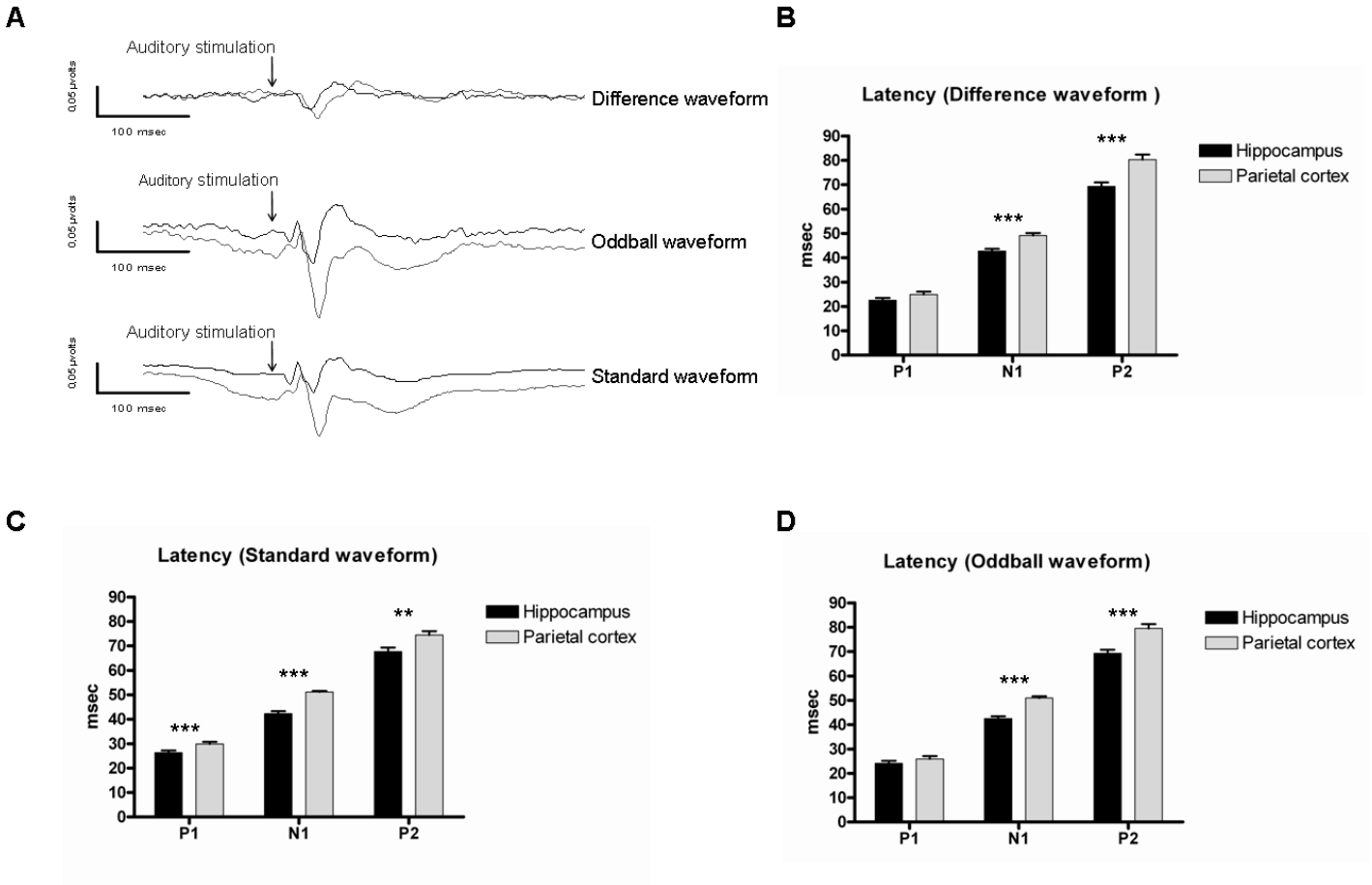

Figure 21. Illustrates absolute data on latencies in hippocampus and parietal cortex. Baseline values are used independent of treatment group, black: hippocampus (n=49); grey: parietal cortex (n=43). (A): Standard, oddball and difference grand average waveforms; (B) Bar chart representation of the absolute P1, N1 and P2 baseline latencies in the difference waveform for both hippocampus and parietal cortex. (C) Bar chart representation of the absolute P1, N1 and P2 baseline latencies in the standard waveform for both hippocampus and parietal cortex. (D) Bar chart representation of the absolute P1, N1 and P2 baseline latencies in the oddball waveform for both hippocampus and parietal cortex. *p<0.05; **p<0.01; ***p<0.001

DISCUSSION

In the present study we investigated the effect of escitalopram (SSRI) on the generation of MMN-like responses utilizing an auditory oddball paradigm. Auditory information is mainly projected from the nuclei cochleares, to the superior olivary nucleus, from where it proceeds upward through the lateral lemniscus to the inferior colliculus, the medial geniculate nucleus to finally terminate in the auditory cortex (Moos and Møller, 2006).

The auditory cortex is responsible for the processing of auditory sensory information to the rest of the brain, whereas hippocampus may play a secondary role in the information processing (Javitt et al., 1997). To this end, auditory cortex has been proposed to constitute a major player in auditory sensory memory, especially in relation to working memory and the discrimination process of incoming auditory stimuli. The interaction between auditory cortex

and hippocampus may be related to the conversion of temporary memory traces generated in auditory cortex into more permanent long-term memory traces in the hippocampus. The discrimination process of incoming auditory stimuli and the use of memory traces to distinguish changes in incoming sensory stimuli during conversation constitute an essential feature, which is likely to be important for social interaction. To this end, auditory MMN interestingly, index the ability of the brain to distinguish changes in the stimuli in the auditory domain.

The aim of the present study was to investigate the effect of increased serotonergic activity on the MMN-like response in rodent brain. Auditory evoked potentials (AEPs) can be recorded in hippocampus and cortex in rats and show identical AEP waveform to humans characterized by a P1-N1-P2 complex. However, the latency of rats AEP subcomponents are approximately 60% shorter than in humans (Siegel et al., 2003). The results from the present study demonstrate a significant increase in AEP latency in parietal cortex compared to hippocampus. This is in accordance with previous findings, which demonstrated that the latency of AEPs increases with the distance from the brainstem (reticular nucleus) (Moxon et al., 1999).

MMN-like responses in rats have been reported by several groups (Ruusuvirta et al., 1998; Tikhonravov et al., 2008; Tikhonravov et al., 2010; Roger et al., 2009). Human MMN has been proposed to be dependent on novelty encoding as well as stimulus-specific adaptation (SSA), whereas rodent MMN-like responses have been suggested mainly to be dependent on SSA (Umbricht et al., 2005; Farley et al., 2010). Very little data exist on the pharmacological sensitivity of MMN-like responses in rodents.

Interestingly, ketamine has been shown to disrupt MMN both in healthy volunteers (Heekeren et al., 2008; Umbricht et al., 2000; Umbricht et al., 2002) and mice (Ehrlichman et al., 2008). Further, Tikhanravov et al. have reported that NMDA receptor antagonists, MK-801, reduce the MMN-like response in rats (Tikhonravov et al., 2008). However, this finding has recently been challenged in a study by Farley et al., who were unable to reduce MMN-like responses using MK-801 in an oddball paradigm mainly dependent on SSA mechanisms (Farley et al., 2010).

In the present study, an acute dose of 3mg/kg escitalopram significantly increased the MMN-like response in rodent hippocampus. This is interesting since it further supports the predictive validity of rodent MMN-like responses in that escitalopram significantly increases MMN in healthy volunteers (Oranje et al., 2008; Wienberg et al., 2009). This finding may lead to the hypothesis that increased serotonergic signaling increases MMN, however, this has been challenged in several studies. Ahveninen et al. (2002) reported decreased MMN in healthy volunteers after acute tryptophan depletion, which decreases the 5-HT synthesis in the brain (Ahveninen et al., 2002), but the investigators were unable to replicate this finding in a later study (Ahveninen et al., 2003). Nevertheless, Kähkönen et al. (2005) reported increased

MMN amplitude following acute tryptophan depletion (Kähkönen et al., 2005). Despite of the inconsistent reports on serotonergic modulation *per se* on MMN in healthy volunteers, the effect of escitalopram on MMN has been replicated in humans.

The effects of drugs selective for subtypes of the serotonergic receptors have also been studied. In healthy volunteers, using 5-HT2A receptor agonists psilocybin and dimithyltryptamine, no evidence of MMN modulation was found (Umbricht et al., 2003b; Heekeren et al., 2008). Atypical antipsychotics (relatively potent 5-HT2A antagonists), such as clozapine (Umbricht et al., 1998) and risperidone (Umbricht et al., 1999) had no effect on MMN deficits in schizophrenic patients. Further, these drugs are relatively insufficient in treating negative and cognitive symptoms in schizophrenia. Several studies indicate that antipsychotic drugs in combination with SSRIs may be efficacious in treating negative and depressive symptoms of schizophrenia (Spina et al., 1994; Jockers-Scherubl et al., 2005; Silver and Nassar, 1992). Consequently, it is likely that increased serotonergic activity is beneficial for schizophrenic patients. Thus, more studies are warranted investigating the effect of SSRIs on symptoms and global functioning in schizophrenia. In connection to this, it would be interesting to investigate, the effect of escitalopram on MMN in schizophrenic patients.

In order to further investigate the relation between MMN-like responses and serotonergic signaling, the combination of SSRI and 5-HT3 inhibition was investigated. Escitalopram was dosed alone or in combination with 5-HT3 antagonist ondansetron in an auditory oddball paradigm in rats. This was done due to the interesting finding by Mørk et al. (2011), who very recently reported that blockade of the 5-HT3 receptors, using ondansetron, significantly enhances the extracellular level of 5-HT induced by SSRI, citalopram or paroxetine. This was demonstrated in both cortex and hippocampus (Mørk et al., 2011). They hypothesized that 5-HT3 receptors were expressed on GABAergic neurons in the raphe nucleus where they would mediate, at least in part, the negative feedback signal produced by SSRI-dependent increase in serotonergic signaling. Consequently, inhibition of 5-HT3 receptors would be hypothesized to block the negative feedback following acute SSRI treatment, thus increase the serotonergic output in the forebrain. In continuation to this, it was hypothesized that ondansetron would boost escitaloprams enhancing effect on MMN. However, in the current study ondansetron in combination with acute escitalopram was not able to produce additive or synergistic effect on MMN-like responses.

The neurobiological basis for the effect of escitalopram on MMN has not been described. One explanation, may come from the interaction between the serotonergic and glutamatergic transmitter systems in that N-methyl-D-aspartate (NMDA) receptors have been implicated in MMN (Heekeren et al., 2008; Umbricht et al., 2000; Umbricht et al., 2002). In a recent study, it was demonstrated that a low dose of escitalopram potently facilitated NMDA receptor-mediated neurotransmission in the cortex of rats and improved recognition memory

(Schilstrom et al., 2011). Escitalopram-mediated facilitation of NMDA receptor-mediated neurotransmission may explain an increased MMN response in that the NMDA antagonist ketamine decrease MMN in both humans and animals (Heekeren et al., 2008; Umbricht et al., 2000; Umbricht et al., 2002; Ehrlichman et al., 2008).

Taken together, the present study translates the human findings and demonstrate that escitalopram increase MMN-like responses in rats. Thus, it is likely that MMN-like responses in rodents show predictive validity for human MMN. Thus may represent a unique tool in pharmacological testing as it is an objective parameter, more closely likely to neurobiological underpinnings of disease biology.

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Discussion

The primary aim of the present master thesis was to back-translate the interesting finding that increased serotonergic signaling mediated by escitalopram increases auditory MMN amplitude in healthy volunteers. This was performed by investigating the effect of increased serotonergic activity by dosing escitalopram alone or in combination with 5-HT3 antagonist ondansetron in an auditory oddball paradigm in rats.

In the article manuscript major issues related to MMN are discussed and the influence of increased serotonergic activity by escitalopram. However, the article manuscript does not discuss methodological consideration in relation to MMN generation, which will be discussed below.

Methodological considerations of mismatch negativity

One parameter that influences the MMN generation is the length of the ISI.

Auditory evoked potentials recorded in response to increasing ISI, demonstrated an association between the P1/N1 amplitude and the ISI. As the ISI increased the P1/N1 amplitude increased equivalently, until a plateau was reached at ISI ≥ 3000msec. This finding is in accordance with previous findings as it has been demonstrated that the N1 amplitude increases dramatically for ISI increases from 0.5sec to 2-3sec and then increases more gradually reaching a maximum at about 10sec (Budd et al., 1998).

In an oddball paradigm with an ISI of 500msec, standard tones demonstrate a peak adaptation of the AEP including the P1/N1 amplitude. Oddballs may theoretically generate maximal P1/N1 amplitudes with similar characteristics as tones presented with an ISI ≥ 3000msec, since the probability of oddballs are low and thus ISI(oddball) is always > 3000msec. The difference in P1/N1 amplitudes in relation to the different ISIs creates a window for the generation of MMN to occur.
It has been shown that the MMN amplitude in humans increases with decreasing ISI (Pekkonen et al., 1993). However, a tendency of increased MMN amplitude in response to increasing ISI was found in the present study.

The ISIs can be controlled in two different ways, either as a fixed ISI or as a variable ISI. In the present study variable ISIs, consisting of a pseudo-randomly fixed interval, were used. For instance, in the oddball paradigm the ISI was fixed to a mean of 500msec, randomized in an interval between 300msec and 700msec. The advantage of using variable ISIs may be that the memory trace of standard tones is exclusively generated to the tone content (frequency), rather than the timing. With the fixed interval, the ISI may contribute to the memory trace, which may result in a decreased AEP to oddball stimuli, since timing is the same between

standard and oddball stimuli, which may lead to a decreased MMN. However, this remains to be a hypothesis. Another reason for using a variable ISI, is the fact that variable ISI is routinely used in human studies (Oranje et al., 2008; Wienberg et al., 2009; Umbricht et al., 1999; Umbricht et al., 1998).

Another parameter which has been reported to influence the MMN generation is the characteristics of the deviant tones used in the oddball paradigm. In the present study frequency oddballs were applied to elicit a MMN response when presented in a homogeneous series of standard tones. This was done based on results from the previous validation study performed at Lundbeck A/S, which demonstrated more prominent MMN amplitudes with frequency oddballs compared to duration oddballs (Hansen, 2010). However, one group have reported that frequency oddballs are less efficient in eliciting MMN compared to duration oddballs (Umbricht et al., 2005; Umbricht and Krljes, 2005). Thus, further validation studies using duration oddballs are warranted. In addition, duration oddballs also produce more robust MMN in humans (Michie, 2001; Umbricht and Krljes, 2005).

A third parameter which may have an impact on MMN is the vigilance state of the animal. Prior to the second escitalopram study (presented in the article manuscript), the circadian rhythm of the rats was changes and MMN was determined during the dark phase, due to speculations as to whether the rats (nocturnal animals) were sleeping during experiments performed within the light phase.

Human findings on MMN during sleep have been quite contradictory. Paavilainen et al. and Nielsen-Bohlman et al. failed to show MMN in humans during sleep (Paavilainen et al., 1987; Nielsen-Bohlman et al., 1991). In contrast, other studies have demonstrated signs of sleep MMN in humans, especially during the REM sleep. Loewy et al. found MMN during REM sleep in humans, which were significantly reduced by approximately 30% compared to the waking MMN. However, no MMN was observed in the non-REM sleep stages (Loewy et al., 1996). In addition, Nashinda et al. found MMN in both drowsiness and REM sleep, with reduced MMN amplitude in sleep compared to in waking (Nashida et al., 2000).

Nevertheless, it is likely that changes or difference in vigilance state during the experiment may bias the data and provide a source of variation. Thus, the second escitalopram study was performed during the dark phase.

In addition, MMN-like responses measured during the active phase of rats, may provide a more valid estimate of the human MMN, as the human MMN usually is measured during daytime (human active phase).

Translational perspective of mismatch negativity

MMN constitute a potential translational assay in schizophrenia. The advantages of using MMN as a translational assay is that it represents an objective parameter, more closely linked to neurobiological underpinnings of disease processes rather than symptomatology, which is normally state-dependent (Gottesman and Gould, 2003). Also, MMN is generated in the absence of attention, which makes it particularly suitable for testing different clinical populations, newborns and animals, which normally would have difficulties in cooperating procedures (Garrido et al., 2009).

One concern regarding the translational perspective of MMN is the methodological differences in "peak detection" for MMN analysis. In humans, MMN is routinely measured as the distance from baseline to the peak of N1 in the difference waveform, whereas it in rodents often is measured as the distance from the peak of P1 to the peak of N1 (P1/N1) in the difference waveform. A possible explanation for this methodological difference may be due to a low signal to noise ratio in rodents, making it difficult to identify the baseline. In the present study both baseline/N1 and P1/N1 amplitudes were assessed. Due to noise and irregularity of waveforms it was difficult to define baseline/N1 precisely. The difficulties in determination of baseline/N1 may in part be one explanation for the fact that no significant differences between treatment groups were obtained in the first escitalopram study (see section: First escitalopram study).

Despite, the methodological differences in MMN determination between species, pharmacological studies report similar effects on baseline/N1 and P1/N1 in response to pharmacological interventions. One example of this is the effect of ketamine (NMDA antagonist) on MMN. In human studies strong reductions of the MMN amplitude, measured as baseline/N1, were obtained in healthy volunteers (Heekeren et al., 2008; Umbricht et al., 2000; Umbricht et al., 2002). Similarly, in rodents ketamine significantly reduced MMN-like responses, measured as P1/N1 (Ehrlichman et al., 2008).

Taken together, it is likely that rodent MMN-like responses, at least in part, have validity as a translational marker for pre-attentive auditory memory processing

Loudness dependence of auditory evoked potentials

The secondary aim of the present master thesis was to validate LDAEP as marker of serotonergic activity in rats.

The LDAEP reflects an increase or decrease in the amplitude of the N1/P2 component of auditory evoked potentials in response to an increase in auditory stimulus intensity. While the exact mechanisms responsible for the generation of LDAEP are unknown, a strong LDAEP

has been associated with decreased serotonergic function, while a weak LDAEP has been associated with increased serotonergic function (Wutzler et al., 2008; Hegerl et al., 2001).

In the present study we examined the relationship between acute enhancement of serotonin mediated by escitalopram and the LDAEP in rat hippocampus. Results did not demonstrate a significantly weakened LDAEP in response to an increased serotonergic activity as expected. A possible explanation for this may be electrode location. In the present study the depth electrode was placed in the hippocampus, whereas the majority of publications reporting a negative correlation between LDAEP and serotonergic activity have been measured in primary auditory cortex.

An animal study demonstrated weaker LDAEP in primary auditory cortex with enhancement of serotonergic activity using spiperone (5-HT1A antagonist) locally in dorsal raphe nucleus (DRN) (Juckel et al., 1999). The same study demonstrated a strong LDAEP in auditory cortex after locally injection of 8-OH-DPAT (5-HT1A agonist) in DRN, which inhibited the firing rate of serotonergic DRN neurons (Juckel et al., 1999). A study in healthy volunteers found that enhancement of serotonergic activity with citalopram (SSRI) significantly weakened the LDAEP in auditory cortex compared to placebo (Nathan et al., 2006).

In relation to usage of LDAEP as a marker of serotonergic activity in psychiatric disorders, a study investigated LDAEP strength among following diseases; major depressive disorder, bipolar disorder, schizophrenia, panic disorder, generalized anxiety disorder and post-traumatic stress disorder. The results showed that LDAEP was significantly weaker in bipolar- and schizophrenic patients compared to healthy volunteers (Park et al., 2010). Gudlowski et al. (2009) investigated the LDAEP in schizophrenic patients with different disease stages. Results showed that LDAEP was significantly weaker in prodromal, first-episode, and chronic schizophrenic patients compared to healthy volunteers. In addition, there was no significant difference between medicated and unmedicated schizophrenic patients (Gudlowski et al., 2009). However, Juckel et al. examined the LDAEP in schizophrenic patients with no medication and healthy volunteers and found significantly weaker LDAEPs in schizophrenic patients compared to healthy volunteers, when measured in the primary auditory cortex. Furthermore it was found that LDAEP tended to strengthen after treatment with clozapine or olanzapine, which partly acts through the antagonistic binding of 5-HT2 receptors, leading to a reduction of serotonergic neurotransmission (Juckel et al., 2003).

Taken together, evidence supports LDAEP as a reliable marker for serotonergic activity in the primary auditory cortex. However, further research need to be done before any conclusions regarding LDAEP as a marker for serotonergic activity in the hippocampus can be made.

Summary

In summary, MMN represents a potential translational assay in schizophrenia and a unique tool in pharmacological testing, as it is an objective parameter, more closely linked to neurobiological foundations of schizophrenic disease processes rather than symptomatology. One advantage of using MMN as a translational assay is that it can be measured independently of attention. This makes it particularly suitable for testing animals and different clinical populations, which normally would have difficulties in cooperating procedures. Furthermore, MMN-like response in rodents shows similar characteristics as the human MMN response, making it attractive for pre-clinical pharmacological testing in efforts to discover new medicines for schizophrenia.

Another advantage of MMN, is that MMN deficits have been reported to be relatively selective for schizophrenia compared to other neuropsychiatric disorders. Moreover, in chronically ill schizophrenic patients the severity of MMN deficits seems to correlate with the severity of negative symptoms.

The influence of serotonergic modulation on MMN has received increasingly attention as schizophrenic patients seem to benefit from the clinical effects of SSRIs. The present study demonstrated that increased serotonergic activity, mediated by escitalopram, significantly increased the MMN-like response in rats, which is in line with previous findings in humans. This indicates that the serotonergic system may be implicated in schizophrenia and the modulation of MMN. However, it is not possible to make specific conclusions on the effect of increased serotonergic activity on the modulation of MMN. Moreover, the specific serotonergic receptor subtypes involved in MMN are currently unknown and need to be investigated. In addition, the modulation of MMN by escitalopram, may involve other neurotransmitter systems such as glutamatergic NMDA receptors.

LDAEP has shown to be a reliable marker of serotonergic activity in the primary auditory cortex in both humans and animals. However, LDAEP measured in the hippocampus, as in the present study, do not constitute a predictable indicator for serotonergic activity.

Conclusion

In conclusion, escitalopram significantly increased the MMN-like response in rodents. However, no synergistic effect on MMN was obtained when administrating escitalopram in combination with ondansetron.

Secondarily, no significantly weakened LDAEP was obtained, in response to an increased serotonergic activity mediated by escitalopram. Thus, it was not possible to use LDAEP as a reliable marker of serotonergic activity, when measured in the hippocampus.

Future perspective

As it is not possible to make specific conclusions on the effect of increased serotonergic activity on the modulation of MMN, other serotonergic subtype receptors and their potential role in MMN generation need to be investigated.

Furthermore, the effect of chronic administration of escitalopram and other serotonergic modulators need to be carried out. Especially, in relation to SSRIs, due to the delayed onset of therapeutic effect.

Finally, it would be of benefit to investigate, whether escitalopram and other serotonergic modulators are able to reverse the reduced MMN amplitude in schizophrenic patients. If increased serotonergic activity is capable of reversing the reduced MMN amplitude, it would be interesting to investigate the symptomatic profile of these schizophrenic patients to see if any improvement in symptoms has occurred.

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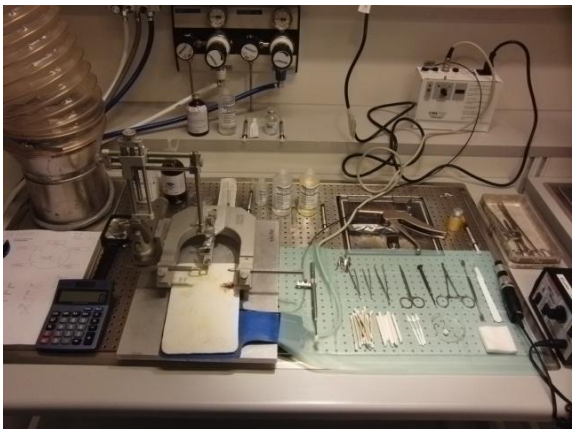
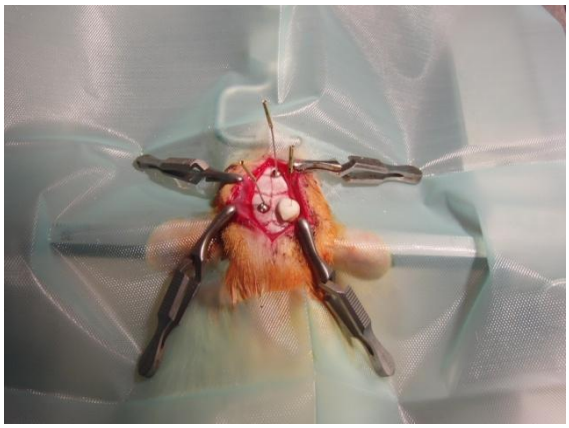
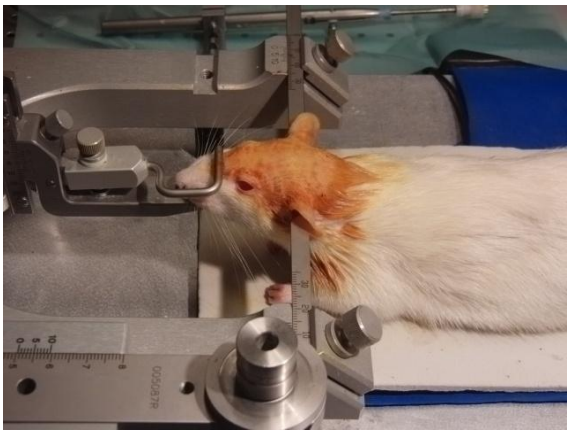
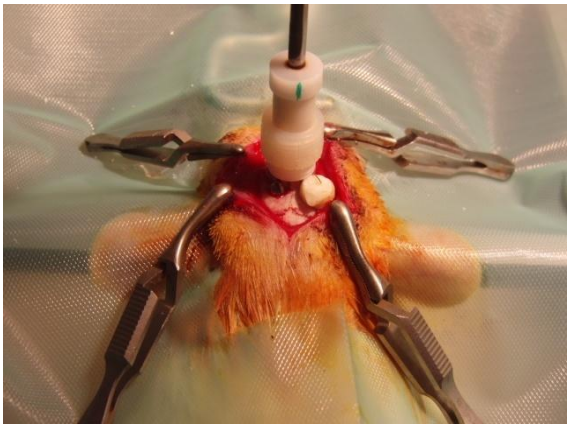
Appendix A

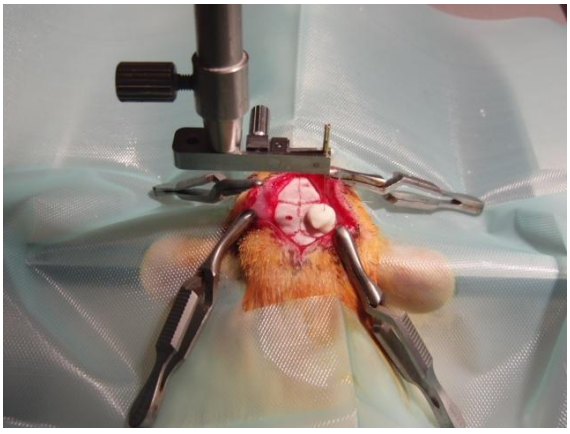
Pictures from the surgical procedures

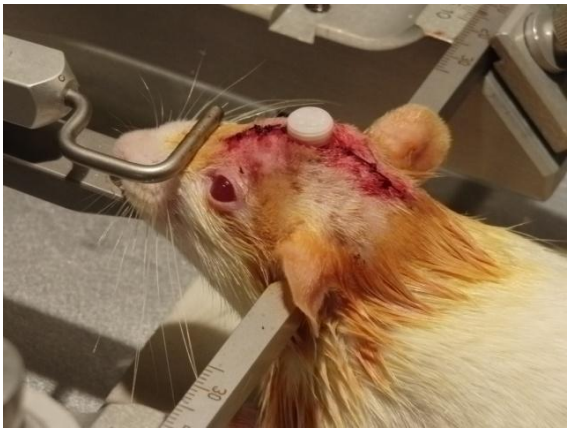












Appendix B

Cortical electrodes:

- Parietal cortex (-4.0mm posterior and +2.0mm lateral relative to bregma)

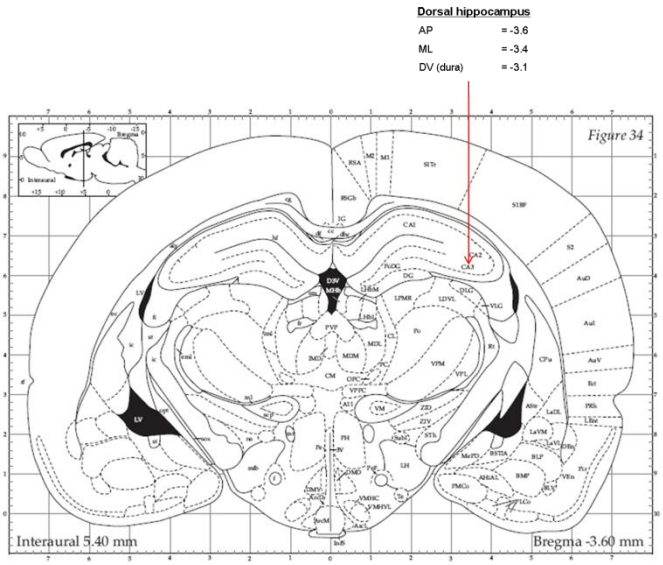
Depth electrodes:

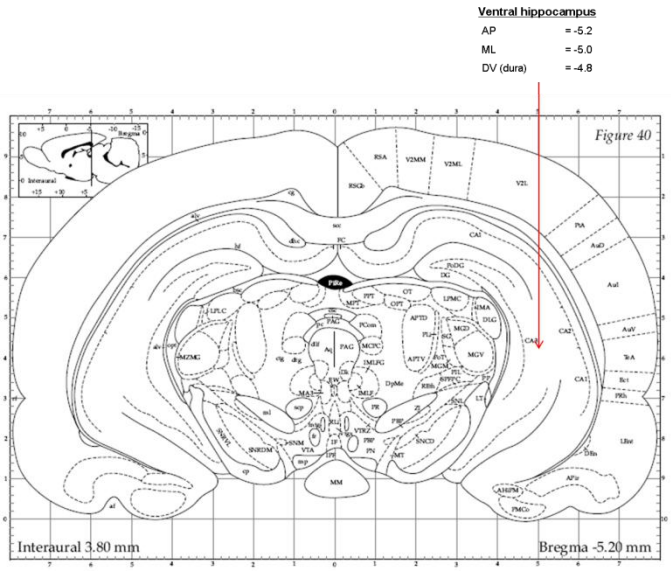
- Dorsal hippocampus (-3.6mm posterior and -3.4mm lateral relative to bregma and -3.1mm deep relative to dura mater).
- Ventral hippocampus (-5.2mm posterior and -5.0mm lateral relative to bregma and -4.8mm deep relative to dura mater).

Test-batch (n=14); 7 with depth electrode placement in the dorsal hippocampus and 7 with depth electrode placement in the ventral hippocampus.

1. batch (n=24); all with depth electrode placement in the ventral hippocampus

Illustration of depth electrode location:





Appendix C

Pictures from the test-location

Appendix D

Drugs used in this study:

Escitalopram

(+)-1-(3-Dimethylamino)propyl)-1-(4'-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, oxalate

Batch 5.

Batch Moleweight: 414.470

Base Moleweight: 324.392

Dissolved in 0.9% isotonic saline, pH >4 and <8

Subcutaneous injection with an injection volume of 5ml/kg

Doses uses in the first escitalopram study: (0.25mg/kg; 0.5mg/kg; 1.0mg/kg)

Doses uses in the second escitalopram study: (1.0mg/kg; 3.0mg/kg)

Ondansetron

9-methyl-3-[(2-methylimidazolyl)methyl]-1,2,3,9-tetrahydro-4aH-carbazol-4-one

Batch 10.

Batch Moleweight: 293.370

Base Moleweight: 293.363

Dissolved in 0.9% isotonic saline, pH >4 and <8

Subcutaneous injection with an injection volume of 5ml/kg

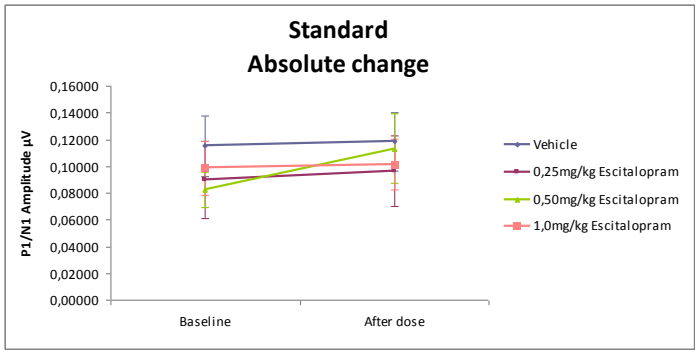
Doses used in the second escitalopram study (0.5mg/kg)

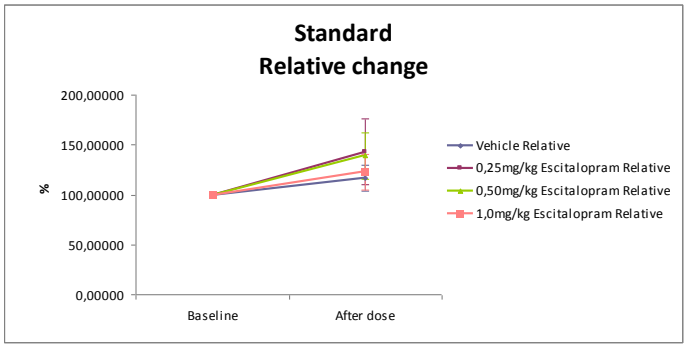
Appendix E

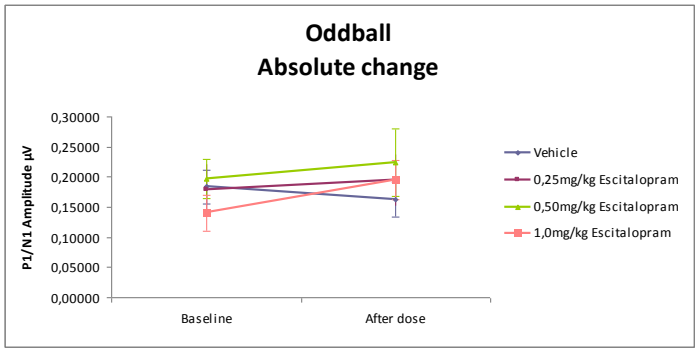
Results from the first escitalopram study

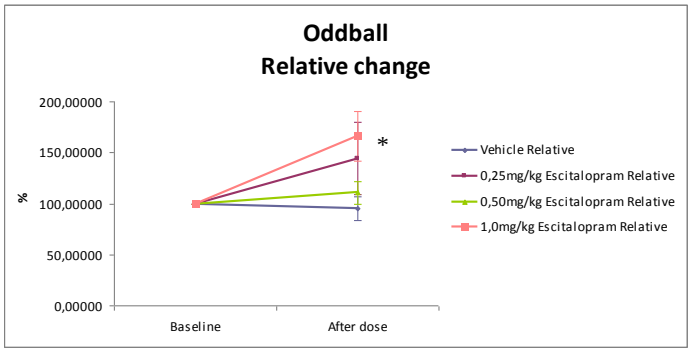
- Hippocampus
  - P1/N1 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - N1/P2 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - Baseline/N1 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - Baseline/P2 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - LDAEP
    - Absolute change
    - Relative change

First Escitalopram study
Hippocampus
P1/N1 Amplitude

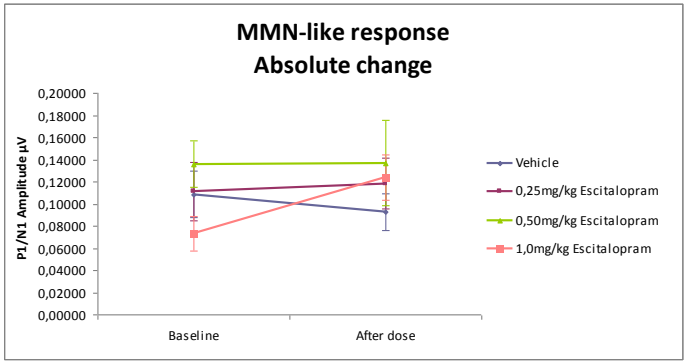


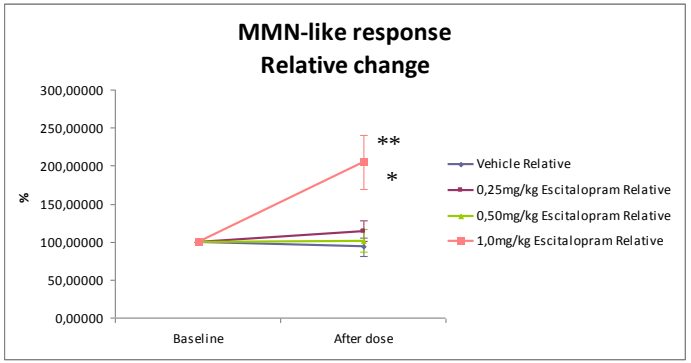






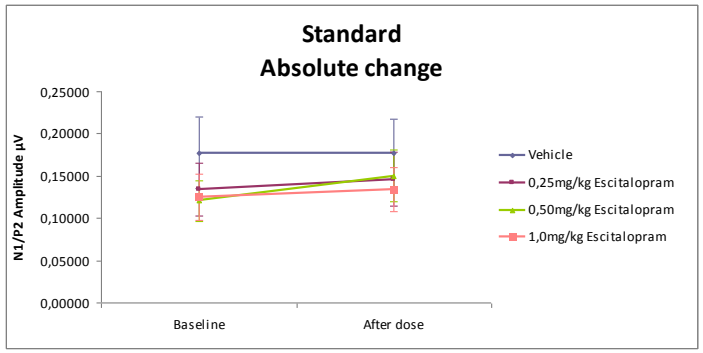
*p<0.05 between vehicle and 1.0mg/kg escitalopram
(Bonferroni t-test)

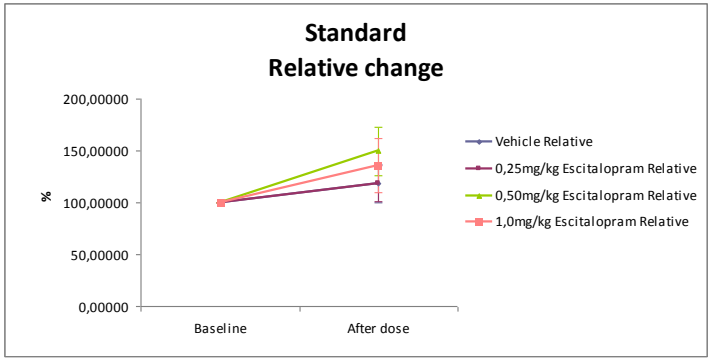


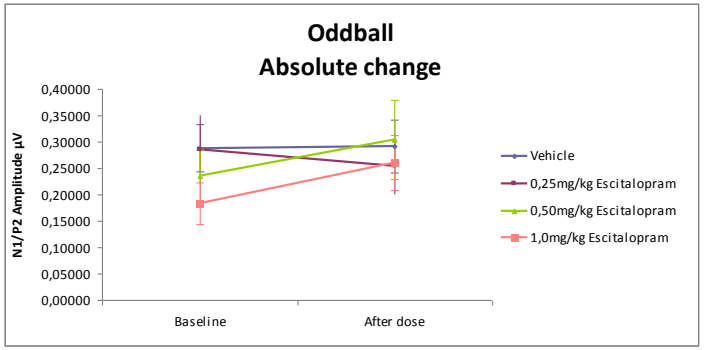


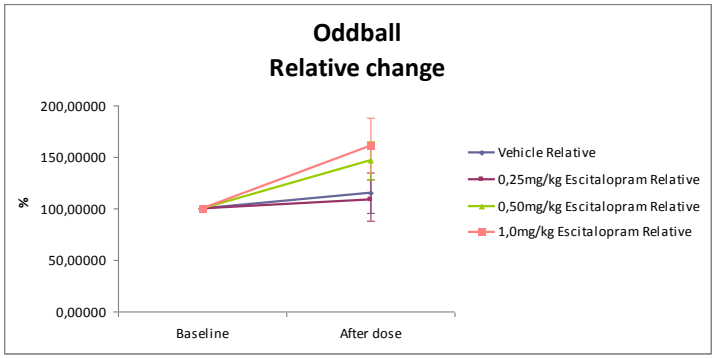
* p<0.05 between 1.0mg/kg escitalopram and 0.5mg/kg escitalopram
**p<0.01 between vehicle and 1.0mg/kg escitalopram
(Bonferroni t-test)

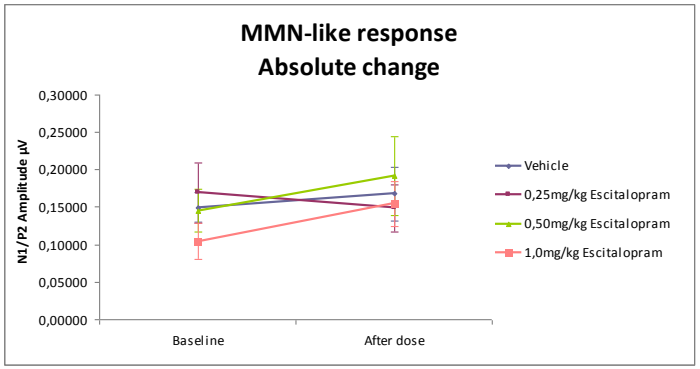
First Escitalopram study
Hippocampus
N1/P2 Amplitude

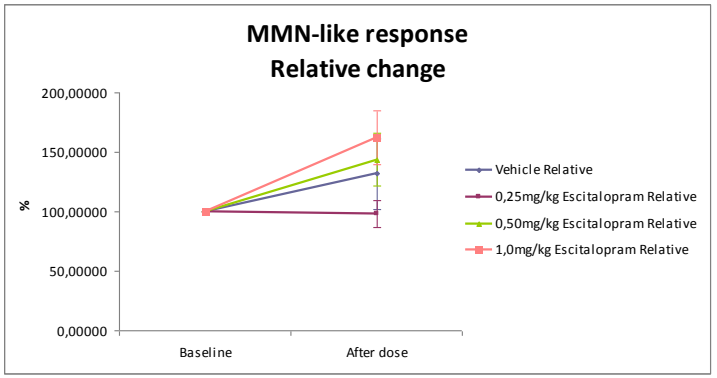




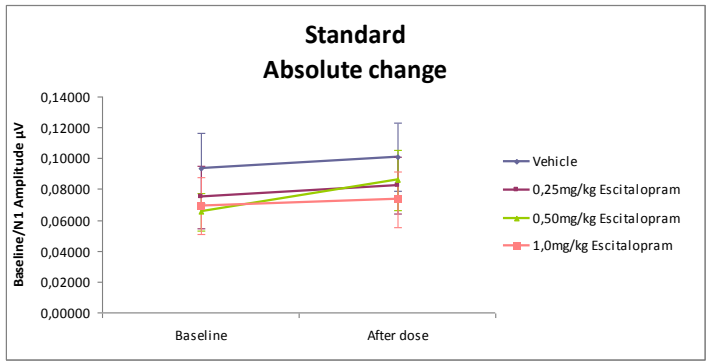


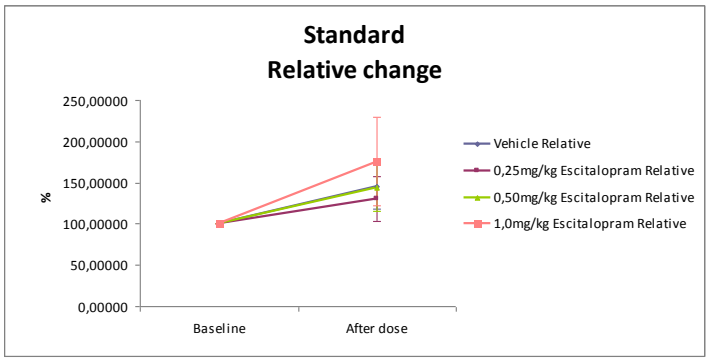


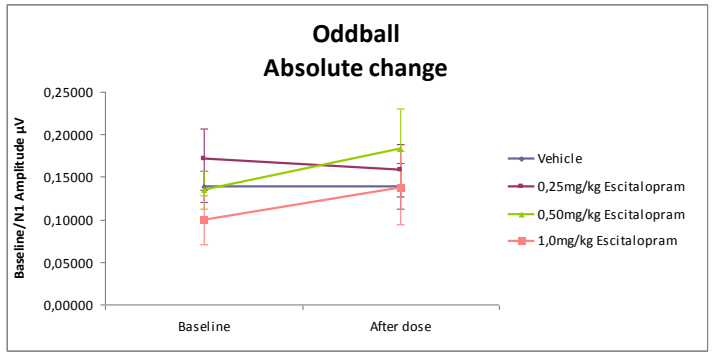


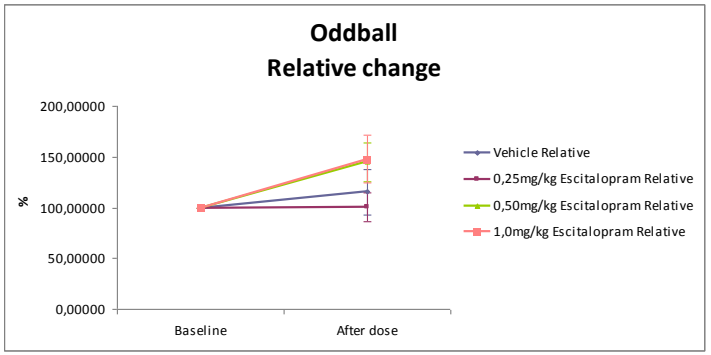


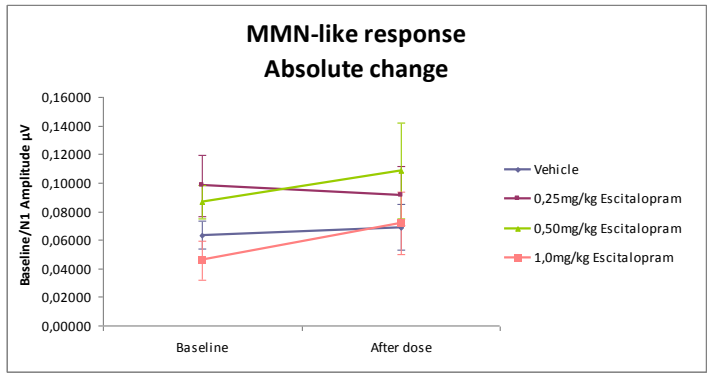
First Escitalopram study
Hippocampus
Baseline/N1 Amplitude

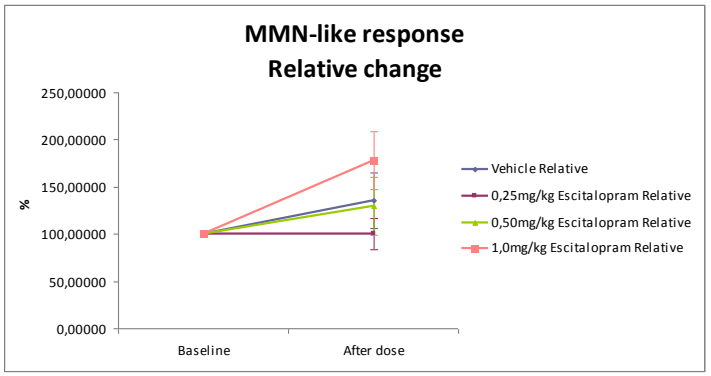




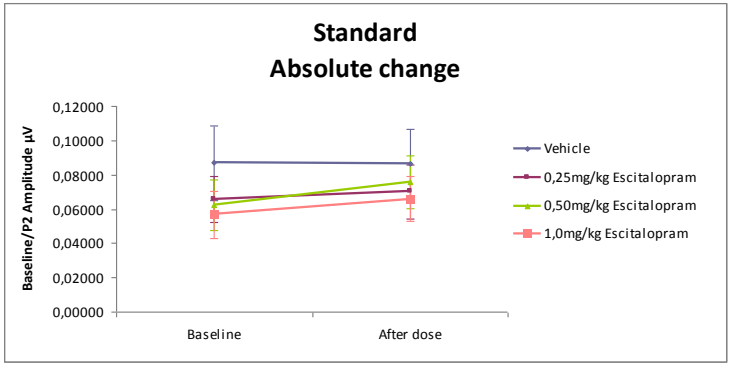


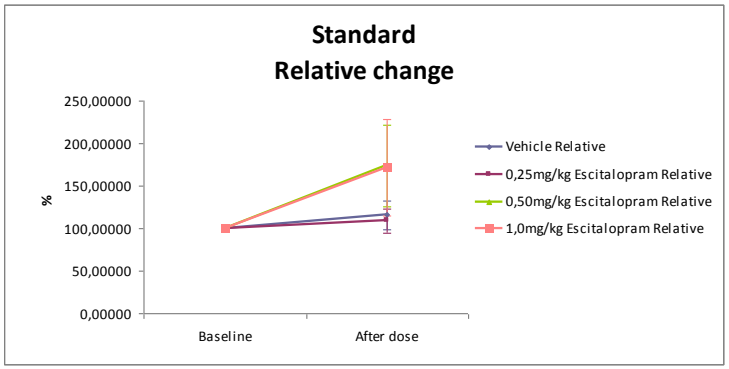


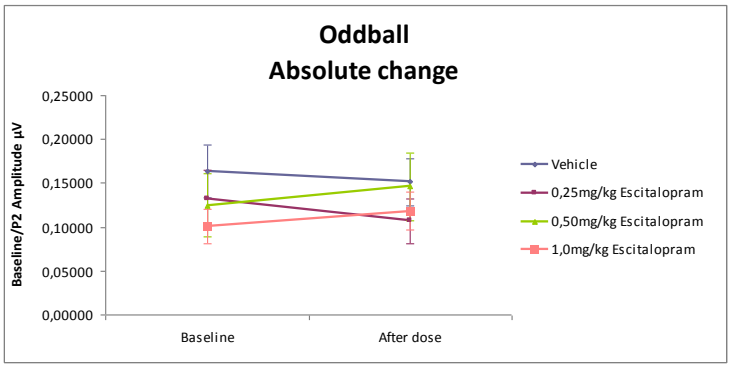


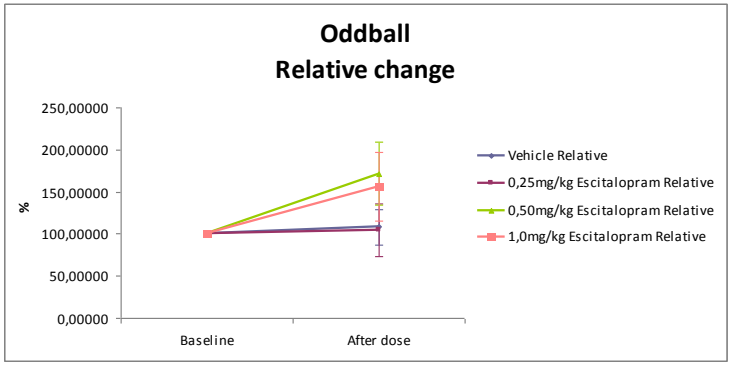


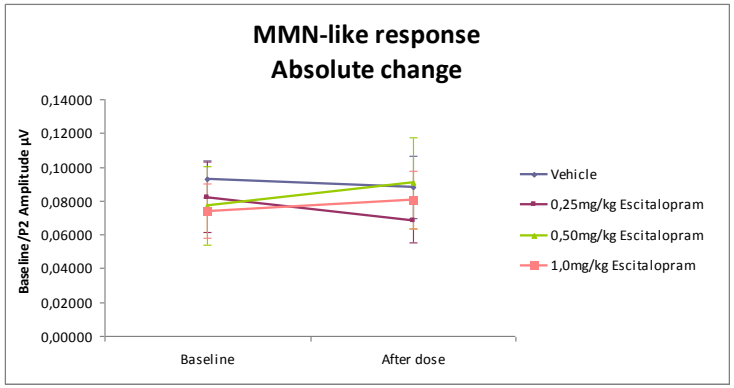
First Escitalopram study
Hippocampus
Baseline/P2 Amplitude

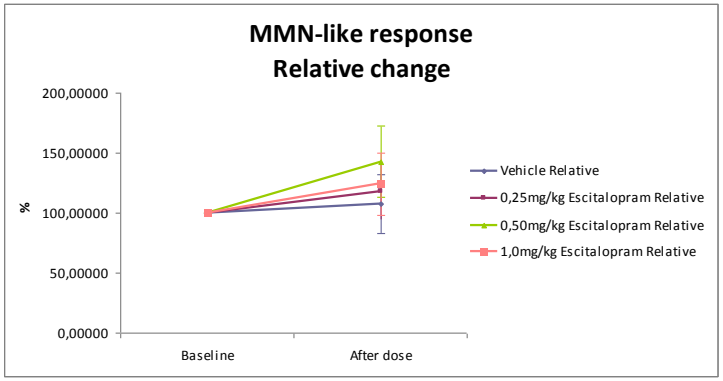




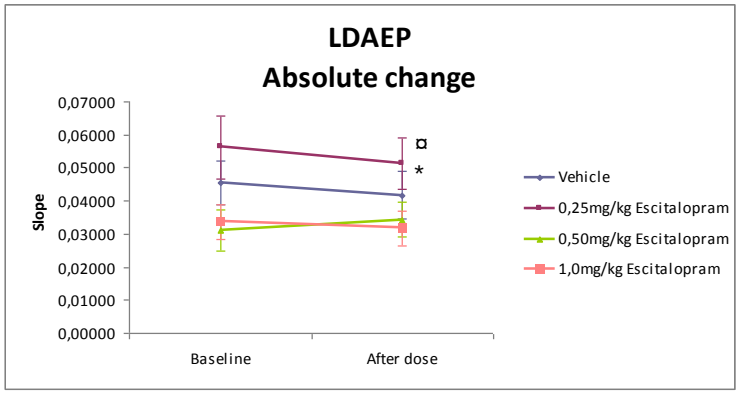


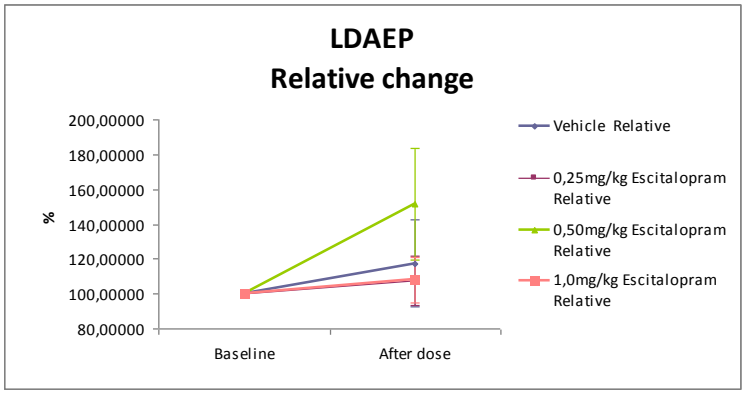






First Escitalopram study
Hippocampus
LDAEP





* p<0.05 between 1.0mg/kg escitalopram and 0.25mg/kg escitalopram

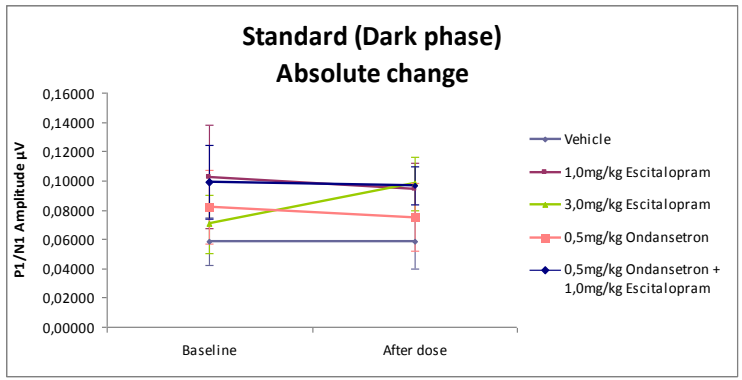
¤ p<0.05 between 0.5mg/kg escitalopram and 0.25mg/kg escitalopram (Bonferroni t-test)

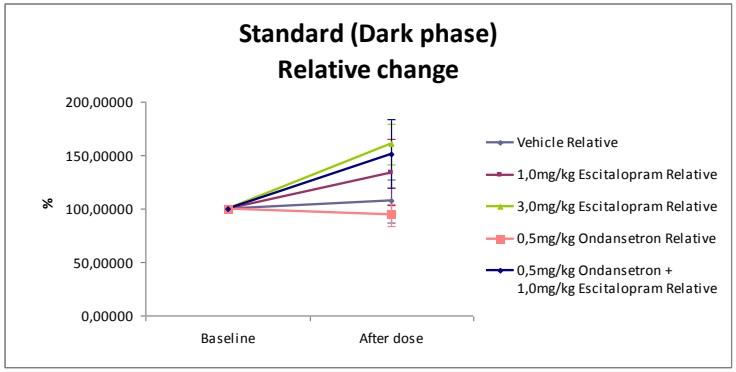
Appendix F

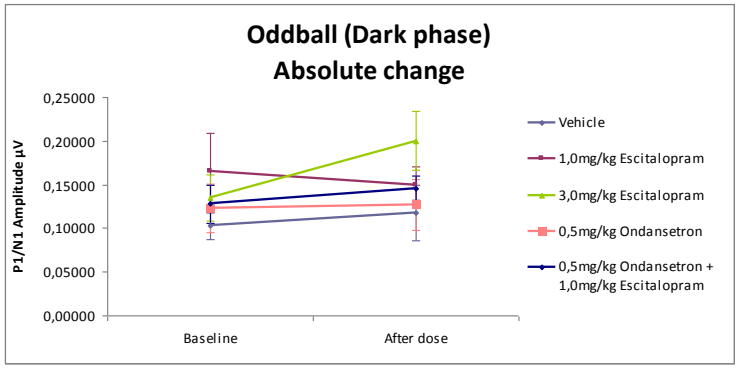
Results from the second escitalopram study

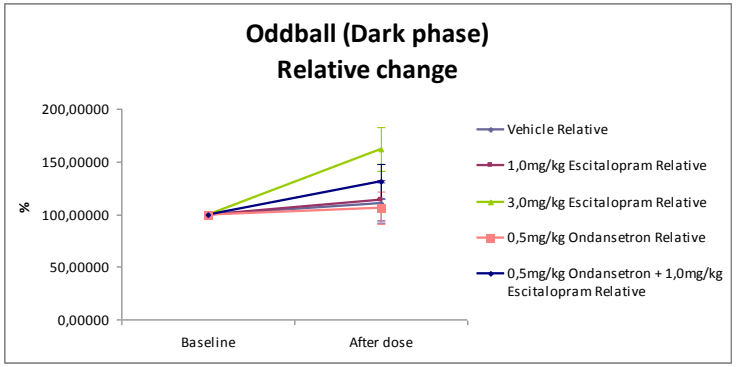
- Hippocampus
  - P1/N1 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - N1/P2 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - Baseline/N1 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - Baseline/P2 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
- Parietal cortex
  - P1/N1 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - N1/P2 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - Baseline/N1 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response
  - Baseline/P2 Amplitude
    - Absolute change: Standard, Oddball and MMN-like response
    - Relative change: Standard, Oddball and MMN-like response

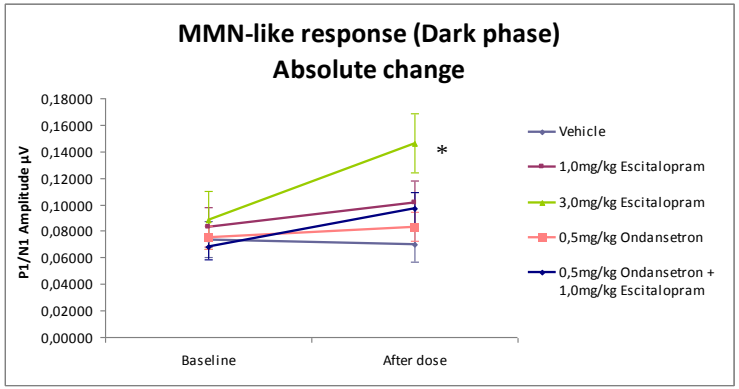
Second Escitalopram study
Hippocampus
P1/N1 Amplitude

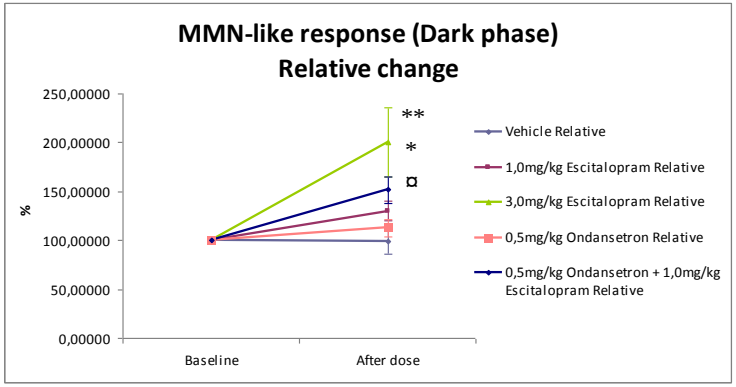








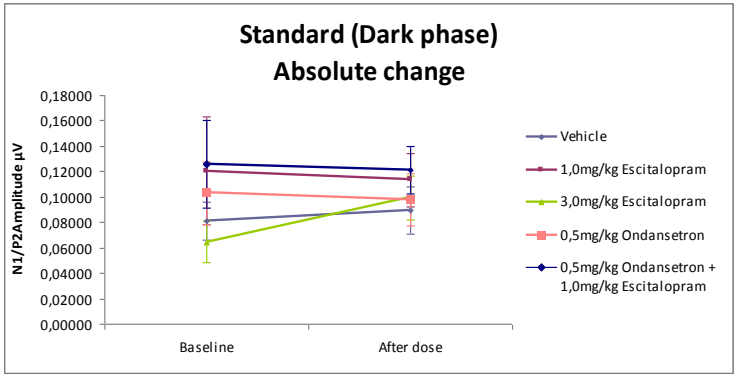


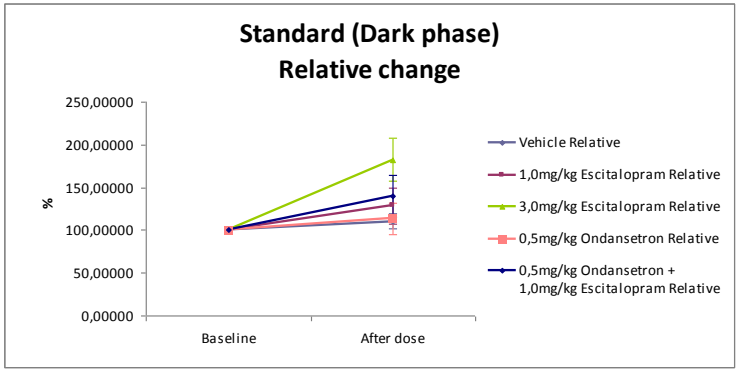


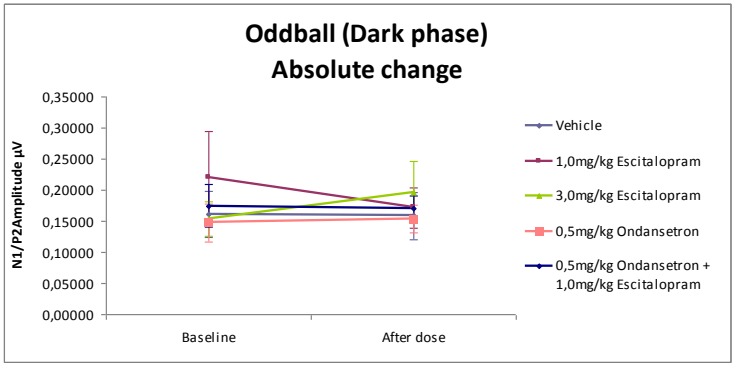
* p<0.05 between vehicle and 3.0mg/kg escitalopram
(Bonferroni t-test)

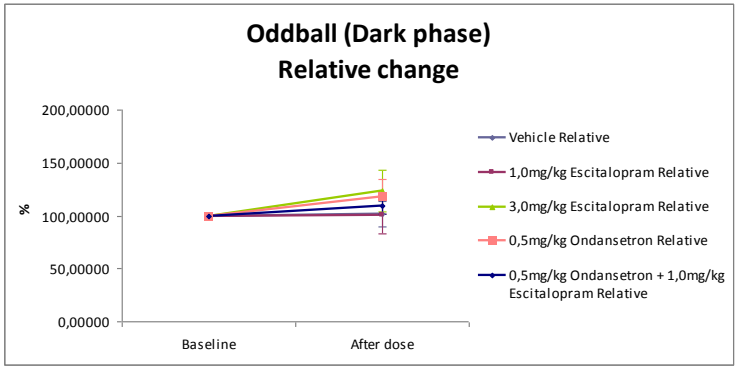
* p<0.05 between 3.0mg/kg escitalopram and 0.5mg/kg ondansetron,
¤ p<0.05 between vehicle and 0.5mg/kg ondansetron+1.0mg/kg escitalopram
**p<0.01 between vehicle and 3.0mg/kg escitalopram
(Bonferroni t-test)

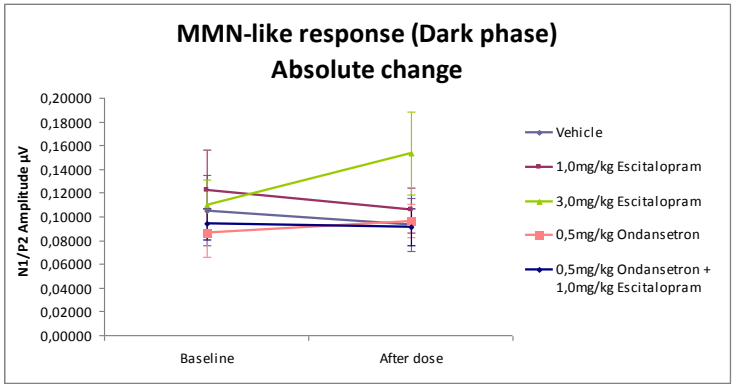
Second Escitalopram study
Hippocampus
N1/P2 Amplitude

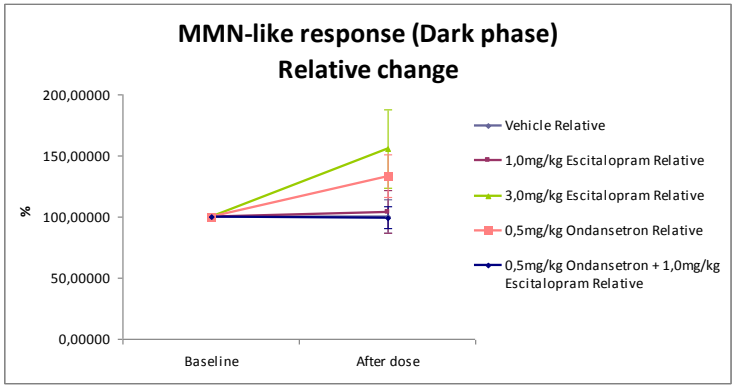




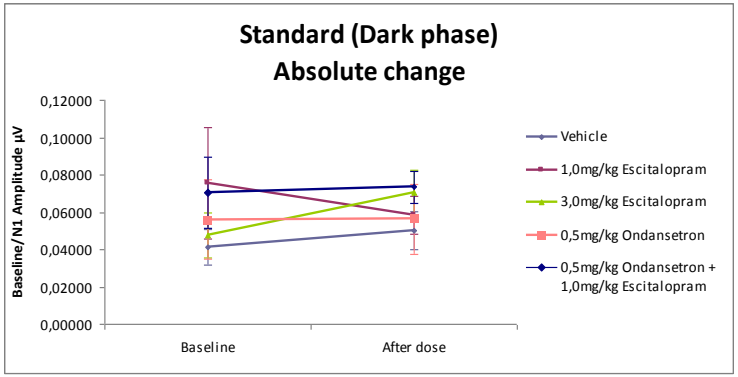


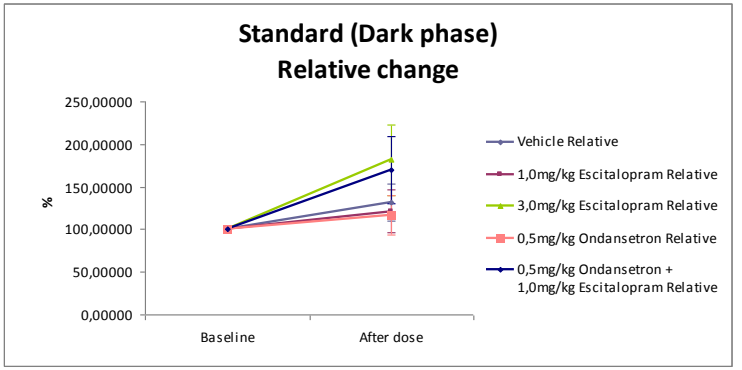


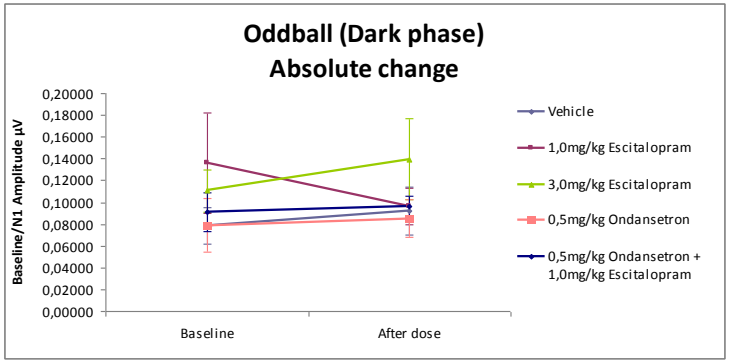


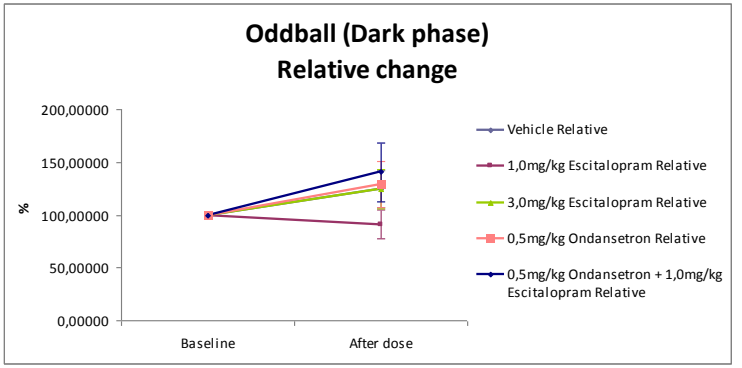


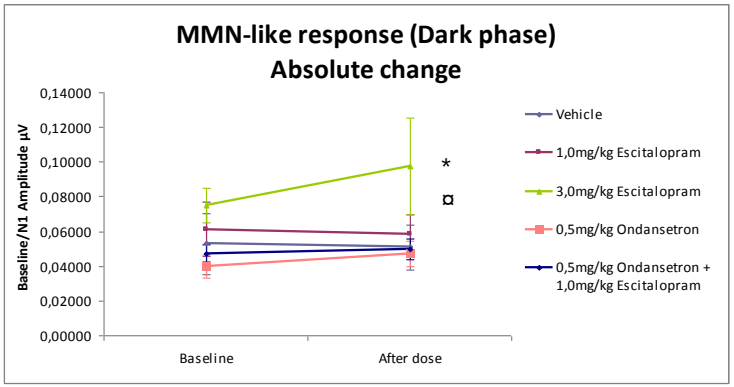
Second Escitalopram study
Hippocampus
Baseline/N1 Amplitude

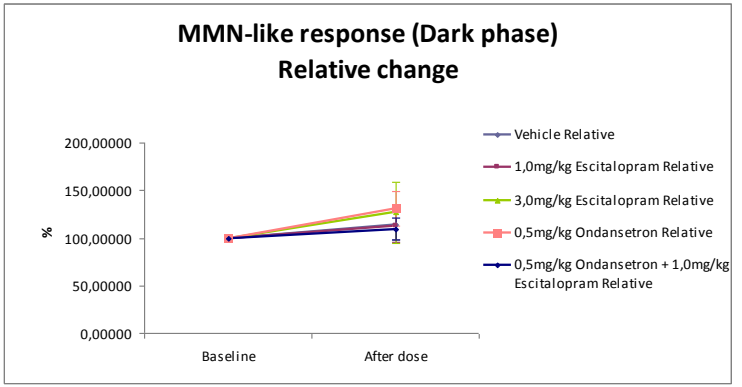








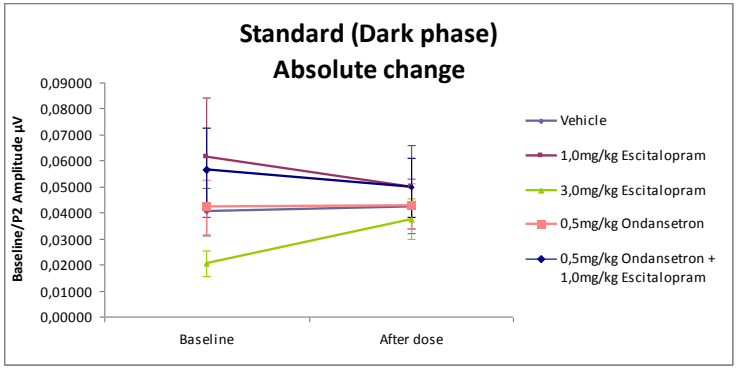


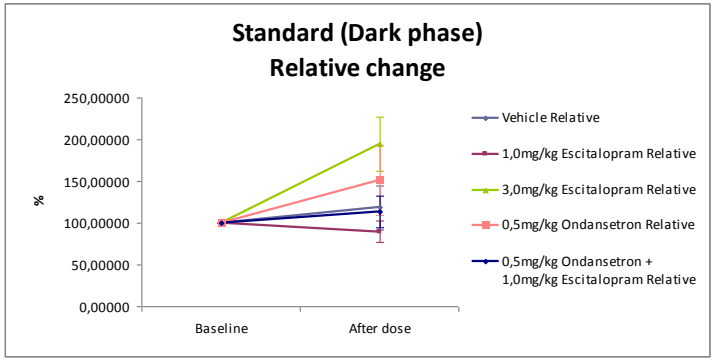


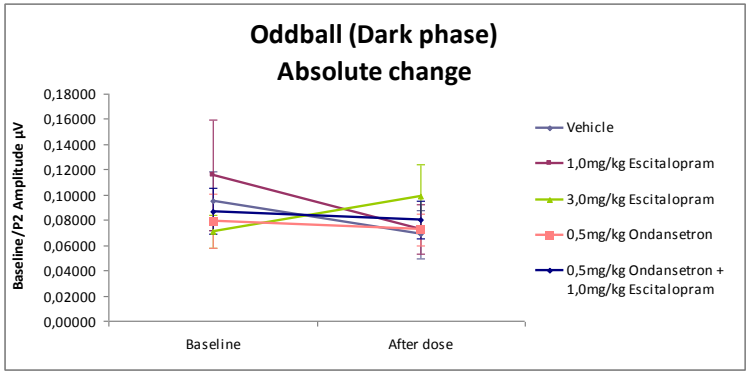
* p<0.05 between 3.0mg/kg escitalopram and 0.5mg/kg ondansetron

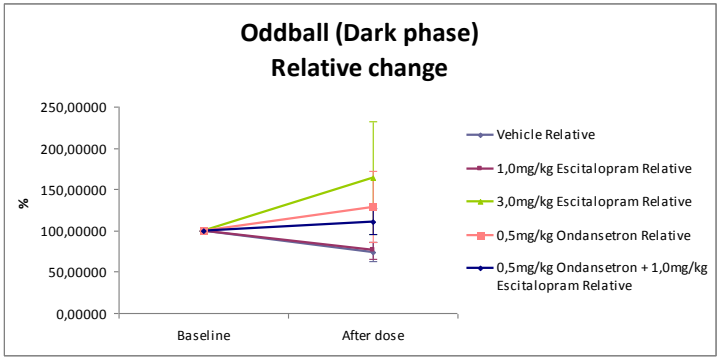
¤ p<0.05 between 3.0mg/kg escitalopram and vehicle (Bonferroni t-test)

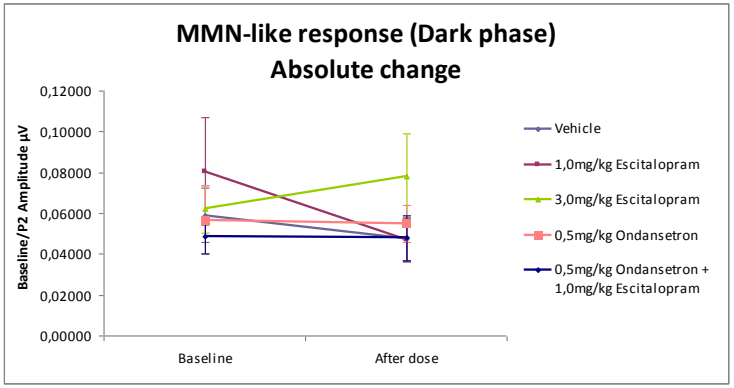
Second Escitalopram study
Hippocampus
Baseline/P2 Amplitude

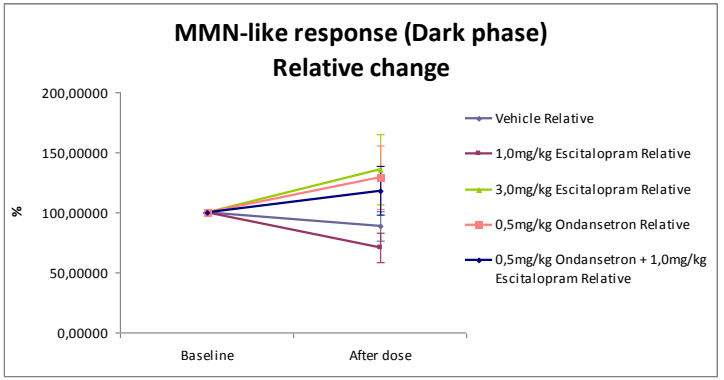




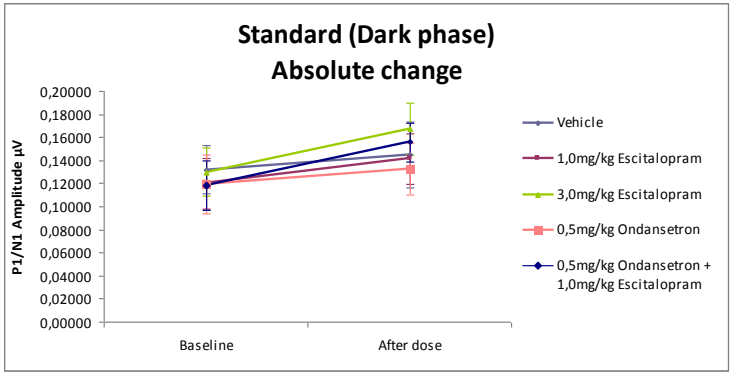


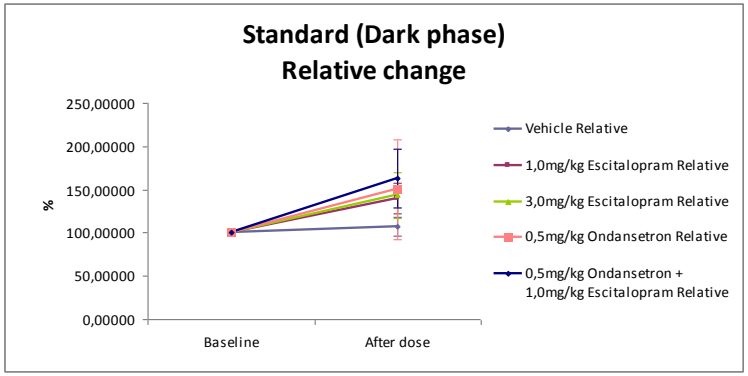


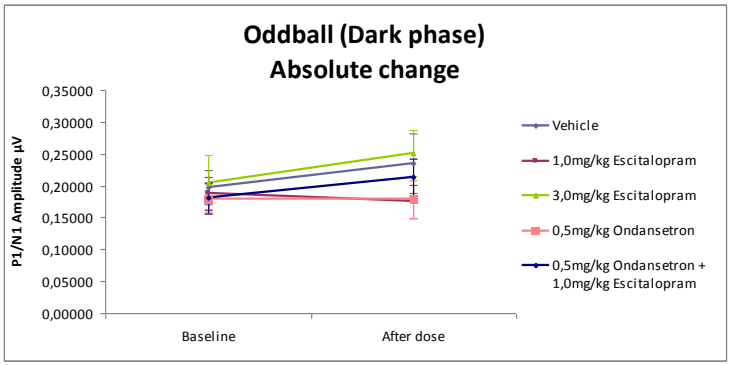


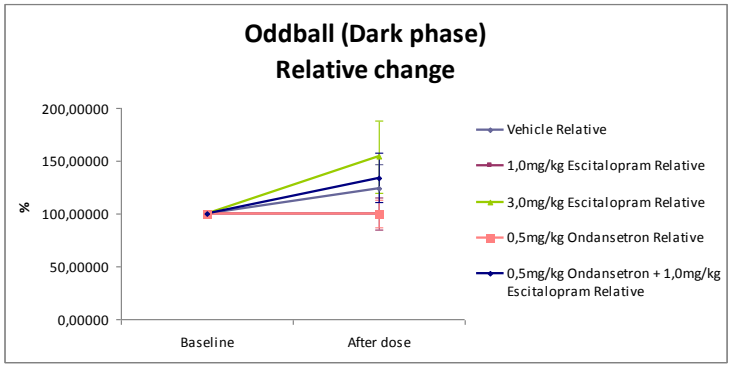


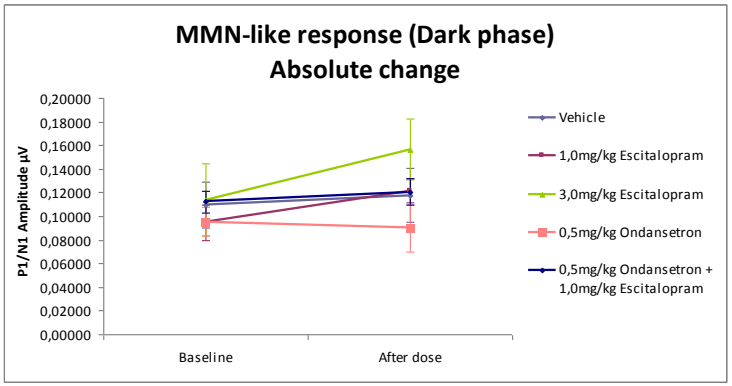
Second Escitalopram study
Parietal cortex
P1/N1 Amplitude

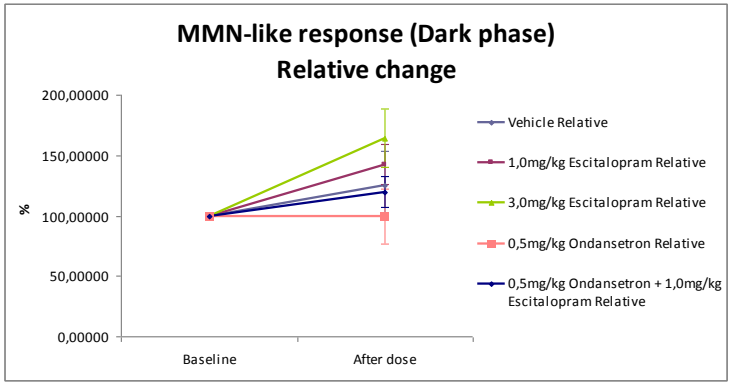




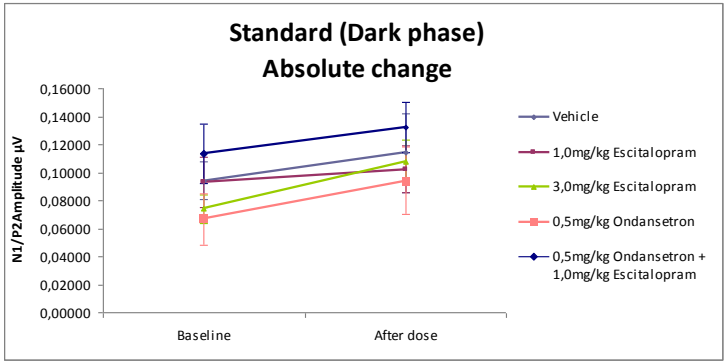


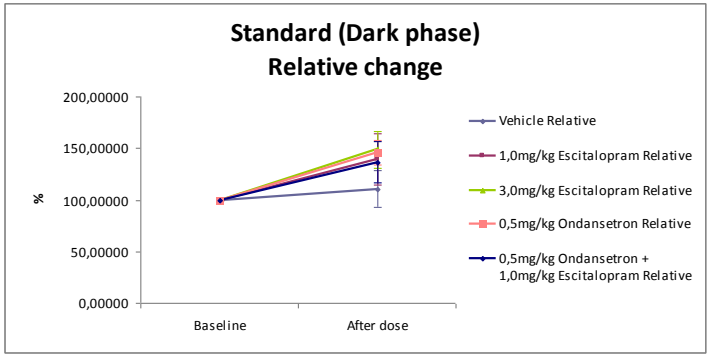


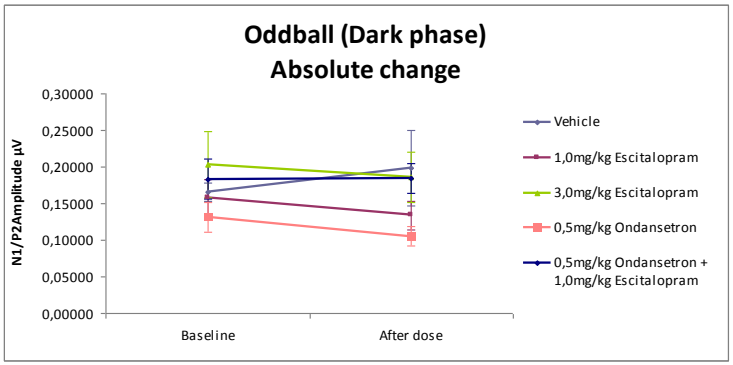


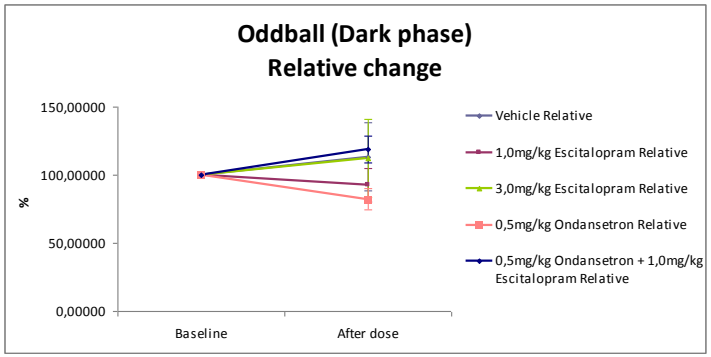


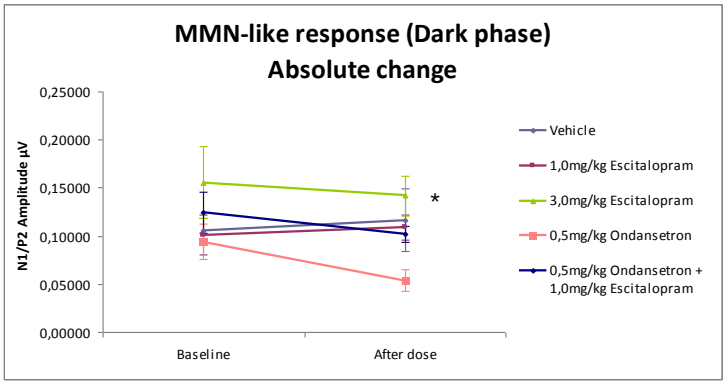
Second Escitalopram study
Parietal cortex
N1/P2 Amplitude

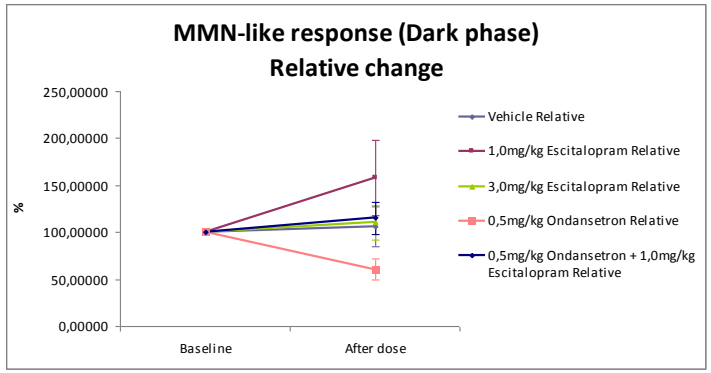






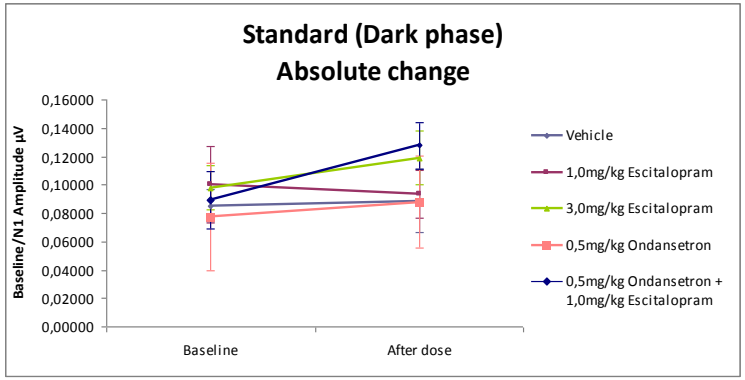


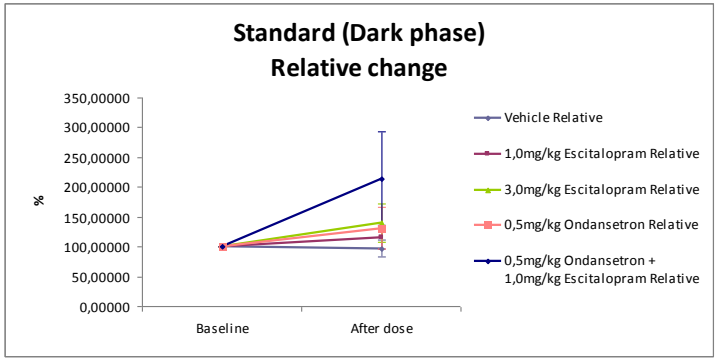


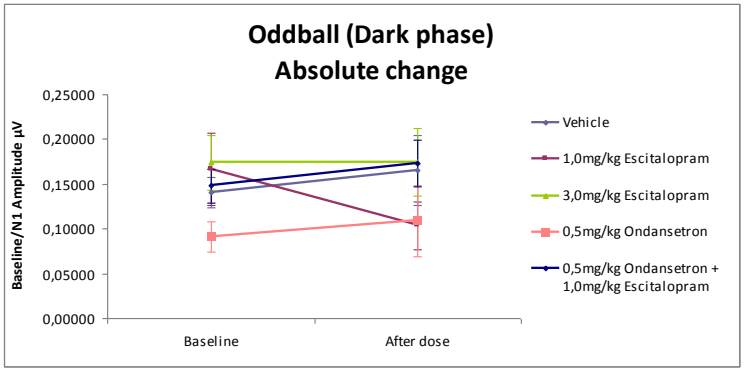


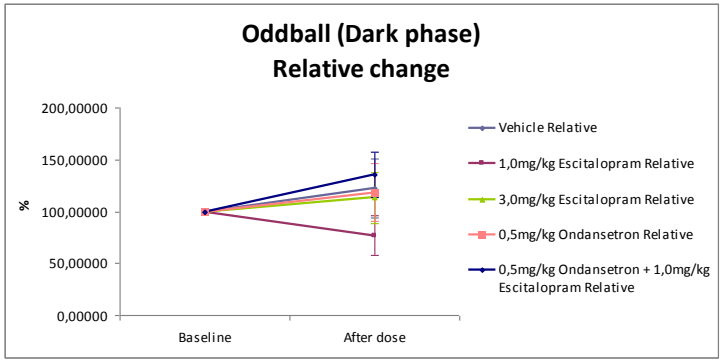
* p<0.05 between 3.0mg/kg escitalopram and 0.5mg/kg ondansetron (Bonferroni t-test)

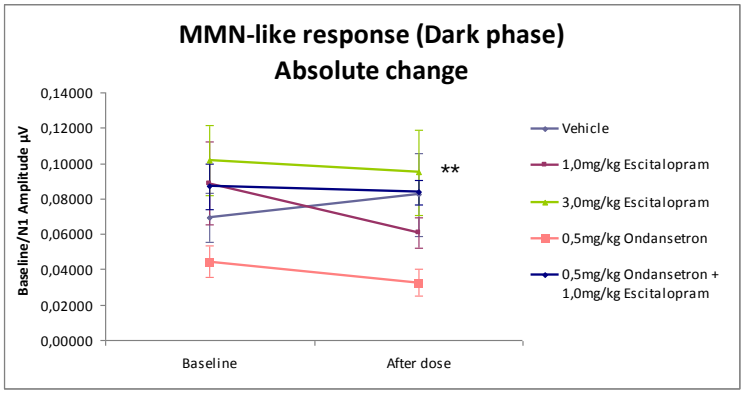
Second Escitalopram study
Parietal cortex
Baseline/N1 Amplitude

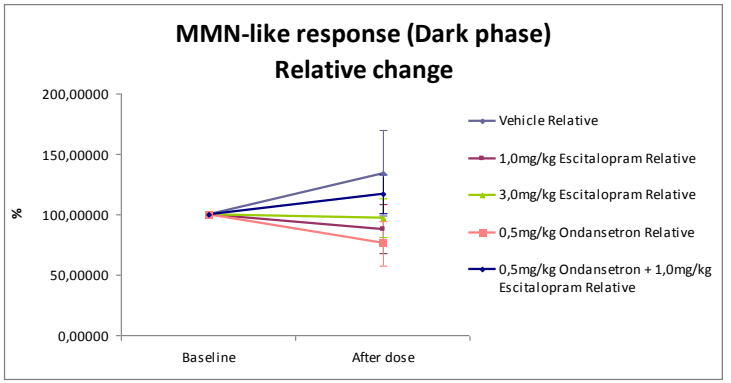






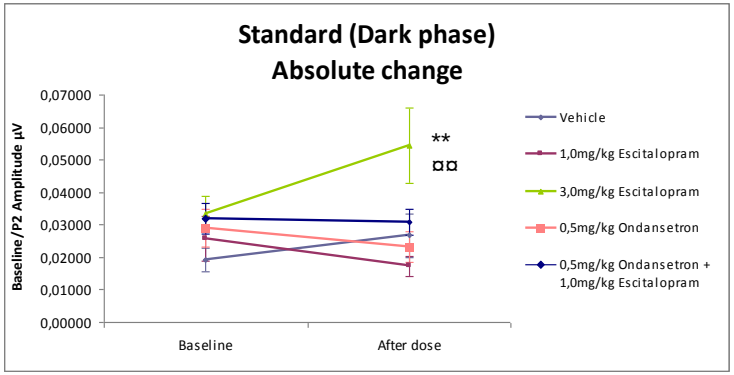






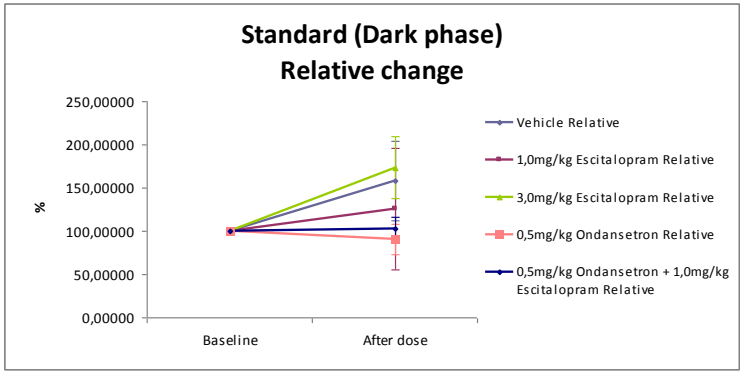
** p<0.01 between 3.0mg/kg escitalopram and 0.5mg/kg ondansetron (Bonferroni t-test)

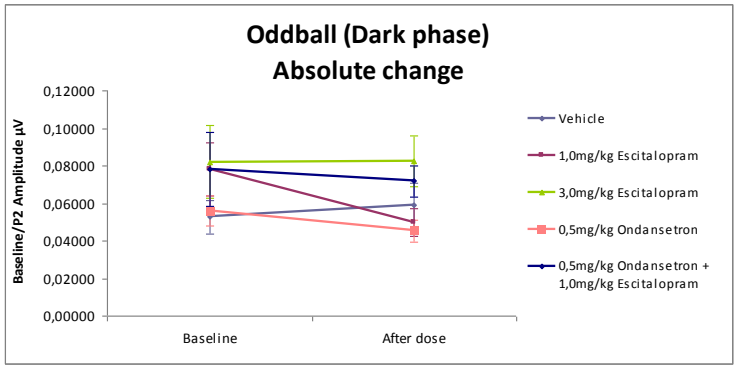
Second Escitalopram study
Parietal cortex
Baseline/P2 Amplitude

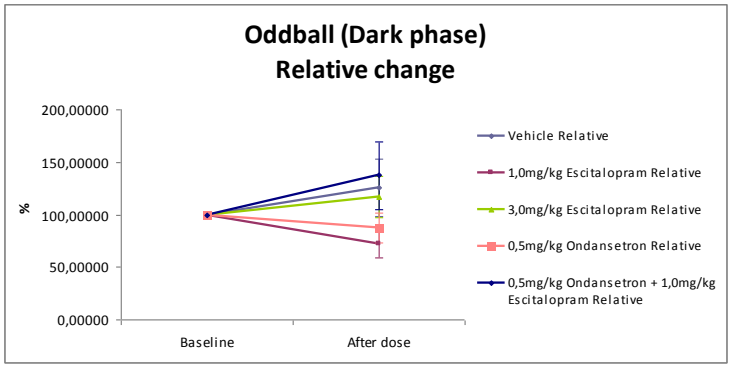


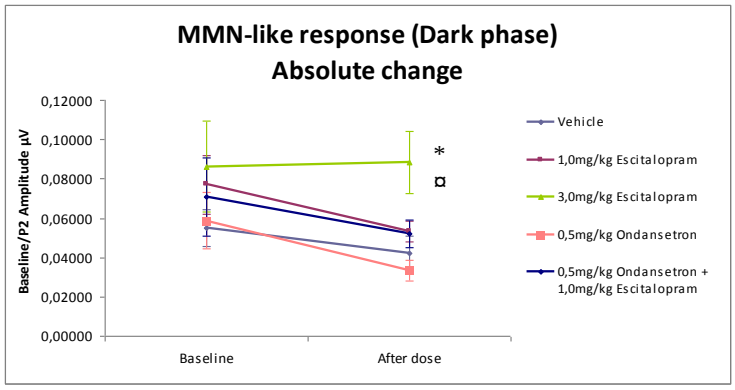
** p<0.01 between vehicle and 3.0mg/kg escitalopram

¤¤ p<0.01 between 1.0mg/kg escitalopram and 3.0mg/kg escitalopram (Bonferroni t-test)









* p<0.05 between vehicle and 3.0mg/kg escitalopram

¤ p<0.05 between 0.5mg/kg ondansetron and 3.0mg/kg escitalopram (Bonferroni t-test)