Statistical analysis of $GABA_A$ receptor modulators' effects in rats with focus on memory improvement and reversing of schizophrenic symptoms

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Abstract

In this project it was aimed to determine if (i) the selective α5 $GABA_A$ receptor inverse modulator α5IA-II would improve memory in the rat; (ii) the selective partial α2 and α3 (and full α5 agonist) $GABA_A$ receptor modulator NS.A would be effective in a putative animal model of schizophrenia; and, finally (iii) to develop a statistical model appropriately describing the pre-clinical data and enable a suitable test of drug effects. The effects of α5IA-II and NS.A in the models utilised were compared in all cases to the non-selective $GABA_A$ receptor modulator alprazolam, a benzodiazepine. All three $GABA_A$ receptor modulators were assessed in male SPRD rats tested in the following models: fear conditioning, trace fear conditioning and pre-pulse inhibition. In the latter model, pre-pulse inhibition, the three $GABA_A$ receptor modulators were tested for their ability to reverse PCP or amphetamine induced deficits which arguably reflect sensorimotor gating deficits seen in schizophrenia.

Whilst initial studies indicated that α5IA-II tended to improve fear conditioning notably during and after the tone period on the test day, this was not reproducible. In the trace fear conditioning experiment, the inclusion of a trace between the offset of the tone and onset of the shock was anticipated to retard memory relative to a normal fear conditioning group (i.e., offset of tone and onset of shock co-terminate). However, animals trained with and without a trace showed equivalent memory 24 hours after training. Therefore, any interpretation of the data for α5IA-II or other $GABA_A$ receptor modulators is equivocal.

In pre-pulse inhibition experiments, NS.A did not affect PCP induced pre-pulse inhibition impairment and surprisingly exacerbated the amphetamine induced pre-pulse inhibition impairment.

From a statistical approach it was found that a bias as well as variation is induced by the experimental equipment which finds expression in heterogeneity across the startle boxes. In order to assess the effect of the drugs in fear conditioning experiment a linear mixed effects model was defined. In order not to violate the model assumptions the heteroscedasticity obtained for the startle boxes was modelled by means of a variance function with the assignment of a variance parameter for each box. An exponential correlation structure was used to model residual autocorrelation.

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1. Introduction

The focus of this masters project is biological as well as statistical, and consequently the biological issues and the statistical analysis will be attached similar importance.

The biological aim of the project is to examine the role of α2, α3, and α5 subtypes of the $GABA_A$ receptor regarding (i) alleviation of symptoms connected to schizophrenia, and (ii) memory enhancement. An important part of the project has been to perform pre-clinical experimental work.

Initially the theoretical part will introduce schizophrenic symptoms and describe neurochemical dysregulations thought to play an important role in the symptomathology of schizophrenia, i.e. the dopamine and the NMDA receptor hypothesis. In the literature evidence of the involvement and dysregulated GABAergic processes in prefrontal cortex and connected areas are accumulating, and thus of special interest in this report. Consequently the most abundant GABAergic interneurons will be described as well as studies focusing at $GABA_A$ α2 receptors and post mortem examinations of dysregulated GABA processes. Studies investigating the role of $GABA_A$ α5 receptor will be discussed with focus on hippocampus-dependent memory improvement.

The pharmacology of the $GABA_A$ modulating compounds tested in fear conditioning and pre-pulse inhibition will be outlined and the experimental procedures utilised will be explained.

In continuation of the experimental section the statistical aspects considered in the development of the statistical model is presented. The aim of this part is to develop a statistical model appropriately describing the data in order to enable a suitable test of the drug effect. In order to reach this the following issues are considered. Possible factors likely to affect the response values are identified and if propitious included in the model in order to enhance estimate precision. Due to the repeated measures a linear mixed effects model is employed and residual autocorrelation is modelled. In addition, variance functions are employed to model heteroscedasticity. The interpretational aspects translating the results into a biological comprehension will be considered as well. The starting point for the development of the statistical model is the considerations presented in the preparatory project, which are assumed known.

Finally the biological theory and the statistically consideration will be utilised in order to discuss and interpret the data obtained in the pre-clinical studies.

2. Theory

2.1. Schizophrenia and cognition

2.1.1. Schizophrenia

Schizophrenia is a mental illness that affects 1% of the population. The manifestations of the illness can vary widely and it is generally believed that schizophrenia is not a single disease but rather a term that covers several symptoms. Patients that suffer from schizophrenia are characterised by loss of contact with reality together with a disruption of thought, perception, mood, and movement (**Bear et al., 2001**).

The symptoms of schizophrenia can be divided into two groups, positive symptoms (additional to normal experience) which include abnormal thought and behaviour and negative symptoms that refer to the absence of responses that normally are present (**Andreasen, 1982; Andreasen et al., 1982; Crow, 1980**). The positive symptoms include for example delusions, hallucinations, thought insertion (the belief that the thought of others are being inserted into one's mind), thought broadcasting (the belief that one's thoughts can be heard by others), disorganised speech and behaviour. Negative symptoms can consist of reduced emotional expression, passivity, depression, deficiency of speech and difficulty in initiating goal-directed behaviour. In addition schizophrenia patients suffer from deficits in cognitive function, which among other symptoms include attention impairment (**Bear et al., 2001;** reviewed by **Morris et al., 2005**). Cognition and the cognitive impairment observed in schizophrenia patients will be described further in section '2.1.2. Cognition and neural circuit deficits involved in schizophrenia'.

Minor volumetric differences have been observed in every cortical and subcortical brain structure of schizophrenic patients, and by the use of magnetic resonance imaging (MRI) structural alterations have been identified in hippocampal and amygdaloid complex, basal ganglia, frontal lobe, and thalamus e.g. The MRI identifications of these structural alterations have been replicated in a broad range of studies as reviewed by Antonova et al., 2004.

The neurobiological basis of schizophrenia is not clear, but physical changes in the fine structure and function of cortical connections have special interest in the

research of schizophrenia. Attention is especially focused on alterations in the chemical synaptic transmission mediated by dopamine (see '2.1.1.1. Dopamine') and glutamate (see '2.1.1.2. The NMDA receptor and glutamate') (**Bear et al., 2001**), and since the neuronal dysfunctions in schizophrenia are not focused to a single region of the brain but involve many regions, neural interactions and circuits in addition have special interest (see '2.1.2.2. Corticolimbothalamic circuit deficits').

2.1.1.1. Dopamine

The parts of the central nervous system that are involved in the regulation of movement, mood, attention and visceral functions all contain catecholaminergic neurons. Catecholaminergic neurons are able to synthesise three different neurotransmitters. These neurotransmitters are synthesised from tyrosine and all contain an identical chemical structure named a catechol, which is a 3,4-dihydroxylated benzene ring.

In the cytosol of the catecholaminergic neurons tyrosine is firstly converted to L-dihydroxyphenylalanine (dopa), which is converted into the neurotransmitter dopamine (DA) (see figure 2.1). In noradrenergic neurons dopamine is transported into synaptic vesicles and synthesised into norepinephrine (NE), which again can be made into epinephrine (adrenaline) in the cytosol of adrenergic neurons. Dopamine, norepinephrine and adrenaline are all neurotransmitters and are collectively called the catecholamines. There are no fast extra cellular enzymes that can degrade the catecholamines after they have been released into the synaptic cleft. Instead, their action is terminated by selective uptake back into the axon terminal via high–affinity Na^+-dependent transporters. Many drugs take advantage of this mechanism and work by

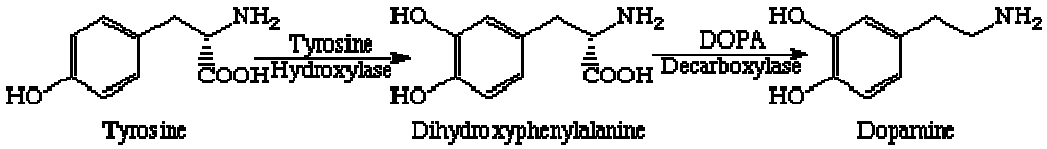


Figure 2.1. Dopamine is a neurotransmitter synthesised from tyrosine in the cytosol of catecholaminergic neurons. Tyrosine is converted to L-dihydroxyphenylalanine (dopa) by the enzyme tyrosine hydroxylase and dopa is thereafter converted to dopamine by the enzyme dopa decarboxylase. Dopamine is synthesized by catecholaminergic neurons in e.g. the ventral tegmental area, which projects to the prefrontal cortex, the nucleus accumbens, and other limbic structures. Enhanced action of dopamine in these systems named mesocortical and mesolimbic dopamine pathways is thought to be important in symptomathology of schizophrenia.

blocking the uptake of the catecholamines, thereby prolonging and enhancing the action of the neurotransmitters in the synaptic cleft.

The catecholaminergic axons and axonal terminals are spread throughout the central nervous system, but the cell bodies of the dopaminergic neurons are primarily placed in two specific regions of the midbrain, the pars compacta of the substantia nigra (a basal ganglia nucleus) and the ventral tegmental area (VTA). The two closely related groups of dopamine-containing neurons in these areas make up the diffuse modulatory dopamine system, which via great branching of the axons influences huge parts of the brain. The dopaminergic neuron in the pars compacta of the substantia nigra project axons to the striatum (a collective name for the two basal ganglia nuclei, putamen and caudate nucleus), where the initiation of voluntary movement is facilitated. Dopamine is in this way involved in the initiation of motor responses by environmental stimuli, and degradation of dopaminergic cells in this area causes the grievous motor disorder Parkinson's disease (**Bear et al., 2001**).

The dopaminergic neurons in the VTA innervate the frontal cortex (**Sasack et al., 1992**) and parts of the limbic system, for example the nucleus accumbens and the prefrontal cortex. These systems are named the mesolimbic and the mesocortical dopamine pathways respectively and are involved in certain adaptive behaviors, motivation and cognition. Enhanced action of dopamine in the dopamine systems is thought to play an important role in the symptomathology of schizophrenia (**Bear et al., 2001; Grace, 1991**). It has been observed in healthy humans that an overdose of amphetamine, which causes elevated concentrations of dopamine in limbic forebrain structures (especially in the nucleus accumbens), can lead to psychotic episodes with symptoms virtually indistinguishable from the positive symptoms of schizophrenia (**Randrup et al., 1972; Bear et al., 2001**). Typical antipsychotic drugs that are potent blockers of D_2 dopamine receptors can manage the positive symptoms of schizophrenia (**Seeman et al., 1976; Crow, 1980**), and it has been observed that their affinity for the D_2 dopamine receptors correlates clearly with their ability to control the symptoms schizophrenia (reviewed by **Seeman, 1980**). Hence an increase of dopamine in limbic forebrain structures is thought to be associated to the positive symptoms of schizophrenia (**Bear et al., 2001**)

2.1.1.2. The NMDA receptor and glutamate

Glutamate (glutamic acids) is an amino acid and one of the main fast excitatory neurotransmitters (**Chizh, 2002**) found in 40 % of all synapses in the mammalian brain (**Tsai et al., 2002**). Pyramidal cells (projecting/principal neurons) which are found in the hippocampus and cerebral cortex release glutamate as their neurotransmitter and are the major excitatory component of the cortex. Their long axons leave the cortex and make glutamatergic synapses in other cortical or subcortical areas (**Nolte, 2002; Shulman et al., 2005**).

Glutamate mediates its effect by binding to one of the four glutamate receptor types found in the central nervous system: the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, the kainate receptors, the metabotropic receptors and the N-methyl-D-aspartate (NMDA) receptors (**Chizh, 2002**). Only the NMDA receptor will be described further, since deficits in the corticolimbic NMDA receptor circuit are believed to be implicated in the pathophysiology of schizophrenia (reviewed by **Tsai et al., 2002**).

The NMDA receptor belongs to the class of heteromeric ion channel receptors, constructed on the basis of 7 different subunits named NR1, NR2A to NR2D, NR3A and NR3B. All NMDA receptors contain at least one NR1 and one NR2 subunit (**Kadieva et al., 2005**), the latter is responsible for the pharmacological characteristics of the NMDA receptor (reviewed by **Tsai et al., 2002**). The opening (activation) of the cation channel is caused by the simultaneous binding of glutamate and glycine to the NR2 and NR1 subunits, respectively (**Kadieva et al., 2005**). The NMDA receptor is not only transmitter-gated, in addition to the binding of glutamate and glycine the membrane also has to be depolarised, which means that the NMDA receptor is both transmitter- and voltage-gated dependent (**Bear et al., 2001**). When the membrane is at normal resting potential the channel is noncompetitively blocked by Mg^{2+}, but when the membrane is depolarised in the presence of glutamate Mg^{2+} is expelled (reviewed by **Tsai et al., 2002**).This channel opening allows efflux of K^+ and influx of Na^+ and Ca^{2+} which effects fast EPSPs (Excitatory PostSynaptic Potential). Additionally, the postsynaptic increase in Ca^{2+} activates a second messenger cascade that increases the synapse transmission and result in Long-Term Potentiation (LTP) (see '2.1.2.1. Learning, memory and LTP') (**Bear et al., 2001**)

For the last three decades the dopamine hypothesis has been mainly investigated in relation to schizophrenia (**Carlsson, 1988**), but in general dopaminergic hyperactivity is associated primarily to the positive symptoms (**Laruelle et al., 1999**), whereas the negative

and cognitive symptoms of schizophrenia might be caused by dysregulation of other systems. In 1980 J. S. Kim and his team reported a reduced concentration of glutamate in the cerebral spinal fluid of patient suffering from schizophrenia (**Kim et al., 1980**). Henceforth it has been observed in a postmortem study that glutamate concentration is decreased in the prefrontal cortex and hippocampus in schizophrenic patients (**Tsai et al., 1995**) and today the focus in many studies of the pathology of schizophrenia also concentrates on the deficits in the neurotransmission of glutamate as reviewed by Tsai et al., 2002. The hypothesis that schizophrenia symptoms result from hypofunction of certain glutamatergic neural systems is supported by studies on the expression of NMDA receptors in the brain of schizophrenics. Schizophrenia patients have decreased expression of NMDA receptor mRNA in frontal cortex (**Sokolov, 1998**). In experiment with genetically engineered mice that only express 5 % of normal level of functional NMDA receptors (**Mohn et al., 1999**), it was shown that the decrease in NMDA receptors caused behaviours similar to pharmacologically induced models of schizophrenia such as repetitive movement, increased motor activity, and altered social interaction with other mice. This behaviour was subsequently ameliorated by antipsychotic drugs that antagonised dopamine receptors, suggesting that inhibition of dopamine receptors may normalise glutamatergic hypofunctions (*Ibid.*).

2.1.1.3. PCP and the glutamate hypothesis, clinically evidence

Phencyclidine hydrochloride (PCP) is a NMDA receptor antagonist which was introduced in the 1950s as a surgical anaesthetic (the structure is shown in table 2.1). But PCP displayed severe postoperative side effect such as hallucination, paranoia, disorganised speech and agitation that lasted for days and today it is only used clinically as an anaesthetic for animals (**Bear et al., 2001**). However PCP is used illicitly and it is of special interest that the abusers of PCP experience and express symptoms that are remarkably similar to the ones of schizophrenia patients (reviewed by **Morris et al., 2005**). Actually PCP abusers are commonly misdiagnosed to suffer from schizophrenia (*Ibid.*), and the psychosis induced by NMDA receptor antagonists includes both the positive and negative symptoms as well as cognitive deficits characteristic of schizophrenia (**Adler et al., 1999**).

PCP is a non-competitive antagonist of the NMDA receptor and binds to a site within the channel pore of the receptor. This site is only accessible when the NMDA

receptor channel is open and after binding of PCP the opened channel is blocked for the influx of Ca^{2+}. The PCP inhibition of the NMDA receptor is often utilised in animal experiments to model the hypofunctions of glutamatergic activity found in schizophrenia patient (reviewed by **Morris et al., 2005**).

The studies and observations presented in '2.1.1.2. The NMDA receptor and glutamate' taken together with the reported symptoms of PCP abusers support the hypothesis that alterations in the chemical synaptic transmission mediated by glutamate causes some of the symptoms of schizophrenia. One of the drug targets in the search for a drug that can normalise the symptoms of schizophrenia is therefore the NMDA receptor, and it is hoped that increasing the responsiveness of these receptors can alleviate the symptoms of schizophrenia (**Bear et al., 2001**).

In summary it is speculated that hyperactivity of dopamine in limbic structures of the forebrain (nucleus accumbens) (reviewed by **Seeman, 1980**), and hypoactivity of glutamatergic corticolimbic NMDA receptors (reviewed by **Tsai et al., 2002; Morris et al., 2005**) may result in impairments in the inter-actions between prefrontal cortex and the limbic system, the basal ganglia and the thalamus (reviewed by **Antonova et al., 2004;** reviewed by **Morris et al., 2005**), which is thought to be the major cause of schizophrenia, see '2.1.2.2. Corticolimbothalamic circuit deficits'.

2.1.2. Cognition and neural circuits involved in schizophrenia

Schizophrenia patients suffer from a broad spectrum of deficits in cognition, which have a conspicuous depressing influence on the social and occupational functions of the patient (**Green, 1996**). Before discussing further with respect to the neural circuit deficits speculated to be responsible for cognitive and the other symptoms of schizophrenia, it will first briefly be discussed what is mean by learning and memory.

2.1.2.1. Learning, memory and LTP

Memory can be categorised in two types; it can be either procedural memory (also referred to as non-declarative or implicit memory) or declarative memory (also referred to as explicit memory). These phrases have been described in the preparatory project, see part '2.1.5. Cognition, Memory and Hippocampus', but briefly procedural memory is the unconscious memory learned through repetition of a certain performance and includes classical conditioning (the association of a reward e.g. a biscuit with a tone,

where the tone provokes the reflex responses associated with the biscuit after a number of tone and biscuit 'pairings'), whereas declarative memory is the conscious memory of facts (sematic memory) or events (episodic memory) (**Delcomyn, 1998**). Declarative memory also includes working memory, which is categorised as a higher level cognitive function (**Baddely, 1982**) that for example includes the ability to create and explain strategies and solve problems. Additionally, memory can be classified as either short-tem or long-term. (**Delcomyn, 1998**). Procedural memory is primarily associated with structures in the cerebellum and basal ganglia (reviewed in **Thompson et al., 1994; Saint-Cyr et al., 1998**), while the hippocampus has an important role in declarative memory (**Squire et al., 1991**) and especially in working memory (**Olton et al., 1979**) together with the prefrontal cortex (**Milner et al., 1985**).

Learning and memory processes alter the structure and function of nerve cells and their connections (**Wenzel et al., 1980**), a phenomenon referred to as plasticity. The nerve cells involved are not specialised 'memory cells', but often the sensory neurons, which following a stimulus via synaptic connection affects motor neurons or interneurons (**Bear et al., 2001**). The simplest learning-processes are named habituation, sensitisation and conditioning (**Bear et al., 2001**). Habituation and conditioning are terms closely connected to the fear conditioning experiment, which has been executed in this project and in which the freezing of the rats is measured. Fear conditioning is observed when an animal over one or a few trials learns to associate a conditioned stimulus (a neutral stimulus) with an unconditioned stimulus (an electric foot shock) and subsequently starts to respond (i.e. freeze) when it is presented with the previously neutral but now conditioned stimuli, see part '2.1.5. Cognition, Memory and Hippocampus' and part '2.3.3. Fear Conditioning' in the preparatory project. The unconditioned stimulus activates interneurons that via axo-axonic connections influence sensory neurons of the conditioned stimuli. If the unconditioned stimulus activates these sensory neurons immediately after they are stimulated by the conditioned stimulus it causes an elevated presynaptic facilitation and an increased presynaptic firing (*Ibid.*).

Declarative learning is an even more complex form of learning than procedural learning and is dependent on the hippocampus, which is important for the storage of declarative memory. In the storage process, different afferent pathways are involved, which starts in the entorhinal cortex, running through the CA1 region and ending in the pyramidal cells of the CA3 region of the hippocampus. If a brief high frequency

stimulus enters these pathways an elevated excitatory postsynaptic potential in the hippocampus will be established, which could last for hours or weeks and provoke sustained presynaptic action potentials (**Bliss et al., 1973; Bear et al., 2001**). This is named Long-Term Potentiation (LTP) and is thought to be the basic mechanism underlying formation and the storage of declarative memory. LTP occurs in many parts of the brain including the hippocampus as mentioned above and the prefrontal cortex (**Laroche et al., 1990**). In addition, the hippocampus is important in spatial memory and the recognition of a familiar environment (**Morris et al., 1982; Morris et al., 1996**).

2.1.2.2. Corticolimbothalamic circuit deficits

The most consistent finding in association to schizophrenia is the impairment of higher cognitive functions (reviewed by **Green, 1996**) that require active information processing, and which include sustained selective attention, executive functions, working memory, language skills, and motor processing (reviewed by **Antonova et al., 2004**). Imaging studies of cerebral blood flow and metabolic activity of schizophrenia patients has shown decreases in activity in the prefrontal cortex, hippocampus, striatum, nucleus accumbens, and thalamus (reviewed by **Morris et al., 2005**). The deficit in processing of memory and working memory is related to the prefrontal cortex and important in the pathology of schizophrenia. The prefrontal cortex does not function individually, but is part of corticolimbothalamic circuits which runs from different parts of the prefrontal cortex to different parts of striatum, pallidum, thalamus and thereafter returning to the prefrontal cortex; the latter are also influenced by the hippocampus (*Ibid.*) (see figure 2.3).

These forebrain circuits are thought to participate in the regulation of pre-pulse inhibition, which is a useful tool in the study of information processing and gating mechanisms (sensorimotor gating) (reviewed by **Braff et al., 2001**). Pre-pulse inhibition of the acoustic startle response is seen as an attenuation of the startle response, when prior to the startle eliciting stimulus a weaker, non-startle-provoking stimulus occurs (reviewed by **Geyer et al., 2001**). The acoustic startle response is depressed for about one second by the pre-pulse and mediated by active neuronal inhibitory processes (**Davis, 1979**). Back in 1978 it was reported that schizophrenia patients showed impairment in the normal inhibition of the acoustic startle response after presentation of a pre-pulse (reviewed by **Hamm et al., 2001**), and today the evidence for impairment of pre-pulse inhibition in schizophrenic patients is accumulating (**Davis, 1979;** reviewed by **Hamm et al., 2001;** reviewed by **Braff et al., 2001;** reviewed by **Geyer et al.,**

2001). See section '2.3.1.2. Pre-pulse inhibition', where pre-pulse inhibition is described further, since the model is used in the experimental part of this project.

Even more interesting than the decreased activity in forebrain structures of schizophrenic patients is the robust and consistent finding of reduced metabolic activity observed in schizophrenic patients when they are executing cognitive tasks, and the correlation of these reductions with the severity of the cognitive and negative symptoms in the individual patients (reviewed by **Morris et al., 2005**).

Chronic exposure to PCP has been reported to produce dopamine hypofunction in the dorsolateral prefrontal cortex of monkeys and long-lasting cognitive deficits. These were ameliorated by the atypical antipsychotic clozapine, which does not have strong D2 receptor antagonist properties (**Jentsch et al., 1997**). Dysfunction of the amygdala is also considered to contribute to cognitive abnormalities. In post mortem studies of schizophrenic patients substantial histopathological alterations in the CA2 and CA3 areas of hippocampus have been observed (**Falkai et al., 1986; Jeste et al., 1989; Benes et al., 1998**), which is suggested to be induced by amygdala dysfunction (**Benes et al., 2000**). Grace and Rosenkranz made in 1999 a study with rats which suggested that the prefrontal cortex inhibits projecting neurons in the amygdala and that this inhibition was induced by activation of dopamine receptors in amygdala (**Rosenkranz et al., 1999**); but more research is needed concerning the role of amygdala in cognition and schizophrenia (**Antonova et al., 2004**).

Overall, it is suggested that schizophrenia and cognitive impairments are associated to dysfunction in the corticolimbothalamic circuit and hypofunction of glutamatergic activity, and that the cognitive deficits include dopaminergic hypoactivity in the dorsolateral prefrontal cortex. The activity of this circuit is strongly regulated by GABAergic interneurons (reviewed by **Morris et al., 2005**), which will be presented in section '2.1.3.1. GABAergic interneurons'.

2.1.3. $GABA_A$ receptors and cognitive deficits in schizophrenia

As mentioned in '2.1.1.2. The NMDA receptor and glutamate' pyramidal cells are the major excitatory component of the cortex, where they receive sensory input from the thalamus (afferents terminate primarily in layer IV) and other cortical areas (afferents terminate in layer II and especially in layer III) and process the information and 'send it'

to the appropriate brain regions. The remaining neurons in the cortex are collectively named nonpyramindal cells. Unlike pyramidal neurons, nonpyramidal neurons compose short axons that are distributed within the cortex, and the majority of the nonpyramidal cells make inhibitory synapses by releasing the neurotransmitter GABA (**Nolte, 2002**).

Eugene Robert postulated in 1972 that a dysfunction of GABAergic mechanisms could contribute to the symptoms of schizophrenia (**Roberts, 1972**) and the disturbances of higher cognitive functions (reviewed by **Benes et al., 2001**). Nonpyramidal GABAergic interneurons ensure that pyramidal neurons have rhythmic inhibitory firing which is thought to be an important neural correlate of higher cognitive processes which are impaired in schizophrenic patients (mentioned in the first part of the section '2.1.2.2. Corticolimbothalamic circuit deficits') (reviewed by **Freund, 2003**).

Reduced levels of parvalbumin in laminae III and IV of the prefrontal cortex (**Beasley et al., 1997; Pierri et al., 1999**) and the hippocampus (**Zhang et al., 2002**) are thought to reflect a dysfunction in GABAergic cells, and dysfunction in the corticolimbothalamic circuits are related to the cognitive deficits of schizophrenia. These observations make the basis of the hypothesis that altered parvalbumin expression (**Cochran et al., 2003**) and deficits in the GABAergic neurotransmission are associated with impaired working memory, information processing and gating of sensory information (reviewed by **Benes et al., 2001**). But before going further into post-mortem studies suggesting dysfunction of GABAergic cells, the GABAergic interneuron will be introduced.

2.1.3.1. GABAergic interneurons

All GABAergic interneurons express and release obviously the neurotransmitter GABA, and the firing of these inhibitory interneurons have the highest frequency compared to other cortical neurons. Consequently GABAergic interneurons have a major influence on the synchronous firing of pyramidal neurons (reviewed by **Guidotti et al., 2005**). GABAergic axon terminals terminate either on the cell body, the dendrites, the dendritic spine necks or the axon hillocks of pyramidal neurons, which means that different GABAergic interneurons differentially modulate pyramidal cell firing rate (*Ibid.*). Additionally, GABAergic interneurons mediate the inhibition of pyramidal neurons either by feedback or feedforward inhibition (reviewed by **Shulmam et al., 2005**). Feedback inhibition appears for example when a pyramidal neuron is activated by an excitatory input. The activated pyramidal neuron then activates a GABAergic interneuron, which

consequently feedback inhibits the pyramidal neuron, which it was activated by. Feedforward inhibition can be exemplified by the information processing in the hippocampus that runs from the CA3 region of the hippocampus and to the CA1 region. The excitatory input in this situation is due to the activated pyramidal cell in the CA3 region of the hippocampus, which in parallel activates both a pyramidal neuron and a GABAergic neuron in the CA1 region. After the activation of these two neurons, the GABAergic neuron feedforward inhibits the activated CA1 pyramidal cell (*Ibid.*). Both feedback inhibition and feedforward inhibition serve to stabilise the activity of pyramidal neurons and regulate the rhythmic responses of pyramidal neurons. By these mechanisms GABAergic interneurons in cortical regions control higher cognitive functions (reviewed by **Benes et al., 2001**). In the prefrontal cortex 'fast-spiking' GABAergic interneurons (neurons found in the cortex, hippocampus, and striatum, which have a short duration action potential and afterhypolarisation and make repetitive firing) receive thalamic excitatory input and mediate fast feedforward thalamocortical inhibition, and finally contribute to the synchronising of firing in the cortex (*Ibid.*).

As mentioned, higher cognitive functions impaired in schizophrenic patients are dependent on synchronous firing of pyramidal cells. The synchronising of the pyramidal output is controlled by perisomatic inhibitory cells that innervate the somata, proximal dendrites and axon initial segment of the pyramidal cell (reviewed by **Freund, 2003**). Involved in this perisomatic inhibition are the GABAergic interneurons: Chandelier cells and basket cells which make axo-axonic and axo-somatic synaptic contact on pyramidal cells, respectively (see figure 2.2). A single perisomatic inhibitory cell is able to synchronise the action potential discharges from many pyramidal cells it innervates (*Ibid.*).

The three most abundant subtypes of GABAergic interneurons are the chandelier cells, the basket cells, and the double bouquet cells (reviewed by **Guidotti et al., 2005**). Every single one of these neurons establishes synapses with hundreds of different pyramidal neurons (reviewed by **DeFilipe et al., 1992**). As mentioned GABAergic interneurons make synaptic connections to different locations on the innervated cells, and the categorisation of the GABAergic interneurons are based on the type of synaptic connections they form (reviewed by **Shulman et al., 2005**).

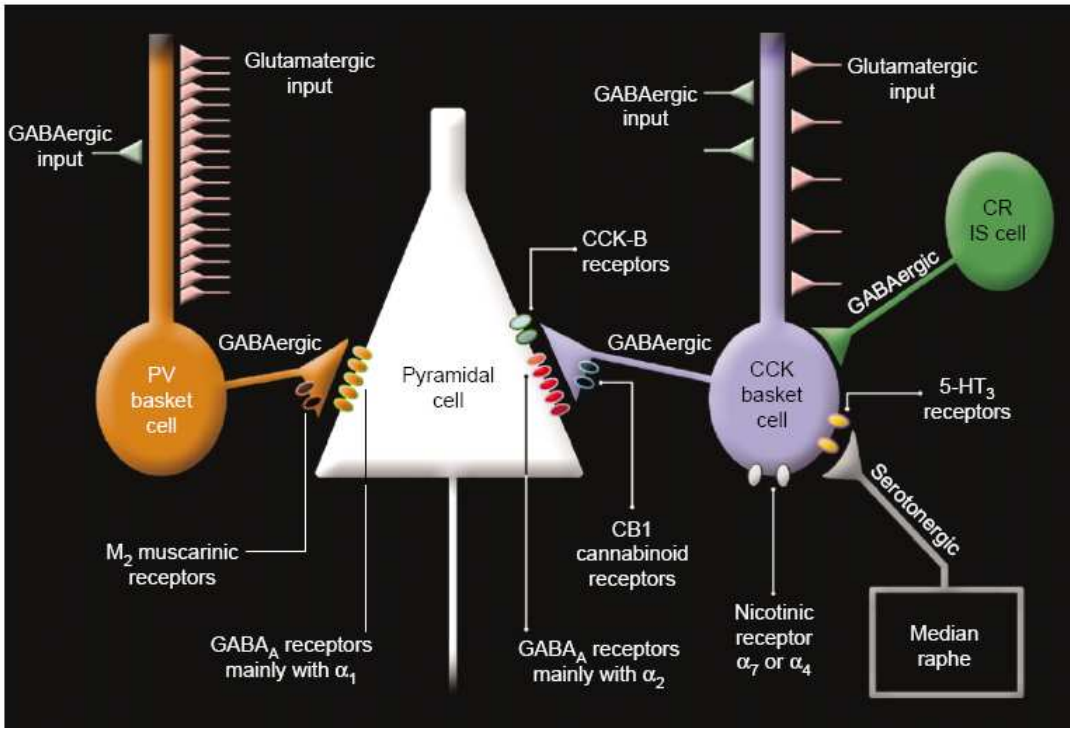


Figure 2.2 (**Freund, 2003**) A schematic view of perisomatic inhibition of pyramidal cell caused by basket cells, which is a GABAergic interneuron. The basket cells make axo-somatic contact on pyramidal cells in order to synchronise the action potential discharges from the pyramidal cells they innervate. Two different types of basket cells are shown. One type expresses cholecystokinin (CCK), whereas the other type expresses parvalbumin (PV). Both CCK and PV are Ca^{2+}-binding-proteins, the latter is as well expressed by chandelier cells

Chandelier cells make axo-axonic contact to the axon initial segment of pyramidal cells. The chandelier cells inhibit the propagation of an action potential in pyramidal cell axons by evoking large IPSPs (Inhibitory PostSynaptic Potential) in the postsynaptic pyramidal cell and consequently modulating the output of the pyramidal cell. The chandelier cells are primarily found in cortex layer II and III and not directly connected with thalamic afferent fibers, and thus chandelier cells indirectly inhibit the responses which are provoked in the cortex by input from the thalamus (reviewed by **Benes et al., 2001**).

Basket cells make axo-somatic contact to the apical dendrites of pyramidal cells and the basket cells can consequently alter the pyramidal cell membrane potential at the cell body level, which make them a very potent inhibitor compared to cells that make contact on a distant point along the dendritic tree. The basket cells are located in layers III to V of the cortex and are innervated by thalamic afferent neurons as the only

interneuron in the cortex (*Ibid.*). After a sensory input has reached thalamic neurons basket cells are subsequently activated and feedforward inhibit pyramidal cells (reviewed by **Shulman et al., 2005**). The basket cells in this way influence the receptive field of the cortex, and subsequently modulate the output of pyramidal cells.

Double bouquet cells make axo-dendritic contact to many dendritic shafts and spines on branches of dendrites of pyramidal neurons. Like chandelier cells, the double bouquet cells are located in layers II and III of the cortex and are not innervated directly by thalamic afferents. Instead of directly controlling the output of the pyramidal cells, dendritic inhibition is suited to controlling the efficacy and plasticity of excitatory synaptic inputs that reach pyramidal cell dendrites (reviewed by **Benes et al., 2001**). The double bouquet cells have a very high density especially in layer III, and are speculated to innervate and inhibit the chandelier and basket cells as well, and in this way provoke disinhibition (**Somogyi et al., 1981; Gabbott et al., 1996**). Innervations of GABAergic interneurons can either be intrinsic or extrinsic, originating either from other cortical areas or other subcortical brain structures, respectively (reviewed by **Benes et al., 2001**).

In addition to the release of GABA, the different subtypes of GABAergic interneurons express different types of intracellular and extracellular proteins (reviewed by **Guidotti et al., 2005**). In postmortem studies some of these proteins are frequently quantified and used as markers for GABAergic interneurons. Many of the GABAergic interneurons express Ca^{2+}-binding-proteins (CBP), which function in order to maintain intracellular calcium homeostasis. The binding of calcium by CBPs can counteract an elevation in calcium concentrations, that otherwise could result in cell death. Parvalbumin is a CBP that is only expressed in chandelier and basket cells (some basket cell types do not contain parvalbumin, but instead they contain cholecystokinin (CCK)) (reviewed by **Shulman et al., 2005**). Parvalbumin positive neurons are mainly found in cortex, hippocampus and striatum and are 'fast-spiking' interneurons. Calbindin is another CBP that is expressed mainly by double bouquet neurons, as well as calretinin, which is another CBP mainly expressed by double bouquet neurons (reviewed by **Benes et al., 2001**).

Reelin is coexpressed and released with GABA, and serves to regulate extrasomatic protein synthesis (**Dong et al., 2003**). It has been shown that reelin can induce LTP in rat hippocampal slices (**Weeber et al., 2002**), and it is speculated that reelin by modulating dendritic spine plasticity affects learning and memory. The GABA signal is

terminated by the reuptake of GABA from the synaptic clefts, and in post mortem studies the GABA membrane transporter 1 (GAT1) is used as a marker for GABAergic activity. A direct marker for GABAergic interneurons is the enzyme glutamic acid decarboxylase (GAD) which is the rate limiting enzyme in the biosynthesis of GABA from glutamate (reviewed by **Guidotti et al., 2005**). GAD occurs in two isoforms; GAD_{65} and GAD_{67}, named in accordance to their molecular weight of 65 kD and 67 kD, respectively (reviewed by **Shulman et al., 2005**). GAD_{65} is primarily found in axon terminals while GAD_{67} is found in the somata and dendrites of GABAergic interneurons (reviewed by **Benes et al., 2001**).

2.1.3.2. GABAergic deficits in man, post mortem studies

Post mortem studies of the brains of schizophrenia patients have in general shown a downregulation of the above mentioned markers of GABAergic interneurons or presynaptic GABA neurotransmission (reviewed by **Guidotti et al., 2005;** reviewed by **Benes et al., 2001;** reviewed by **Shulman et al., 2005**). Decreases in the concentrations of GAD_{67}, reelin, GAT1 and parvalbumin have been measured to be as high as 30 - 50 % in schizophrenic patients compared to normal volunteers (**Guidotti et al., 2000; Impagnatiello et al., 1998**). It is speculated that such decreases are not only related to neuronal loss of non-pyramidal cells, but rather related to downregulation of gene expression in GABAergic neurons (reviewed by **Guidotti et al., 2005**). Still it has been shown that the density of GABAergic neurons is reduced, particularly in prefrontal cortex layer II (**Benes et al., 1991**), the limbic system, and the CA2 region of the hippocampus (**Benes et al., 1998**). Decreases in the expression of GAT1 in the prefrontal cortex (**Volk et al., 2001**) mirror the reduction in reduced GABA uptake sites measured in amygdala, hippocampus and temporal cortex of schizophrenia patients (**Simpson et al., 1989**); a decrease in GABA reuptake sites speculated to be a compensatory response to a decrease in synaptic GABA concentration. In many of the studies it is not the proteins that are measured directly, but instead the mRNA levels. The highest reduction in mRNA levels has been observed in the levels of parvalbumin-mRNA in all regions of hippocampus and in the prefrontal cortex of schizophrenia patients, and consequently the greatest deficit in schizophrenia patients is speculated to be related to the chandelier cells and basket cells (reviewed by **Shulman et al., 2005**). In addition to reduced GABAergic neuronal density in the prefrontal cortex, the evidence for GABA deficits in schizophrenia has in post mortem studies been found in many other brain regions; low

GABA concentrations have been found in amygdala, nucleus accumbens and thalamus and low concentrations of GAD have been measured in nucleus accumbens, amygdala, hippocampus and putamen (*Ibid.*).

The literature concerning the above mentioned measurements of GABAergic mechanisms are marked by failures to replicate findings, potentially due to different types of methodology employed or heterogeneity in patient population (reviewed by **Benes et al., 2001**). Still in general the literature agree that GABA deficits may be a important factor in the pathophysiology of schizophrenia (reviewed by **Guidotti et al., 2005;** reviewed by **Shulman et al., 2005;** reviewed by **Benes et al., 2001**). As mentioned before the GABAergic interneurons regulate the input and output of pyramidal neurons of the cortex and hippocampus. Failures in such GABAergic mechanisms can consequently be speculated to result in elevated and uncoordinated firing of pyramidal excitatory cells in the cortex and following such elevated and uncoordinated firing may spread to other areas of the corticolimbothalamic circuits (**Morris et al., 2005**). Subsequently other mechanisms may start to compensate for the missing suppression of elevated excitability in the forebrain, which may result in comprehensive suppression of GABAergic interneurons in the circuit and connected brain areas and finally result in metabolic hypoactivity in cortical and subcortical structures (*Ibid.*). This is in agreement with the depressed metabolic activity observed in schizophrenic patients as described in part '2.1.2.2. Corticolimbothalamic circuit deficits', and the cognitive deficits (see figure 2.3).

Finally the resulting decreases in synaptic GABA concentrations are thought to be counteracted by postsynaptic increases in $GABA_A$ receptors on both non-pyramidal and pyramidal neurons (reviewed by **Benes et al., 2001;** reviewed by **Shulman et al., 2005;** reviewed by **Guidotti et al., 2005**). In post mortem studies of the prefrontal cortex and hippocampus of schizophrenia patients it has been found that the postsynaptic expression of $GABA_A$ receptors on pyramidal neurons is increased up to 100 % (**Volk et al., 2002**). In the literature it is widely agreed, that this upregulation is a direct consequence of a decreased GABAergic tone. New treatments for schizophrenia are consequently focussed on GABAergic synaptic transmission and normalising of their function. Enhancing $GABA_A$ receptor function by an agonist or selective positive allosteric modulator is consequently a logical suggestion for treating schizophrenia (reviewed by **Guidotti et al., 2005**). The $GABA_A$ receptor and the

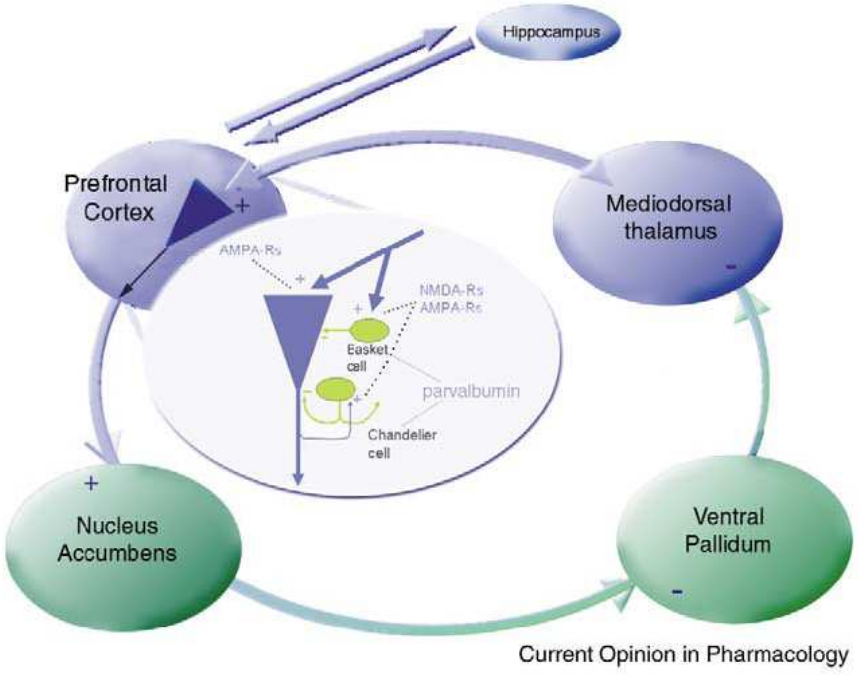


Figure 2.3. (**Morris et al., 2005**) Schematic drawing of the corticolimbothalamic circuits which run from different parts of the prefrontal cortex to different parts of striatum/nucleus accumbens, pallidum, thalamus and thereafter returning to the prefrontal cortex; the latter is also influenced by the hippocampus. It is suggested that schizophrenia and cognitive impairments are associated to dysregulations in these circuits such as hypofunction of glutamatergic activity or increased dopamine concentration in nucleus accumbens. Failures in GABAergic mechanisms are thought to cause elevated and uncoordinated firing of pyramidal excitatory cells in the cortex and following spread to other areas of the corticolimbothalamic circuits

different subtypes will be discussed further in part '2.2. The role of different $GABA_A$ receptor subtypes in schizophrenia'.

2.1.4. Dopamine, NMDA-receptors and GABA mechanisms

It has been shown that GABAergic neuronal systems in the prefrontal cortex appear to influence dopamine release in the dorsolateral striatum (**Matsumoto et al., 2005**). Matsumoto et al. have measured the concentrations of GABA and dopamine in the prefrontal cortex and dorsolateral striatum in rats after exposure to contextual fear conditioning (CFC). They found elevated GABA and dopamine concentrations in the prefrontal cortex together with increased freezing behavior of the animals. By contrast, the same CFC procedure had no effect on the concentrations of GABA and dopamine in the striatum. Injection of a $GABA_A$ receptor antagonist into the prefrontal cortex before exposure to CFC caused attenuation in the freezing behavior and increased dopamine release in the dorsolateral striatum. These authors concluded that $GABA_A$ receptors in the prefrontal

cortex modulate dopamine release in dorsolateral striatum under CFC conditions (*Ibid.*). Amphetamine (dopamine releaser) which causes enhanced action of dopamine in the mesolimbic system, as mentioned earlier, has been shown to result in decreases of extracellular GABA concentration and expression of GAD_{67} mRNA in the nucleus accumbens of rats after repeated injections (**Lindefors et al., 1992**). In another study it was observed that administration of a D_2 receptor agonist to rats also caused lowering of GAD_{67} mRNA in the striatum (**Laprade et al., 1995**). It has in addition been shown that some of the dopamine afferents from the VTA project directly to GABAergic interneurons in of rat prefrontal cortex (**Verney et al., 1990**), and parvalbumin positive GABAergic interneurons have been observed to express dopamine receptors (**Vincent et al., 1993; Vincent et al., 1995; Davidoff et al., 1998**).

Since it is known that NMDA-receptors are involved in the activation of parvalbumin positive neurons and calretinin positive cells (**Jones et al., 1993; Goldberg et al., 2003**), it is likely that a NMDA blocker such as PCP would suppress the activation of these GABAergic interneurons. Administration of NMDA receptor antagonists to rats has been observed to cause a reduction of the parvalbumin level in the prefrontal cortex (**Morris et al., 2005**) as well as in the hippocampus (**Keilhoff et al., 2004**). In addition it has been observed in a hippocampal slice preparation that GABAergic interneurons are more sensitive to the actions of NMDA-inhibitors compared to pyramidal neurons (**Grunze et al., 1996**). Inhibition of NMDA-receptors is therefore speculated to cause decreases of GABA inhibition in both the cortex and hippocampus.

In summary dysregulation of the dopaminergic mesolimbic and mesocortical systems, dysregulation of excitatory NMDA-receptor neurotransmissions and alterations in inhibitory GABAergic tone, contribute to dysregulation of the corticolimbothalamic system and such changes may lead to the symptomathology of schizophrenia as expounded in previous chapters. It is widely agreed in the literature that these components and systems modulate each other. By administration of either PCP or amphetamine to rodents it is possible to directly provoke dysregulation of NMDA-receptor and dopaminergic functions, respectively. These effects are anticipated to result in a broader dysregulation of GABAergic tone in the corticolimbothalamic systems. Consequently, the administration of amphetamine and PCP to rats in this project can plausibly be argued to mimic some aspects of schizophrenia symptomathology, with

alterations in GABAergic tone potentially the common underlying neural basis for such symptoms.

2.2. The role of different $GABA_A$ receptor subtypes in schizophrenia

As described the expression of $GABA_A$ receptors are enhanced in schizophrenic patients, which might be a result of a decrease in GABA concentrations, and thus these receptors may be an interesting target in the development of new drugs that can alleviate the symptoms of schizophrenia. The structure of $GABA_A$ receptors and the functions of different subtypes have been presented in the preparatory project, see part '2.1.6. $GABA_A$ receptor, a GABA-gated Cl^- channel'.

It has been observed that $GABA_A$ receptor subunit density changes with respect to the strength of GABAergic transmission, and postmortem studies of the prefrontal cortex of schizophrenia patients have revealed 30-35 % increase of the α1 subunit (**Impagnatiello et al., 1998; Guidotti et al., 2005**) and increases of up to 100 % of the α2 (**Volk et al., 2002**) and α5 (**Impagnatiello et al., 1998; Guidotti et al., 2005**) expressed postsynaptically on pyramidal neurons. The α2 and α5 subtypes of the $GABA_A$ receptor exhibit higher affinities for GABA compared to the α1 $GABA_A$ receptor subtype. (**Levitan et al., 1988; Lavoie et al. 1997; Costa et al., 1996**). It has been suggested that the predominant elevation in expression of α2 and α5 subunits may be a consequence of $GABA_A$ receptor subtypes containing these subunits having higher affinity for GABA (**Guidotti et al., 2005**).

Benzodiazepines have been shown to reduce positive and negative symptoms in 33-50% of schizophrenia patients (reviewed by **Wolkowitz et al., 1991; Carpenter et al., 1999**). Benzodiazepine is a collective name for a large group of psychotropic agent which were introduced into clinical practice in the early 1960s. Benzodiazepines act at $GABA_A$ receptors that contain an α and a γ subunit, between which the benzodiazepines bind and allosterically modulate the interaction between the neurotransmitter GABA and the $GABA_A$ receptor. The benzodiazepines enhance the effect of GABA and consequently elevate GABA stimulated Cl^- flux that results in a hyperpolarisation of the neurons (**Enna et al., 1997**). Today these drugs are still used frequently in the treatment of anxiety, insomnia, and epilepsy, because of their anxiolytic, sedative and anticonvulsant effects, respectively. Especially the action of the α2 and α3 GABA receptor subtypes is

connected to the anxiolytic and the anticonvulsant activity, whereas the α1 subtype is linked to the sedative effect (**Haefely et al., 1993**). In the above mentioned use of benzodiazepines in the treatment of schizophrenia symptoms, the patients also display many side effects including sedation, amnesia, tolerance, and dependence, because the tested benzodiazepines acted as non-selective full positive allosteric modulators of $GABA_A$ receptor subtypes (i.e. α1, α2, α3 and α5) and influenced the α1. Additionally benzodiazepines only alleviate symptoms of schizophrenia for a short time (reviewed by **Guidotti et al., 2005**). The α1 subtype is the most abundant type of $GABA_A$ receptor in the cortex, where it is found both on pyramidal and GABAergic postsynaptic membranes (*Ibid.*). Interestingly, drugs that selectively influence the α2, α3 and α5 subtypes of the $GABA_A$ receptor, have in studies with monkeys and rodents shown profiles, which are interesting in relation to the treatment of schizophrenia symptoms (reviewed by **Guidotti et al., 2005**). Imidazenil which acts as a selective positive allosteric modulator of GABA at the three subtypes of the $GABA_A$ receptor: α2, α3 and α5 have pre-clinically been shown to reduce auditory gating deficits and social interaction deficits in rodents. In addition imidazenil did not exhibit sedation, tolerance and amnesia and it is speculated to be effective in the clinic to alleviate the symptoms of schizophrenia (*Ibid.*).

$GABA_A$ agonists or inverse agonists can show either binding- or functional selectivity. Binding selectivity refers to drugs that have higher affinity for one sort of receptor subtype and low for the other, while functional selectivity refers to drugs, which bind all receptors subtypes with equal affinity, but have differential functional efficacy at subtypes of receptor (**Maubach, 2003**).

Below, the three $GABA_A$ receptor subtypes α5, α2 and α3, and their roles in relation to schizophrenia and cognition, will be discussed further.

2.2.1. $GABA_A$ α5 receptors

$GABA_A$ receptors expressing the α5 subunit have been found to have the highest density in the hippocampus. They are expressed in the cortex, the striatum and thalamus as well, but in much lesser amounts (**Quirk et al., 1996**). Both in the hippocampus and in the cortex the α5 $GABA_A$ receptors are located on the somata and aprical dendrites of the pyramidal neurons (reviewed by **Guidotti et al., 2005**). The preferential hippocampal location suggests that the α5 subtypes of $GABA_A$ receptors may play an important role in

hippocampus dependent functions of learning and memory, and that an inverse agonist, which modulates the inhibitory effects of the $GABA_A$ receptor negatively would enhance neuronal activity in the hippocampus. In order not to provoke anxiety, sedation and convulsions, the inverse agonist should be α5-selective (**Maubach, 2003**) (see also the part '2.3.2.4. $GABA_A$ α5-selective inverse agonist (α5IA-II)'). These speculations are justified by an experiment executed by Collinson and colleagues in 2002, where they tested α5 knock out mutated mice in regard to cognitive performance. The α5 knock out mice did not showed any phenotypic changes or compensational increased expression of other α-subunits. When spatial learning was tested in a water maze, the mutated mice showed a significantly enhanced performance in regard to control mice (**Collinson et al., 2002**). In addition α5 $GABA_A$ receptor knock out mice show impairment in pre-pulse experiments (see part '2.3.1.2. Pre-pulse inhibition'). Hauser and his team executed in 2005 an experiment with 80 mice, 40 wild type mice and 40 mice having a point mutation in the α5 subunit of $GABA_A$ receptors expressed in the CA1, CA2, and CA3 regions of the hippocampus. In order to avoid compensatory changes which often are seen in experiment with full knockout, the mutation was made as a region-specific partial knockout. The mutation was speculated to alter the synaptic transmission from the hippocampus to the nucleus accumbens, areas thought to control mechanisms underlying pre-pulse inhibition (see part '2.3.1.2. Pre-pulse inhibition'). In addition the mutation was thought to modify the neuronal transmission from the hippocampus to limbic structures including the amygdale, prefrontal cortex, and nucleus accumbens. The alteration and modification of these mechanisms was thought to be involved in the enhancement of excitatory activity and neuronal transmission in the pyramidal cells of the hippocampus as a direct consequence of the reduced effect of the α5 $GABA_A$ receptors. The team observed that α5 knock-in mice showed elevated locomotor activity and an attenuation of pre-pulse inhibition. Hauser and colleges concluded that these observations suggest that pre-pulse inhibition is highly sensitive to imbalance between excitatory and inhibitory hippocampal neurotransmission (**Hauser et al., 2005**). Impairment of pre-pulse inhibition in response to a reduction of α5 $GABA_A$ receptor function in the hippocampus has been observed in other studies as well (**Bast et al., 2003; Bast et al., 2001**). Furthermore Hauser speculated that pre-pulse inhibition deficits might be associated to some forms of hyperdopaminergic state in the striatum, based on the observed elevation

in locomotor activity. But if pre-pulse inhibition is only due to a partial reduction of GABA activity of the hippocampus, Hauser suggested that benzodiazepines (see '2.3.2.3. Alprazolam, an anxiolytic drug') could be effective in alleviating the pre-pulse inhibition impairment seen in schizophrenia patient by enhancing the $GABA_A$ receptor mediated inhibition (**Hauser et al., 2005**).

2.2.2. $GABA_A$ α2 receptors

In addition to the anxiolytic effect of benzodiazepines via the α2 $GABA_A$ receptor, this receptor subtype is very interesting in relation to schizophrenia, since its expression as already mentioned is upregulated 100 % in the prefrontal cortex of schizophrenic patients compared to controls. More precisely Volk et al. (**Volk et al., 2002**) showed in post-mortem brain tissue that the number of α2 $GABA_A$ receptors was upregulated 113 % in the axon initial segment of the prefrontal cortex cells of schizophrenic subjects. In the same experiment brain tissue from a subject suffering from major depressive disorder with psychosis was assessed, but no difference from control was observed, suggesting that the α2 $GABA_A$ receptor upregulation might be characteristic for schizophrenia and not other psychotic conditions (*Ibid.*). The α2 $GABA_A$ receptor subtype constitutes only 15 % of all $GABA_A$ receptors found in the cortex of the rat (**Fritschy et al., 1995**), but it has been shown mainly to be localised at the axon initial segment of pyramidal neurons in the human brain (**Loup et al., 1998**), and in 80 % of the inhibitory synapses at pyramidal cells of rat hippocampus (**Nusser et al., 1996; Nyíri et al., 2001**). Thus, it is speculated that the α2 subtype is specialised in the inhibition of pyramidal cell output and the synchronising of pyramidal cell firing (**Volk et al., 2002**). This is in agreement with the observation, that primarily the greatest reductions in schizophrenia have been found in parvalbumin-positive neurons (reviewed by **Shulman et al., 2005**). Since chandelier cells innervate the axon initial segment of pyramidal cells (reviewed by **Benes et al., 2001**). This may mean that the downregulation of chandelier cells in the prefrontal cortex could be counteracted by upregulation of the α2 $GABA_A$ receptor. Hence it can be hypothesised that an enhancement of the α2 $GABA_A$ receptor inhibition of the pyramidal cell firing could alleviate some symptoms of schizophrenia. But as mentioned earlier it is not an uncoordinated inhibition of the pyramidal cells that is of interest with regard to higher cognitive deficits observed in schizophrenic patients, but instead the synchronisation of

pyramidal cell firing. Thus, the drug of therapeutic interest should be a $GABA_A$ α2 selective agonist (reviewed by **Lewis et al., 2005**).

2.2.3. The α3 $GABA_A$ receptor and modulation of the dopaminergic system

As presented in '2.1.1.1. Dopamine' overactivation in the dopaminergic system is thought to be a contributing factor in the development of schizophrenia. Dopaminergic neurons express α3-containing $GABA_A$ receptors (**Fritschy et al., 1995**) and consequently it has been postulated that GABAergic hypofunction could lead to dopaminergic hyperfunction (**Roberts, 1972**). Yee and colleagues have executed an experiment with $GABA_A$ α3 receptor knockout (α3KO) mice. In pre-pulse inhibition experiments the α3KO mice showed deficits in sensorimotor gating (**Yee et al., 2005**), typical for schizophrenia (**Braff et al., 1990**). Such deficits can be normalised by treating the α3KO mice with a D_2 dopamine receptor antagonist, and Yee et al. concluded that the sensorimotor-gating deficit was dopamine dependent. This experiment supports the idea that the dopamine producing neurons are under inhibitory GABAergic control, primarily mediated by the α3 $GABA_A$ receptor, and that a hypofunction in GABA mediated inhibition leads to overactivation in the dopaminergic system (**Yee et al., 2005**).

2.3. Methodology and Pharmacological tools used in the current research

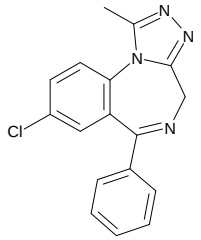
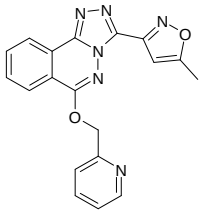
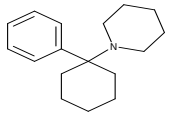
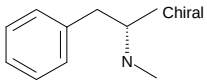
In this project two experimental methods have been used: The fear conditioning model and the pre-pulse inhibition model. These two methods have been used to investigate the pharmacology of the three drugs: alprazolam, α5IA-II, and NS.A (a completely novel NeuroSearch compound). In addition the two compounds PCP and amphetamine have been used to model schizophrenic symptoms in rats. The structures of all the drugs are shown in the table 2.1.

2.3.1. Methods

2.3.1.1. Fear Conditioning

The fear conditioning model is described in the preparatory project; see part '2.3.3. Fear Conditioning'. In this project we executed three different types of fear conditioning learning experiment, where rats were trained to associate context/tone stimulus with a

Table 2.1. The structures of the GABAA receptor modulating compounds alprazolam and α5IA-II. The structure of the completely novel NeuroSearch compound NS.A is not shown. The structures of PCP and amphetamine is as well presented, since these drugs have been used to induce impairment in pre-pulse inhibition.

Compound	Structure
Alprazolam	
α5IA-II	
NS.A	Structure not shown
PCP	
Amphetamine	

footshock stimulus. The experimental protocols are described in detail in part '3. Material and Methods'.

In the first type of experiment rats are tested 1 day and one week after training. The re-test is identical to the original test, and both tests examine the rats' ability to associate the 10 seconds long tone stimulus/context, with the electric shock delivered coincident with the offset of the tone stimulus during training, by recording the freezing of the rats. The re-test is interesting, since rats on re-test day have been presented with the tone stimulus/context in association with an electric shock (i.e., training) and without the shock (i.e., test day). It is widely agreed that it is possible to inhibit learned fear response after the conditioning by extinction training, e.g. presenting the tone stimulus/context consistently without shock resulting in loss of freezing (reviewed by **Leslie et al., 2004**). An extinction of the association of the tone stimulus/context with the electric

shock might be speculated to take place under the second appearance of the tone stimulus/context alone. A general thesis is that extinction is not solely an eradication of original memory, because a wide number of experiments have shown that memory can be re-kindled even after apparent extinction (*Ibid.*). Instead it is widely agreed that extinction is a form of new learning (reviewed by **Yee et al., 2004; Wasserman et al., 1997**). Even though the understanding of the neural mechanism of extinction is poor, studies have shown that GABAergic mechanisms directly influence extinction learning and that benzodiazepines facilitate the extinction of prior learning (**McGabe et al., 2004**). In addition benzodiazepines are known to engender anterograde amnesia in man (**Enna et al., 1997**), i.e., forgetting of events being learned after taking a benzodiazepine. The re-test can following be used to evaluate how strong the memory of the learned response at the conditioning day is even after a new learning or extinction have taking place at the test day.

In the second type of fear conditioning experiment the tone on the test day was extended to 90 seconds, which made it possible to observe if the rats froze throughout the tone or starts moving again before the tone ends. This is interesting in order to maximise the chances of ascertaining if the tested drugs specifically altered memory for the tone stimulus. It could be speculated that the length of the freezing could reflect how strong the animals' memory of the conditioning day is. The extended tone was presented two times on the test day, with a 40 seconds interval between the two occasions.

It should be kept in mind under the interpretation of the test data from the two types of fear conditioning learning experiments described above, that the ability to remember the context is hippocampus dependent (reviewed by **Wallenstein et al., 1998**), whereas the anxiety that the rats may associate with the tone stimulus is amygdala dependent (reviewed by **Medina et al., 2002**) (described in the preparatory project in the parts '2.1.5. Cognition, memory and hippocampus' and '2.1.2. Fear and Anxiety'), which means that the drugs may influence the memory of the context before the tone, the memory of tone, and the memory of the context/tone after the tone period differently.

In the third type of the fear conditioning learning experiment, named trace fear conditioning, a time gap of 60 seconds separates the appearance of the tone after the electric shock on the training day (in the two other experimental types described above

the tone and shock co-terminate on the training day). The association of events that occurs separated in time are hippocampus dependent (**Rawlins, 1985;** reviewed by **Wallenstein et al., 1998**) (the association of a tone and a shock that overlaps are not hippocampus dependent (reviewed by **Wallenstein et al., 1998; Medina et al., 2002**)) and involves the hippocampal α5 $GABA_A$ receptors (**Crestani et al., 2002; Yee et al., 2004**). In mice with point mutations in the α5 subunit of the $GABA_A$ receptor of the hippocampus trace fear conditioning is facilitated (**Crestani et al., 2002; Yee et al., 2004**). Yee and colleagues showed in 2004 that this point mutation did not affect no-trace conditioning (i.e., normal fear conditioning) but resulted in a prolonged freezing at the test day when there was a gap of 20 seconds between the tone and the shock during training (**Yee et al., 2004**). It is speculated that α5 $GABA_A$ receptors normally inhibit the processing of associative learning, and that this hippocampal receptor plays a critical role in learned associations (**Crestani et al., 2002; Yee et al., 2004**). It is also worth noting that these α5 mutated mice showed retarded extinction of fear conditioning (**Yee et al., 2004**).

2.3.1.2. Pre-pulse inhibition

Pre-pulse inhibition, introduced in part 'Corticolimbothalamic circuit deficits', is an active information processing and automatic sensorimotor gating mechanism, which is thought to selectively secure the processing of a weak stimulus (reviewed by **Hamm et al., 2001**). This is an extremely important mechanism for the organism in order to screen out trivial stimuli from awareness and be able to focus attention on the most important stimuli in an environment with many stimuli. Sensorimotor gating refers to the mechanisms involved in the process of inhibiting or 'gating' the startle motor response to a startling stimulus after the presentation of a weak pre-pulse (reviewed by **Geyer et al., 2001**). The inhibitory influence of the pre-pulse is regulated by the limbic system among others regions (e.g., cortex, striatum and thalamus), whereas the hippocampus is involved in sensorimotor gating. Thus pre-pulse inhibition is regulated by neural activity in the forebrain (*Ibid.*).

Pre-pulse inhibition is observed in more than 90 % of healthy humans, and regarded as a very robust model of sensorimotor gating (reviewed by **Hamm et al., 2001**). Additionally it occurs across a variety of species (**Davis, 1979**) and there is a high degree of homology between pre-pulse inhibition in rodents and man. In humans pre-pulse inhibition or sensorimotor gating is calculated by measuring the eyeblink component of

the startle response whereas the whole-body flinch is measured in rodents. The stimulus used to provoke the acoustic startle response is the same in humans as well as in rodents, e.g. a 115 dB noise burst (reviewed by **Braff et al., 2001**).

In this project the movement level of rats was measured using the same apparatus for pre-pulse inhibition and fear conditioning experiments, i.e. startle chambers.

Disruption of pre-pulse inhibition of the acoustic startle response can experimentally be induced in rats by different pharmacological manipulations that affect neurotransmitter systems thought to be dysregulated in schizophrenic patient (reviewed by **Braff et al., 2001**). For example pre-pulse inhibition can be impaired by stimulation of dopamine receptors by e.g. apomorphine and amphetamine which are a dopamine agonist and a dopamine releaser, respectively, or by blocking the NMDA receptor with e.g. PCP (reviewed by **Geyer et al., 2001**). Additionally it is possible to recover the pre-pulse inhibition deficits induced by such drugs in rats by pretreatment with antipsychotics. Deficits provoked by direct or indirect dopamine agonists are most readily counteracted both by typical and atypical antipsychotic compounds like haloperidol and clozapine, respectively, whereas only atypical antipsychotic compounds e.g. clozapine (not a D2 receptor antagonist) have been reported to alleviate PCP induced pre-pulse inhibition deficits (reviewed by **Hamm et al., 2001**).

Finally it is important to emphasise that experimentally provoked deficits in pre-pulse inhibition can not be used as an animal model of schizophrenia, but can only provide a model of the sensorimotor gating deficits related to schizophrenia, since pre-pulse inhibition deficits are well characterised in other diseases e.g. Huntington's disease, Tourette's syndrome and obsessive-compulsive disorders (reviewed by **Geyer et al., 2001**).

2.3.2. Pharmacological tools

2.3.2.1. PCP and modelling schizophrenia pre-clinically

PCP is a common drug of choice to model schizophrenia in rodent. Acute administration of PCP has been reported to impair social interactions in rats (**Sams-Dodd, 1997**) as well as inducing cognitive deficits that reflect schizophrenia (**Adams et al., 1998**). In addition it has been reported that acute PCP administration (both at 0.86 and 8.6 mg/kg) after 24 hours causes depression of cerebral glucose metabolism (**Gao et al., 1993**), but in

another study it has been reported that 2.58 mg/kg of PCP 2 hours after i.p. injection caused increased expression of immediate-early genes (thought to reflect neuronal activity) in the prefrontal cortex which had returned to control levels 24 hours after administration (**Cochran et al., 2002**). In general measurements of glutamatergic and GABAergic neurotransmitter systems after acute dosing of PCP are not consistent in the literature as reviewed by Morris et al., 2005. Chronic administration of PCP in rodents is often considered to be more relevant because chronic administration in humans is more likely to induce schizophrenia-like psychosis. Chronic dosing (> five days) of low-dose (2.58 mg/kg) PCP in rats has been shown to decrease the glucose metabolism in the prefrontal cortex, the thalamus and in the auditory system (**Cochran et al., 2003**). In addition it has been observed to lead to deficits in working memory, changes in hippocampus and thalamus and decrease in prefrontal cortex parvalbumin expression. Chronic low-dose administration of PCP in rats is frequently reported to cause neurochemical and metabolic changes that closely reflect those observed in schizophrenia patients (reviewed by **Morris et al., 2005**), which has been described in part '2.1. Schizophrenia and cognition'.

2.3.2.1.1. Pre-pulse inhibition and acute PCP treatment

In regard to disrupting pre-pulse inhibition in experiments with rats, the PCP-treatment in contrast to the above should be acute rather than chronic. Myers and colleagues have reported that injection of 3 mg/kg PCP i.p. in Sprague-Dawley rats caused disruption in pre-pulse inhibition, when PCP was administered a quarter of a hour before testing (**Myers et al., 2005**). Similar results had been obtained in Wistar rats after chronic injections of 2 mg/kg PCP s.c. 10 minutes prior to the pre-pulse inhibition test. In the experiment PCP was administered daily for 11 days. PCP was shown to induce pre-pulse inhibition deficits on all eleven days. On day 12 PCP was not administered before the pre-pulse inhibition test and no pre-pulse inhibition deficits was observed (**Schwabe et al., 2004**). In 2004 Teen et al. treated Sprague-Dawley rats with PCP by injecting 3 mg/kg PCP i.p. nine times spread over a period of 19 days. On the 19th day pre-pulse inhibition was measured 15 minutes after the injection, and a significant pre-pulse inhibition deficit was registered. Pre-pulse inhibition was measured again after 3, 21 and 60 days from last PCP administration, but here no deficits in pre-pulse inhibition were noted. Latent

inhibition (LI) of active avoidance and taste aversion, used to model attentional impairments in schizophrenia, was also executed 28 and 48 days after the last administration of PCP. These experiments showed that LI was normal in the PCP-treated rats. Twenty-five days after the final PCP-injection a test of locomotor activity was undertaken, which demonstrated a sensitised locomotor response after injections with 1.5 mg/kg PCP compared to non-PCP treated rats. Finally Teen and colleagues concluded on the basis of these experiments, that repeated administration of PCP did not produce schizophrenia-like deficits in pre-pulse inhibition as well as in LI, even though the chronic exposure to PCP induced long lasting neuroadaptive and functional changes expressed by the sensitised locomotor activity (**Teen et al., 2005**).

In summary the above experiments suggest that acute administration of PCP is sufficient to induce impairment in pre-pulse inhibition in rats.

2.3.2.2. Amphetamine

Amphetamine has in the clinic been observed to cause elevated concentration of dopamine in nucleus accumbens and in other limbic forebrain structures. Overdosing of amphetamine has as well been shown to induce psychotic symptoms in man which reflects the positive symptoms of schizophrenic patients (**Randrup et al., 1972; Bear et al., 2001**). Chronic administration of amphetamine to rats has been shown to result in decrease of extracellular GABA concentration and GAD_{67} mRNA expression in nucleus accumbens (**Lindefors et al., 1992**), which reflects the findings in post mortem studies of schizophrenic patients (see '2.1.3.2. GABAergic deficits in man, post mortem studies'). Acute administration of amphetamine impairs pre-pulse inhibition in rats. The threshold dose that disrupts pre-pulse inhibition does in addition elevate locomotor activity of rats (reviewed by **Geyer et al., 2001**). Pre-clinically acute administration of amphetamine has been shown to induce psychotic activity (**Langen et al., 2006**).

2.3.2.3. Alprazolam, an anxiolytic drug

Alprazolam belongs to the benzodiazepines and is a triazolobenzodiazepine (the structure is shown in table 2.1 and it produces its pharmacological effect by binding non-selectively to the benzodiazepine-binding sites at $GABA_A$ receptors containing either α1, α2, α3 or α5 subunits (**Biggio et al., 1990**). The GABA-agonistic effect of alprazolam is clinically utilised to treat anxiety, panic disorders, insomnia, and

depression (**Casacalenda et al., 1998; Molewijk et al., 1995**). In the clinic alprazolam has a rapid onset of action in the alleviation of these disorders and in behavioural studies of animals, it has frequently been reported to be effective in reversing experimentally induced states of behavioural despair (**Casacalenda et al., 1998**). Molewijk and colleagues executed an experiment in 1995 where they conditioned rats to associate a specific environment with electric foot shocks. Following this they evaluated the ultrasonic vocalization (ultrasounds emitted by rats for example when pups are separated from their mother or adult rats are presented to a dominant male) produced by the rats, placed back in the environment where they received foot shocks. Alprazolam administered i.p. at a dose of 3 mg/kg was shown to decrease the number of ultrasonic vocalizations produced by the rats, and Molewijk and colleagues concluded that alprazolam suppressed panic attacks. Additionally, they recorded that the same dose also lowered the locomotor activity of the rats in open field (**Molewijk et al., 1995**).

2.3.2.4. $GABA_A$ α5-selective inverse agonist (α5IA-II)

In 2004 Sternfeld et al. identified a $GABA_A$ α5 subtype selective inverse agonist, which in rats enhanced cognition in the delayed matching to position test in the water maze without anxiolytic, convulsant, or proconvulsant side effects. The drug is a triazolophthalazines and named α5IA (**Sternfeld et al., 2004**). α5IA binds with equivalent affinity to the BZ binding sites of the $GABA_A$ receptor subtypes: α1, α2, α3 and α5. But α5IA only has inverse agonist efficacy for the α5 subtype, whereas it is an antagonist at α1 receptors and a weak agonist at α2 and α3 receptors. So the effect of α5IA is primarily mediated via $GABA_A$ receptors containing a α5 subunit (**Dawson et al., 2005**). Dawson and his team have investigated the function of α5IA in more detail. They administered α5IA orally to rats and subsequently tested the rats in the Delayed-Matching-to-Position version of the Morris water maze among other tests. They reported that the rats had improved performance and concluded that α5IA significantly enhanced performance in rat contextual conditioning, i.e. a hippocampal dependent learning and memory process. Furthermore it was shown that α5IA was devoid of anxiogenic behavior and convulsant, proconvulsant, sedative, kindling, or motor-impairing activities (**Dawson et al., 2005**).

Collinson and colleagues performed in 2006 an experiment that also tested a compound selective for the $GABA_A$ receptors containing an α5 subunit in the Morris

water maze. The drug they tested was structurally very closely related to α5IA and named α5IA-II. The structure is presented in table 2.1. α5IA-II was administered i.p. to male hooded Listar rats at a dose of 1 mg/kg and 0.5 hours prior to the trials. In order to test whether the drug modulated the effect of the encoding phase, the consolidation phase or the recall phase, α5IA-II was administered before the trial, after the trial and before the trial was repeated four hours after the first trial, respectively. To limit the action of α5IA-II to the desired memory processing phase, the action of the drug was subsequent blocked with an antagonist (flumazenil). On the basis of these experiments they concluded that the $GABA_A$ α5 selective inverse agonist improved the encoding and recall processes in the Morris water maze but did not improved the consolidation process. Based on the preferential location of the α5 subtype in the hippocampus, the effect of α5IA-II on recall is assumed to be hippocampally mediated (**Collinson et al., 2006**).

In the light of these experiments it is plausible to imagine that the properties of $GABA_A$ α5-selective inverse agonists can be of utility in the treatment of cognitive deficient. But as discussed in '2.2.1. $GABA_A$ α5 receptors' a reduction in α5 $GABA_A$ receptor mediated neurotransmission might also disrupt pre-pulse inhibition.

2.3.2.5. NS.A, a selective α2 and α3 partial $GABA_A$ receptor agonist

As indicated in the sections '2.2.2. $GABA_A$ α2 receptors' and '2.2.3. The α3 $GABA_A$ receptor and modulation of the dopaminergic system' an α2/α3 $GABA_A$ receptor selective agonist might be interesting as a new therapeutic in the treatment of schizophrenia. Yet there has not been much research focusing on α2 and α3 selective benzodiazepine agonists and schizophrenia (reviewed by **Lewis et al., 2005**).

ELB139 is an affinity selective α3 $GABA_A$ receptor agonist, showing more than 30-70 fold selectivity over receptors containing either α1, α2 or α5 subunits (**Rabe et al., 2006**). It should howeer be noted that although affinity selective, ELB139 attains a higher maximum efficacy at $GABA_A$ receptors containing an α1 or α2 subunit compared to receptors with either an α3 or α5 subunit; although it is a partial agonist relative to diazepam at all receptor subtypes (*Ibid.*). In 2005 Langen and colleagues tested OLB139 *in vivo* (**Langen et al., 2005**). The rats were administered ELB139 orally and at both 10 and 30 mg/kg, anxiolytic effects where observed in three different tests of anxiety. They did not observe significant sedation or tolerance, not even after 6 weeks of treatment (*Ibid.*). ELB139 (30 mg/kg p.o.) has in addition showed a significant antipsychotic activity in

both MK-801 induced psychotic activity and amphetamine induced psychotic activity when administered o.p. to female Wister rats (**Langen et al., 2006**). Because ELB139 exhibits selectivity for the α2 and α3 subtypes of the $GABA_A$ receptor, but does not exhibit sedation or tolerance, it is really interesting to test in regard to the development of new treatments for schizophrenia. In this project the novel drug NS.A has been tested in both fear conditioning experiments and in a pre-pulse inhibition experiments. NS.A does not share any structural similarities with ELB139, but it is also a selective α2 and α3 partial $GABA_A$ receptor agonist (as well as a full α5 agonist), so it might exhibit some of the same properties as ELB139 without provoking sedation, and consequently it is very interesting to test in relation to schizophrenia models.

2.4. Aim of the project

Based on the above, agonistic effects on the α2 and α3 subtypes of the $GABA_A$ receptor are interesting with regard to alleviation of sensorimotor gating deficits in schizophrenia, whereas selective inverse agonistic effects of a compound of the α5 subtype of the $GABA_A$ receptor are interesting with regard to hippocampus-dependent memory improvement. Consequently it is logical to investigate the properties of a α2 and α3 selective agonist of the $GABA_A$ receptor and an α5 selective inverse agonist in animal models. In this project the two experimental models pre-pulse inhibition and fear conditioning have been used to investigate effects of both types of drugs. As a control a full non-selective agonist, alprazolam, was tested as well.

The aim of this project is to determine the role of α2, α3, and α5 subtypes of the $GABA_A$ receptor in animal models of sensorimotor gating deficits as well as in memory.

Below is speculated a priori on the potential effects of the pharmacological tools in the animal model utilised:

The control drug alprazolam, the full nonselective $GABA_A$ receptor agonist, might be speculated to impair hippocampus-dependent associations – i.e. impaired freezing response in the fear conditioning experiment, since it has full α5 agonistic properties and benzodiazepines are known of engender anterograde amnesia in man. Alprazolam is as well anxiolytic and sedative, which may be confounding factors. In regard to disrupted pre-pulse inhibition, alprazolam may be anticipated to normalise

pre-pulse inhibition deficits, since benzodiazepines have been observed to alleviate schizophrenic symptom in some patients. However again, non specific effects may interfere with any possible observation.

α5IA-II, the selective α5 inverse agonist, might improve the ability of the rats to associate the tone with the shock, especially in the trace fear conditioning experiment, since this type of fear conditioning is hippocampus-dependent. In addition to the cognition enhancing effects, α5IA-II might impair pre-pulse inhibition as shown in the experiments with knock-in α5 mutated mice.

NS.A is most interesting with regard to pre-pulse inhibition, since its selective partial α2 and α3 agonistic effects are hypothesised to influence schizophrenic symptoms positively, based on the previous research introduced earlier. NS.A also shows full agonistic α5 properties, and it might therefore be anticipated to impair the ability of the rats to associate a tone with shock in the fear conditioning experiments. With reference to its α2 and α3 selectivity and anxiolytic properties of such a compound may be a confounding factor as in the case for alprazolam.

3. Materials and methods

Standard procedures for the experiments performed in the period between August the 14th and December the 1st are described in the following sections. A summary table of the different individual experiments is presented in appendix 3.1., where each experiment has been given a short reference number e.g A1. Details of the individual experiments are listed in the laboratory journals (see appendix 3.2.-3.18.) and the experimental protocols (see appendix 3.19.-3.34.). In addition short descriptions of the behaviour of the animals during the experiments are noted in the laboratory journals, and unintended deviations from the standard procedure are noted in the experimental protocols.

3.1. Animals

Male Sprague-Dawley rats were used (Harlan Scandinavia). The rats were housed in and habituated to the laboratory three days before fear conditioning experiments and one day prior to pre-pulse inhibition experiments. During the experiments the rats were housed in Macrolon III cages (20 X 40 X 18 cm) with two rats per cage or in Macrolon III cages (40 X 50 X 20 cm) with four rats per cage. (See appendix 3.1.).

Female Naval Medical Research Institute (NMRI) mice were used (Harlan Scandinavia) in the dose-response pre-pulse inhibition experiment with methylphenidate (experiment D1). The mice were housed in and habituated to the laboratory one day before the experiment. During the experiment the mice were housed in Macrolon III cages (20 X 40 X 18 cm) with eight mice per cage.

In all of the experiments Food (Altromin 1324, Lage, Germany) and tap water were available ad libitum. The animals were housed on a 12 hours light/dark cycle (light on at 4:00 AM and off at 4:00 PM) and the room temperature was maintained at 21-24^{o}C. The experiments were performed during the light periods and in accordance with the guidelines of the Danish Committee for Experiments on Animals and 'Principles of Laboratory Animal Care' (NIH publication NO. 85-23, revised 1985).

3.2. Drugs and solutions

Prior to the experiments the three drugs under investigation were prepared: Alprazolam, α5IA-II and NS.A as well as the drugs used to model sensorimotor gating deficits: methylphenidate, PCP and amphetamine.

3.2.1. Alprazolam

In the fear conditioning experiment (A1), the extended-tone fear conditioning experiment (B1) and the pre-pulse inhibition with PCP-deficit experiment (F1) the drug alprazolam was administered i.p. in dose volume of 2 ml/kg and at the different doses 0.4, 1.33 and 4 mg/kg. Consequently the following alprazolam concentrations were made: 0.2, 0.67 and 2 mg/(ml cremophor 5 %).

Reagents

Alprazolam, Cambrex Profarmaco, Italy

Cremophor RH40 (Polyoxyl 40 hydrogenated caster oil), BASF, Germany

Procedure

Alprazolam was dissolved in cremophor 5 %. In order to dissolve the drug the solution was standing on a 50 ^{o}C hot plate and stirred. To destroy any lumps the solution was thereafter treated with ultrasound (Sonifier) for 14 minutes and then stirred before the solution were diluted in cremophor 5 % to the different concentrations. The solutions were protected against light throughout the animal experiments by tinfoil.

3.2.2. α5IA-II

In the fear conditioning experiment (A2), the extended-tone fear conditioning experiment (B2) and the pre-pulse inhibition with PCP deficit experiment (F2) the drug α5IA-II was administered i.p. in dose volume of 2 ml/kg and at the different doses 1, 3 and 10 mg/kg. Consequently the following α5IA-II concentrations were made: 0.5, 1.5 and 5 mg/(ml tween 5 %). In the trace fear conditioning experiment (C) α5IA-II was administered i.p. in dose volume of 2 ml/kg and at the different doses 1 and 3 mg/kg. Consequently the following α5IA-II concentrations were made: 0.5 and 1.5 mg/(ml tween 5 %). In the dose-response with PCP deficit experiment (H) α5IA-II was

administered i.p. in dose volume of 2 ml/kg and with the two possible doses: 30 and 10 mg/kg. Consequently the following α5IA-II concentrations were made: 15 and 5 mg/(ml tween 5 %).

Reagents

α5IA-II ($C_{19}H_{14}N_6O_2$), Blocona Company, India

Tween ($C_{58}H_{114}O_{26}$), Merck-Schuchardt, Germany

Ringer-klorid SAD (NaCl 0.9 %), Sygehusapotekerne i Danmark, Denmark

Procedure

α5IA-II was dissolved in pure tween. In order to dissolve the drug the solution was placed at a 50 ^{o}C hot plate and stirred. Hereafter tween and α5IA-II was diluted in NaCl 0.9 % in order to reach a tween 5 % solution, containing 5 mg/ml α5IA-II (1,5 mg/ml α5IA-II in the trace fear conditioning experiment and 15 mg/ml α5IA-II in the dose-response with PCP deficit experiment). To destroy any lumps the solution was treated with ultrasound (Sonifier) for a quarter of a time and then stirred before the solution was diluted in tween 5 % to the different concentrations. It was not possible to dissolve α5IA-II totally. To maintain the drug in a homologous suspension, the solutions at the concentrations of 5 and 15 mg/ml was constantly stirred and maintained at 50 ^{o}C hot plate throughout the animal experiments.

3.2.3. NS.A

In the fear conditioning experiment (A3), the extended-tone fear conditioning experiment (B3), the pre-pulse inhibition with PCP deficit experiment (F3) and the pre-pulse inhibition with amphetamine deficit experiment (G) the drug NS.A was administered i.p. in dose volume of 2 ml/kg and at the different doses 0.3, 1 and 3 mg/kg. Consequently the following NS.A concentrations were made: 0.15, 0.5 and 1.5 mg/(ml tween 5 %).

Reagents

NS.A, Syngene, Blocona, India

Tween ($C_{58}H_{114}O_{26}$), Merck-Schuchardt, Germany

Ringer-klorid SAD (NaCl 0.9 %), Sygehusapotekerne i Danmark, Denmark

Procedure

NS.A was dissolved in pure tween. In order to dissolve the drug the solution was placed on a 50 ^{o}C hot plate and stirred for 5 minutes. Hereafter tween and NS.A was diluted in NaCl 0.9 % in order to reach a tween 5 % solution, containing 3 mg/ml NS.A and stirred 5 minutes before the solution was diluted in tween 5 % to the different concentrations.

3.2.4. Methylphenidate

In the dose-response pre-pulse inhibition experiments (D1 and D2) the drug methylphenidate was administered s.c. in the scruff of the neck in dose volume of 10 ml/kg for mice (D1) and of 1 ml/kg for rats (D2). In both of the experiments methylphenidate was administered at the different doses 5, 10 and 20 mg/kg. Consequently the following methylphenidate concentrations were made: 0.5, 1 and 2 mg/(ml NaCl 0.9 %) for the D1 experiment and 5, 10 and 20 mg/(ml NaCl 0.9 %) for the D2 experiment.

Reagents

Methylphenidate HCl, Orgamol The Swiss Fine Chemical Company, Switzerland
Ringer-klorid SAD (NaCl 0.9 %), Sygehusapotekerne i Danmark, Denmark

Procedure

Methylphenidate was dissolved in NaCl 0.9 % and stirred. Hereafter the solution was diluted further in NaCl 0.9 % to the different concentrations.

3.2.5. PCP

Reagents

Phencyclidine HCl (PCP), NeuroSearch, Denmark
Ringer-klorid SAD (NaCl 0.9 %), Sygehusapotekerne i Danmark, Denmark

Procedure

In the dose-response pre-pulse inhibition experiments (72 hours pre-treatment time in experiment E1, 24 hours pre-treatment time in experiment E2 and 10 minutes pre-treatment time in experiment E3) PCP was administered s.c. in the scruff of the neck in dose volume of 1 ml/kg and at the different doses 1.25, 2.5 and 5 mg/kg. Consequently the following PCP concentrations were made: 1.25, 2.5 and 5 mg/(ml NaCl 0.9 %).

PCP was dissolved in NaCl 0.9 % and stirred. Hereafter the solution was diluted further in NaCl 0.9 % to the different concentrations

Procedure

In the pre-pulse inhibitions with PCP deficit experiments (F1, F2 and F3) and dose-response with PCP deficit experiment (H) PCP was administered s.c. in the scruff of the neck in dose volume 1 ml/kg and at dose 2.5 mg/kg. Consequently the following PCP concentration was made at 2.5 mg/(ml NaCl 0.9 %). PCP was dissolved in NaCl 0.9 % and stirred.

3.2.6. Amphetamine

Reagents

S(+)-Methamphetamine HCl, Research Biochemicals International, USA

Ringer-klorid SAD (NaCl 0.9 %), Sygehusapotekerne i Danmark, Denmark

Procedure

In the pre-pulse inhibition experiments with amphetamine deficit (G) amphetamine was administered s.c. in the scruff of the neck in dose volume 1 ml/kg and at dose 4 mg/kg. Consequently the amphetamine concentration was made at 4 mg /(ml NaCl 0.9 %). Amphetamine was dissolved in NaCl 0.9 % and stirred.

3.3. Fear conditioning (A1, A2 and A3)

(NS Intern experiment procedure Fear Conditioning, see appendix 3.35.)

Three fear conditioning experiments were executed with the three different drugs: alprazolam (experiment A1), α5IA-II (experiment A2) and NS.A (experiment A3).

Alprazolam was tested with the use of 40 animals which randomly were classified into five groups: Vehicle (cremophor 5 %) no electric shock, vehicle (cremophor 5 %)

with electric shock, 0.4 mg/kg alprazolam with electric shock, 1.33 mg/kg alprazolam with electric shock, and 4 mg/kg alprazolam with electric shock. The terms no/with electric shock refers to the conditioning experiment at day 3 and these terms will be used through out the report. Prior to the conditioning experiment the rats were injected with either vehicle (cremophor 5 %) or 0.44 mg/kg, 1.33 mg/kg or 4 mg/kg of alprazolam according to the group they belonged to (see appendix 3.19.).

α5IA-II was tested with the use of 64 rats in a full factorial design with eight different groups: Vehicle (tween 5 %) with electric shock, vehicle (tween 5 %) no electric shock, 1 mg/kg α5IA-II with electric shock, 1 mg/kg α5IA-II no electric shock, 3 mg/kg α5IA-II with electric shock, 3 mg/kg α5IA-II no electric shock, 10 mg/kg α5IA-II with electric shock, and 10 mg/kg α5IA-II no electric shock. The rats were randomly classified into these eight different groups and injected prior to the conditioning experiment with either vehicle (tween 5 %) or 1 mg/kg, 3 mg/kg or 10 mg/kg of α5IA-II according to the group they belonged to (see appendix 3.20.).

NS.A was tested with the use of 40 animals which randomly were classified into five groups: Vehicle (tween 5 %) no electric shock, vehicle (tween 5 %) with electric shock, 0.3 mg/kg with electric shock, 1 mg/kg with electric shock, and 3 mg/kg with electric shock. Prior to the conditioning experiment the rats were injected with either vehicle (tween 5 %) or 0.3 mg/kg, 1 mg/kg or 3 mg/kg of NS.A according to the groups they belonged to. (see appendix 3.21.).

Equipment

8 TSE StartleResponse Box (TSE Technical & Scientific Equipment GmbH, Germany). The 8 TSE startle boxes were separated in two systems named system 1 and system 2

8 rat cages (Aluminium, plexi glass, integrated floor grid) (270 X 100 X 125 mm) (TSE Technical & Scientific Equipment GmbH, Germany)

3.3.1. Day 1: Handling of animals and calibration

Program

Trial (system 1): Soren test, 110dB

Trial (system 2): Soren test

The sampling interval was 2 ms

Procedure

The rats were handled in at least one minute in order to get familiar with the operators. When the rats became calm they were weighed and marked, see experimental protocols (see appendix 3.19., 3.20. and 3.21.).

The transducers were calibrated to 150 (±2) Maximum g values with the use of an artificial rat and the programs: 'Soren test, 110dB' and 'Soren test' in system 1 and system 2, respectively. The programs included the same trials, but were named differentially in system 1 and system 2.

3.3.2. Day 2: Habituation

Program

Trial: FC Habituation 45dB KBT

Habituation: 180sec (with background noise, 45dB)

Baseline: 30sec (with background noise, 45dB)

The sampling interval was 4 ms

The rats were placed in the startle boxes and exposed to the background noise (white noise at 45 dB). See appendix 3.37. for details regarding the program

Procedure

The computer program was started and the startle boxes were tested to ensure that the noise stimulation was functional. Hereafter it was entered into the program which rat was allocated to which startle box. See appendix 3.35. for details about the upstart of the computer program.

The rats were placed in the rat cages and inserted into the startle boxes whereupon the habituation program was started. Eight animals were habituated simultaneously in system 1 and system 2.

Throughout the rest of the experiment the individual animal was placed in the same startle box in every experiment to ensure that the animals were presented for the exact same noise and tones during the different stages of the experiment.

3.3.3. Day 3: Conditioning experiment

Program

Trial: FC conditioning 2006-04

Habituation: 5sec (with background noise, 45dB)

Baseline: 30sec (with background noise, 45dB)

The sampling interval was 4 ms.

In the conditioning experiment, the rats were exposed to the background noise (45 dB) for 2 x 30 seconds, separated by an Inter-Trial-Interval at 60 seconds, where after they were presented to a tone at 5 kHz and 80 dB for a period of 10 seconds. In the last second of the period the rat simultaneously received an electric foot shock at 0.6 mA through the steel grid, which makes up the floor in the startle cages. This was followed by a period of 180 seconds with background noise. See appendix 3.37. for details regarding the program

Procedure

The rats were prior to the conditioning experiment injected in agreement with the dose groups they belonged to.

The computer program was started and the startle boxes were tested in regard to ensure that the noise stimulation and the electric stimulation were functional. Hereafter it was entered into the program which rat was put in which startle box. See appendix 3.35. for details about the upstart of the computer program.

The rats were 30 minutes prior to the conditioning test injected i.p.

Four animals were injected simultaneously. After the injections the rats were placed in their cages again and maintained there for approximately 30 minutes before they were placed in the rat cages and inserted into the startle boxes. The rat cages were connected to electricity through sockets placed inside the startle boxes, if the tested rat belonged to a 'with electric shock' group. Hereafter, the conditioning program was started. Four animals were tested at the same time, alternately in system 1 and system 2.

The same drug dose was not tested in the same box as the experiment was repeated; see experimental protocols (appendix 3.19., 3.20. and 3.21.)

3.3.4. Day 4: Testing the animals

Program

Trial: FC Test 2005-12

Habituation: 5sec (with background noise, 45dB)

Baseline: 30sec (with background noise, 45dB)

The sampling interval was 4 ms.

The test experiment was carried out after approximately 24 hours. The rats were presented to the same tone as they were at day 3, but without the additional foot shock. See appendix 3.37. for details regarding the program

Procedure

The computer program was started and the startle boxes were tested in regard to ensure that the noise stimulation was functional. Hereafter it was entered into the program which rat was assigned to which startle box. See appendix 3.35. for details about the upstart of the computer program.

The rats were placed in the rat cages and inserted into the startle boxes whereupon the test program was started. Four animals were tested at the same time, alternately in system 1 and system 2.

3.3.5. Day 10: Re-testing the animals

Program

Trial: FC Test 2005-12

Habituation: 5sec (with background noise, 45dB)

Baseline: 30sec (with background noise, 45dB)

The sampling interval was 4 ms.

The experiment was a repetition of the day 4 test experiment. At the end of the experiment the rats were sacrificed by suffocation with CO_2. The fear conditioning experiments were executed by Merete Hansen and Line Sørensen.

3.4. Extended-tone fear conditioning (B1, B2 and B3)

(**NS Intern experiment procedure Fear Conditioning, see appendix 3.35.**)

Three extended-tone fear conditioning experiments were executed with the drugs: alprazolam (experiment B1), α5IA-II (experiment B2) and NS.A (experiment B3). All the drugs were tested with the use of 40 animals, and consequently the groups were the same for alprazolam and NS.A as the groups presented in the fear conditioning experiments (See part '3.3. Fear conditioning (A1, A2 and A3)'). The five groups for α5IA-II were: Vehicle (tween 5 %) with electric shock, vehicle (tween 5 %) no electric shock, 1 mg/kg α5IA-II with electric shock, 3 mg/kg α5IA-II with electric shock, and 10 mg/kg α5IA-II with electric shock. See experimental protocol, appendix 3.22., 3.23. and 3.24.

The procedure was exactly the same as the one used in the fear conditioning experiments described above, except there was no re-testing and the test program used on day 4 differed. The tone on day 4 is the same in both of the fear conditioning models, but in the extended-tone fear conditioning model, the tone last for 90 seconds instead of only 10 seconds and appears twice. Only the procedure for day 4 will be described in the following.

3.4.1. Day 4: Extended-tone testing of the animals

Program

Trial: FC test 2006-10 Tone ext twice

Habituation: 5sec (with background noise, 45dB)

Baseline: 30sec (with background noise, 45dB)

The sampling interval was 4 ms.

The test experiments were carried out approximately 24 hours after the conditioning experiments. None of the rats received an electric foot shock, but was only presented to the tone. The rats were exposed to the background noise (45 dB) for 40 seconds where after they were presented to the tone at 5 kHz and 80 dB for a period of 90 seconds. The two trials were repeated, and the trials were separated by an Inter-Trial-Interval at 10 seconds. See appendix 3.38 for details regarding the program.

Procedure

The procedure was the same as the one described above in part '3.3.4. Day 4: Testing the animals'. At the end of the experiment the rats were sacrificed by suffocation with CO_2. The extended-tone fear conditioning experiments were executed by Merete Hansen and Line Sørensen.

3.5. Trace fear conditioning (C)

(**NS Intern experiment procedure Fear Conditioning, see appendix 3.35.**)

One trace fear conditioning experiment was executed with the drug α5IA-II (experiment C). α5IA-II was tested with the use of 40 animals which randomly were classified into five groups: Vehicle (tween 5 %) no electric shock (nTFC), vehicle (tween 5 %) with electric shock (nTFC), vehicle (tween 5 %) with electric shock (TFC), 1 mg/kg α5IA-II with electric shock (TFC), and 3 mg/kg α5IA-II with electric shock (TFC). The abbreviate nTFC and TFC refers to the programs used, see below at section '3.5.3. Day 3: Conditioning Experiment'. Prior to the conditioning experiment the rats were injected with either vehicle (tween 5 %) or 1 mg/kg or 3 mg/kg of α5IA-II according to the groups they belonged to. See experimental protocol, appendix '3.25.'.

The procedure was similar to the one used in the extended-tone fear conditioning experiments described above ('3.3. Fear conditioning (A1, A2 and A3)'), but the programs used differed and there was no re-testing.

3.5.1. Day 1: Handling of animals and calibration

Program

Trial (system 1): Soren test, 110dB

Trial (system 2): Soren test

The sampling interval was 2 ms

Procedure

See section '3.3. Fear conditioning (A1, A2 and A3)'.

3.5.2. Day 2: Habituation

Program

Trial: FC Habituation 45dB MKH
Habituation: 180sec (with background noise, 45dB)
Baseline: 30sec (with background noise, 45dB)
The sampling interval was 4 ms

The rats were placed in the startle boxes and exposed to the background noise (white noise at 45 dB). See appendix 3.39. for details regarding the program

Procedure
See section '3.3. Fear conditioning (A1, A2 and A3)'.

3.5.3. Day 3: Conditioning experiment

Program
Trial: TFC tone shock no trace KBT (abbreviated to nTFC)
Trial: TFC tone shock 60 sec trace KBT (abbreviated to TFC)
Habituation: 5sec (with background noise, 45dB)
Baseline: 30sec (with background noise, 45dB)
The sampling interval was 4 ms.

In the conditioning experiment two different programs were used. The nTFC program exposed the rats to the background noise (45 dB) for 10 seconds, where after they were presented to a tone at 5 kHz and 80 dB for a period of 10 seconds. In the last second of the period the rats simultaneously received an electric foot shock at 0.6 mA through the steel grid. This was followed by a period of 30 seconds with background noise. In the TFC program the tone and the electric shock was separated by a time gap at 60 seconds. First the rats were exposed to background noise (45 dB) for 10 seconds, where after they were presented to a tone at 5 kHz and 80 dB for a period of 10 seconds. The tone was followed by a period of background noise lasting for 50 seconds and then a period of 10 seconds without any noise. In the last second of this quiet period the rat received an electric foot shock at 0.6 mA through the steel grid. See appendix 3.39. for details regarding the programs.

Procedure

The computer program was started and the startle boxes were tested in regard to ensure that the noise stimulation and the electric stimulation were functional. Hereafter it was entered into the program which rat was put in which startle box. See appendix 3.35 for details about the upstart of the computer program.

30 minutes prior to experiment the rats were injected i.p. with either vehicle (tween 5 %), 1 mg/kg α5IA-II or 3 mg/kg α5IA-II according to the group they belonged to (see '3.25'). Four rats were injected simultaneously and after the injections, they were placed back in their cages and maintained there for approximately 30 minutes before they were placed in the rat cages and inserted into the startle boxes. The rat cages were connected to electricity through sockets placed inside the startle boxes, if the tested rat belonged to a 'with electric shock' group. Hereafter one of the two trace fear conditioning program was started according to which groups the rats placed in the startle boxes belonged to (see appendix 3.25). Four animals were tested at the same time, alternately in system 1 and system 2. The same drug dose was not tested in the same box as the experiment was repeated and in order to balance the differences between system 1 and system 2, both programs were used equal amounts of times in system 1 and system 2.

3.5.4. Day 4: Testing the animals

Program

Trial: TFC LIS MKH

Habituation: 5sec (with background noise, 45dB)

Baseline: 30sec (with background noise, 45dB)

The sampling interval was 4 ms.

The test experiment was carried out after approximately 24 hours. The rats were presented to the same tone as they were at day 3, but without the additional foot shock. See appendix 3.39. for details regarding the program.

Procedure

See section ‘3.3. Fear conditioning (A1, A2 and A3)’ At the end of the experiment the rats were sacrificed by suffocation with CO_2.

The trace fear conditioning experiment were executed by Merete Hansen and Line Sørensen.

3.6. Pre-pulse inhibition, dose-response, methylphenidate (D1 and D2)

3.6.1. Pre-pulse inhibition, dose-response, mice (D1)

(**NS Intern experiment procedure Pre-pulse inhibition, see appendix 3.36.**)

Equipment

8 TSE StartleResponse Box (TSE Technical & Scientific Equipment GmbH, Germany).
The 8 TSE startle boxes were separated in two systems named system 1 and system 2
8 mice cages (Aluminium, plexi glass, integrated floor grid) (100 X 60 X 90 mm) (TSE Technical & Scientific Equipment GmbH, Germany)

Program

Trial (system 1): PPI, pp4, pp8, pp16, pp24 pulse all + 5dB
Trial (system 2): PPI, pp4, pp8, pp16, pp24 pulse
Habituation: 180s (with background noise, 70dB)
Baseline: 30sec (with background noise, 70dB)
The sampling interval was 2 ms.

In the pre-pulse inhibition experiment, the animals were exposed to an adaptation period for 3 minutes and 50 seconds in which no startle stimulus were presented. The startle pulse consisted of 110 dB noise bursts of 40 mseconds duration, whereas the pre-pulses consisted of noise bursts at 4, 8, 16 or 24 dB above background noise and with duration of 20 mseconds. The pre-pulses were presented 100 mseconds prior to the startle pulse. There were 5 types of stimulus trials, and each trial type was presented 12 times. The trial types were presented in a random order to avoid order effects and habituation. Inter-trial intervals ranged randomly from 10 to 20 seconds. Trial types included 110 dB stimuli (startle pulse alone trial), 110 dB stimuli preceded by pre-pulses, and a trial with

no discrete stimulus other than the background noise. The testing period lasted for approximately 22 minutes. The programs 'PPI, pp4, pp8, pp16, pp24 pulse all + 5dB' and 'PPI, pp4, pp8, pp16, pp24 pulse' included the same trials, but were named differentially in system 1 and system 2. See appendix 3.40. for details regarding the program.

Procedure

The transducers were calibrated exactly as described above in section '3.3. Fear Conditioning (A1, A2 and A3)'. The computer program was started and the startle boxes were tested in regard to ensure that the noise stimulation were functional. Hereafter it was entered into the program which mouse was put in which startle box. See appendix 3.36 for details about the upstart of the computer program (This system test and the calibration were performed before each of the pre-pulse inhibition experiments described in the following sections). The 32 mice were randomly classified into four methylphenidate dose groups: Vehicle (NaCl 0.9 %), 5 mg/kg, 10 mg/kg, 20 mg/kg. Substances were administered s.c. in the scruff of the neck 15 minutes prior to start of pre-pulse experiment. The mice were weighed (25 – 30 g) and injected with either vehicle (NaCl 0.9 %) or 5 mg/kg, 10 mg/kg or 20 mg/kg of methylphenidate according to the groups they belonged to. Four mice were injected simultaneously and after the injection the mice were placed back in their cages and maintained there for approximately 15 minutes before they were placed in the mice cages and inserted into the startle boxes. Hereafter, the pre-pulse inhibition program was started. Four mice were tested at the same time, alternately in system 1 and system 2. The same drug dose was not tested in the same box as the experiment was repeated. At the end of the experiment the mice were sacrificed by suffocation with CO_2. The pre-pulse inhibition experiment was executed by Jesper Tobias Andreasen, Merete Hansen and Line Sørensen.

3.6.2. Pre-pulse inhibition, dose-response, rat (D2)

Equipment

The equipments used was the same as used in the fear conditioning experiments, see part '3.3. Fear Conditioning (A1, A2 and A3)'.

Program

The program used was the same as the one used in pre-pulse inhibition dose-response experiment with mice. See above in part ‘3.6.1. Pre-pulse inhibition, dose-response, mice, D1’.

Procedure

The transducers were calibrated exactly as described above in section ‘3.3. Fear Conditioning (A1, A2 and A3)’ 24 rats were randomly classified into four methylphenidate dose groups: Vehicle (NaCl 0.9 %), 5 mg/kg, 10 mg/kg, 20 mg/kg. The rats were marked, weighed (280 – 330 g) and injected with either vehicle (NaCl 0.9 %) or 5 mg/kg, 10 mg/kg or 20 mg/kg of methylphenidate according to the groups they belonged to. See experimental protocol in appendix 3.27. Substances were administered s.c. in the scruff of the neck 15 minutes prior to beginning of pre-pulse experiment. Hereafter the procedure was exactly the same as the procedure described for the pre-pulse inhibition experiment with mice described above in part ‘3.6.1. Pre-pulse inhibition, dose-response, mice, D1’. At the end of the experiment the rats were sacrificed by suffocation with CO_2. The pre-pulse inhibition experiment was executed by Merete Hansen and Line Sørensen.

3.7. Pre-pulse inhibition, dose-response, PCP (E1, E2 and E3)

Three pre-pulse inhibition dose response experiments (E1, E2 and E3) were executed with PCP administered at the different doses: 1.25 mg/kg, 2.5 mg/kg and 5 mg/kg. These three different concentrations were all tested in three individual pre-pulse inhibition dose response experiments and with the use of 32 rats in each of the three experiments. Consequently the rats were randomly classified into four dose groups: Vehicle (NaCl 0.9 %), 1.25 mg/kg PCP, 2.5 mg/kg PCP, and 5 mg/kg PCP (see experimental protocols ‘3.28’, ‘3.29’ and ‘3.30’). Before the start of each of the three pre-pulse inhibition experiments the rats were marked and weighted and the transducers were calibrated exactly as described above in section ‘3.3. Fear Conditioning (A1, A2 and A3)’.

In experiment E1 PCP or vehicle (NaCl 0.9 %) was administered s.c. in the scruff of the neck 72 hours prior to pre-pulse inhibition experiment, whereas in experiment E2 PCP or vehicle (NaCl 0.9 %) was administered 24 hours prior to pre-pulse inhibition experiment, and finally PCP or vehicle (NaCl 0.9 %) was administered 10 minutes prior to pre-pulse inhibition experiment in experiment E3.

3.7.1. Pre-pulse inhibition (E1, E2 and E3)

(**NS Intern experiment procedure Pre-pulse inhibition, see appendix 3.36.**)

Equipment

The equipments used were the same as used in the fear conditioning experiments, see part '3.3. Fear Conditioning (A1, A2 and A3)'.

Program

The program used were the same as the one used in pre-pulse inhibition dose-response experiment with mice. See above in part '3.6.1. Pre-pulse inhibition, dose-response, mice, D1'.

Procedure

72 hours after PCP or vehicle injections the 32 rats in experiment E1 were injected s.c again. This time all the rats received NaCl 0.9 %. In experiment E2 the 32 rats also were injected with NaCl 0.9 %, but only 24 hours after injections of PCP or vehicle. The injections of NaCl 0.9 % in both experiments were done simultaneously for four rats and 10 minutes later the rats were exposed to the pre-pulse inhibition tests. In the E3 experiment PCP or vehicle was injected s.c 10 minutes prior to the pre-pulse inhibition test and the rats in experiment E3 only had this single injection.

After the last injection the rats were placed in their cages again and maintained there for approximately 10 minutes before they were placed in the rat cages and inserted into the startle boxes. The procedure was hereafter similar to the one used in the pre-pulse inhibition experiment described above in part '3.6.1. Pre-pulse inhibition, dose-response, mice, D1'. Immediately after the end of each round of the pre-pulse inhibition tests, the brains of the rats were removed and frozen. The pre-pulse inhibition experiments were executed by Merete Hansen and Line Sørensen, and the brains

removed by Helle Hvorup Knudsen (experiment E1), Rigmor Jensen (experiment E2) and Britta Karlsson (experiment E3).

3.8. Pre-pulse inhibition, PCP deficits (F1, F2 and F3)

(NS Intern experiment procedure Pre-pulse inhibition, see appendix 3.36)

Three pre-pulse inhibition experiments with PCP deficit were executed. PCP was administered s.c. in the scruff of the neck at dose 2.5 mg/kg and 10 minutes prior to pre-pulse experiments (The dose of PCP and the pre-treatment time was chosen after evaluation of the three dose response pre-pulse inhibition dose response experiments: E1, E2 and E3). In the three pre-pulse inhibition experiments with PCP deficit the three drugs under investigation was tested: alprazolam (experiment F1), α5IA-II (experiment F2) and NS.A (experiment F3). The three experiments were sat up as full factorial designs with eight groups of each eight animals. Consequently each drug was tested with the use of 64 rats, which randomly were classified into the following groups:

Alprazolam experiment (F1): Vehicle (NaCl 0.9 % s.c) and vehicle (cremophor 5 % i.p.), vehicle (NaCl 0.9 % s.c) and 0.4 mg/kg alprazolam, vehicle (NaCl 0.9 % s.c) and 1.33 mg/kg alprazolam, vehicle (NaCl 0.9 % s.c) and 4 mg/kg alprazolam, PCP and vehicle (cremophor 5 % i.p.), PCP and 0.4 mg/kg alprazolam, PCP and 1.33 mg/kg alprazolam, PCP and 4 mg/kg alprazolam.

α5IA-II experiment (F2): Vehicle (NaCl 0.9 % s.c) and vehicle (tween 5 % i.p.), vehicle (NaCl 0.9 % s.c) and 1 mg/kg α5IA-II, vehicle (NaCl 0.9 % s.c) and 3 mg/kg α5IA-II, vehicle (NaCl 0.9 % s.c) and 10 mg/kg α5IA-II, PCP and vehicle (tween 5 % i.p.), PCP and 1 mg/kg α5IA-II, PCP and 3 mg/kg α5IA-II, PCP and 10 mg/kg α5IA-II.

NS.A experiment (F3): Vehicle (NaCl 0.9 % s.c) and vehicle (tween 5 % i.p.), vehicle (NaCl 0.9 % s.c) and 0.3 mg/kg NS.A, vehicle (NaCl 0.9 % s.c) and 1 mg/kg NS.A, vehicle (NaCl 0.9 % s.c) and 3 mg/kg NS.A, PCP and vehicle (tween 5 % i.p.), PCP and 0.3 mg/kg NS.A, PCP and 1 mg/kg NS.A, PCP and 3 mg/kg NS.A.

See experimental protocols ‘3.31.’, ‘3.32.’ and ‘3.33.’. Before the start of each of the three pre-pulse inhibition experiments the rats were marked and weighted and the transducers were calibrated exactly as described above in section ‘3.3. Fear Conditioning (A1, A2 and A3)’.

Equipment

The equipments used was the same as used in the fear conditioning experiments, see part ‘3.3. Fear Conditioning (A1, A2 and A3)’.

Program

The program used was the same as the one used in pre-pulse inhibition dose-response experiment with mice. See above in part ‘3.6.1. Pre-pulse inhibition, dose-response, mice, D1’.

Procedure

30 minutes prior to pre-pulse inhibition experiment the rats were injected i.p. with either the drug under investigation or the matching vehicle. Simultaneously four animals were injected according to the dose groups they belonged to. After the injections the rats were placed back in their cages and maintained there for approximately 20 minutes before they were injected again with either PCP or vehicle (NaCl 0.9 %). Again the rats were placed back in their cages and after further teen minutes the rats were placed in the rat cages and inserted into the startle boxes. Hereafter the procedure was exactly the same as the procedure described above for the pre-pulse inhibition experiment with mice; see part ‘3.6.1. Pre-pulse inhibition, dose-response, mice, D1’. At the end of the experiment the rats were sacrificed by suffocation with CO_2.

Unfortunately the experiment with α5IA-II (F2) was discontinued by a power failure, and the rats numbered 29 to 44 had consequently to be executed from the experiment. The rats 45 to 64 were tested the following day. The drugs were stored in tinfoil in refrigerator over night. These changed are listed in the experimental protocols ‘3.32.’. The three pre-pulse inhibition experiments were executed by Merete Hansen and Line Sørensen.

3.9. Pre-pulse inhibition, amphetamine deficit and NS.A (G)

A single pre-pulse inhibition experiment with amphetamine deficit was executed with NS.A. Amphetamine was administered s.c. in the scruff of the neck at the dose 4 mg/kg, and the experiment was sat up as full factorial designs with 64 rats randomly divided into eight NS.A dose groups: Vehicle (NaCl 0.9 % s.c) and vehicle (tween 5 % i.p.),

vehicle (NaCl 0.9 % s.c) and 0.3 mg/kg NS.A, vehicle (NaCl 0.9 % s.c) and 1 mg/kg NS.A, vehicle (NaCl 0.9 % s.c) and 3 mg/kg NS.A, amp and vehicle (tween 5 % i.p.), amp and 0.3 mg/kg NS.A, amp and 1 mg/kg NS.A, amp and 3 mg/kg NS.A. See experimental protocol '3.26.'. Before the start of the pre-pulse inhibition experiment the rats were marked and weighted and the transducers were calibrated exactly as described above in section '3.3. Fear Conditioning A1, A2 and A3)'.

Equipment

The equipments used was the same as used in the fear conditioning experiments, see part '3.3. Fear Conditioning (A1, A2 and A3)'.

Program

The program used was the same as the one used in pre-pulse inhibition dose-response experiment with mice. See above in part '3.6.1. Pre-pulse inhibition, dose-response, mice, D1'.

Procedure

30 minutes prior to the pre-pulse inhibition experiment the rats were injected in rounds of four. Amphetamine and NS.A was injected s.c. and i.p, respectively 30 minutes before the pre-pulse inhibition tests. Initially the rats were injected s.c. and then turned around and injected i.p. After the two injections the rats were placed back in their cages and maintained there until they were placed in the rat cages and inserted into the startle boxes. Hereafter the procedure was exactly the same as the procedure described above for the pre-pulse inhibition experiment with mice. See part '3.6.1. Pre-pulse inhibition, dose-response, mice, D1'. At the end of the experiment the rats were sacrificed by suffocation with CO_2. The pre-pulse inhibition experiment was executed by Merete Hansen and Line Sørensen.

3.10. Dose-response with PCP deficits, α5IA-II (H)

Procedure

In the dose-response experiment with PCP deficit the behaviours of the rats after injections of PCP and/or α5IA-II was observed. Before the experiment 10 rats were

marked and weighted and classified into the four following groups: Vehicle (NaCl 0.9 % s.c) and vehicle (tween 5 % i.p.), PCP and vehicle (tween 5 % i.p.), vehicle (NaCl 0.9 % s.c) and α5IA-II, PCP and α5IA-II. See experimental protocol '3.34'. 30 mg/kg α5IA-II or vehicle (tween 5 %) was injected 30 minutes prior to the beginning of the observations of the rats' behaviour and subsequently PCP or vehicle (NaCl 0.9 %) was administered 10 minutes prior to the start of the observation s.c. in the scruff of the neck at dose 2.5 mg/kg. The rats were after the two injections observed in their cages and after the observation the rats were sacrificed by suffocation with CO_2 (the observations of the behaviours of the animals during the experiments are noted in the laboratory journals appendix 3.18). The experiments were executed by Line Sørensen and Karin Sandager Nielsen and Naheed Mirza helped with the observations.

4. Statistical considerations and analyses

In this section the statistical issues are considered. The objective is to obtain a suitable analysis of the experiments performed. Three main types of experiments have been carried out: fear conditioning experiments, pre-pulse inhibition experiments and a dose-response experiment. The latter have been of observational character with no response values recorded and consequently no statistics applied. Regarding the two former types, they share some common properties. First, the two experiments are performed in the same room with utilisation of the same experimental equipment, i.e. startle boxes, control boxes, computers and software, and consequently the output is similar. In both cases the movement level for each animal is measured for a given period of time with a fixed interval between observations. The similarities in the information obtained in different experiments can be utilised in the statistical analysis. A complete analysis is developed for test day data in one of the fear conditioning experiment. The model is used as a starting point in the analyses of remaining fear conditioning experiments. Due to the points of resemblance between fear conditioning and pre-pulse inhibition some of the information is also reusable in the analysis of pre-pulse inhibition and the knowledge of the relevant terms are utilised in this framework.

In the development of the statistical model the character of the experiments and data are considered in order to identify all possible factors that are conceivable to affect the response values and, thus, is advantageous to include in the model. Due to the repeated measures on each animal a linear mixed effects model is employed. In order to avoid violation of model assumptions it proves to be a requisite to allow for heteroscedasticity and residual autocorrelation in the model. Some interpretational aspects of the results are subsequently considered. Finally, the model is modified in order to attain an appropriate analysis of pre-pulse inhibition experiments and the interpretation of the results is considered.

All statistical analyses are performed using R, version 2.4.0 (**R Development Core Team, 2006**). Some graphs are made in SigmaPlot 2000 for Windows, Version 6.00.

4.1. Analysis of fear conditioning experiments

In the analysis of the fear conditioning experiment some practical and biological issues are regarded before assessment of actual data. The obtained output from the fear conditioning experiments is extensive due to high sampling frequency. An average is therefore calculated every fifth second, i.e. low-pass filtering, and these values are used in the further data processing. Furthermore, the data is analysed one day at a time although the data of one experiment is collected over three or four days. In addition, one of the fear conditioning experiments is a full factorial design with 'treatment' and 'shock' as the factors and in this case the data obtained for the animals receiving 'shock' and 'no shock', respectively, are analysed separately. This is due to the fact that 'shock' is a prerequisite for a reasonable interpretation of the experiment and therefore the 'no shock' group probably will obscure the trends of biological interest. However, the 'no shock' group is of interest in order to see if the drug is accountable for any effect per se, e.g. sedation or shift of attention. If this is the case 'treatment' may be given a misleading interpretation. Due to these considerations, there will be 2-6 separate analyses.

As a starting point of the analysis the experiment, with the administration of α5IA-II and a conditioned stimuli on conditioning day, was chosen since this was the most extensive of any of the experiments performed. It was carried out as a full factorial design with 'treatment' and 'shock' as factors on the contrary to the other fear conditioning experiments. Furthermore, the animals were retested at day 10 of the experiment opposed to some of the other experiments. The experimental day of main importance is the test day and for that reason the data obtained this day was analysed first.

Several factors in the fear conditioning experiment have been identified to possibly influence the response. These are 'treatment', 'time', 'period', 'animal', 'system', 'box', 'round' and 'operator'. The meaning of these factors will at some point be clear from the description of the experimental protocol seen in section '3.3. Fear conditioning (A1, A2 and A3)' but is summarised shortly: 'Treatment' is the drug tested in the experiment and consists most frequently of three different doses and a drug-free vehicle group acting as control. The experimental measurement period was approximately 250 seconds and 'time' is a continuous index variable with time points

every fifth second interrupted a few times by a prolonged interval. During the experiment a tone occurs and ‘period’ indicates if the response value is measured before or after the tone. Various animals are used in the experiment and ‘animal’ indicates which animal the response values are obtained for. The animals are tested in eight different boxes with the first four boxes addressed to system 1 and the remaining boxes to system 2. The factors ‘system’ and ‘box’ is an index reflecting the box and system the response values are obtained from. Eight boxes were available when the 64 animals were tested and consequently eight rounds were performed. Round is an index with the number of the round the response values were obtained in. The experiment was performed by two operators but opposite the other factors the experimental design was not balanced with respect to this factor. The laboratory work was equally distributed between the operators, but it was assigned randomly and the factor is not included in the analysis. The primary experimental motivation is to test the effect of ‘treatment’ and possible interactions between ‘treatment’ and the experimental factors. The other terms should be included in order to eliminate variation from the residual variation and/or to correct for possible biases.

Due to the inherent behaviour of the animals a development due to ‘time’ is expected since the animals in general habituate to their environment. Moreover, ‘period’ is expected to be influential at the groups receiving a foot shock at the conditioning session the previous day. The variations due to ‘animals’ will be referred to as the biological variation and will presumably be causing a certain amount of variation that is impossible to eliminate in experimental testing. The remaining factors, ‘system’, ‘box’, ‘round’ and ‘operator’, should ideally not cause any substantial amount of variation or bias.

4.1.1. Graphical exploration of fear conditioning data

In order to attain an initial impression of the structure of data the response values can be depicted against the different factors. In this way patterns may be revealed that may prove to be useful in the model development.

First, the average response values for each dose are depicted against ‘time’ in figure 4.1. Here it is seen that the response values for the two lowest doses behave like anticipated in that a higher response is seen especially after the occurrence of the tone.

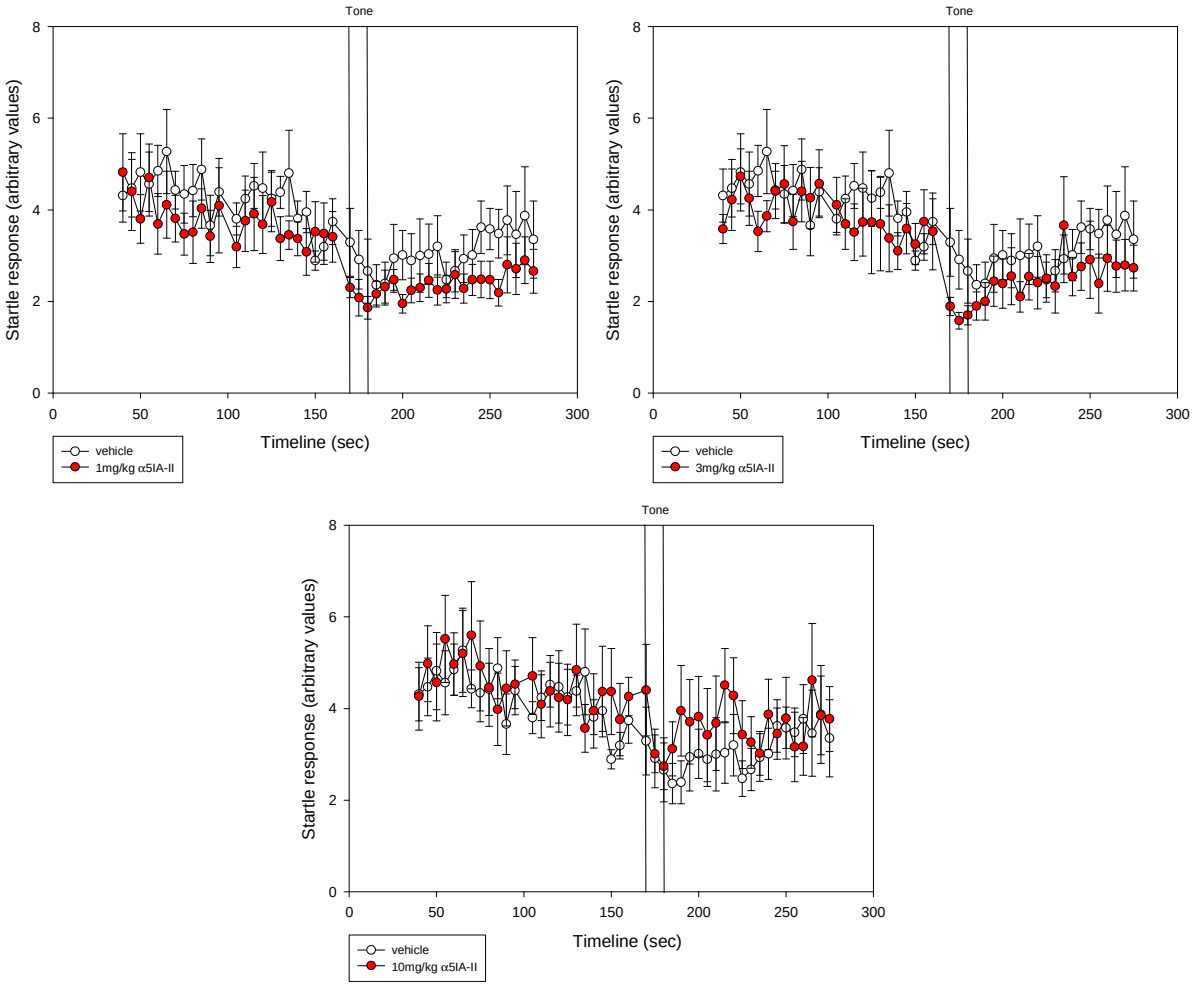


Figure 4.1. The average values depicted against 'time' for each of the three doses 1 mg/kg, 3 mg/kg and 10 mg/kg of the drug α5IA-II. The vehicle group is the same in all three plots.

Though, for the highest dose 10 mg/kg it is seen that response values are located close to the vehicle group and that they even tend to exceed them at some time spans.

The response values for each dose are depicted in figure 4.2 with a separate line for each animal. In order to diminish the correlation between the intercept and the slope the time points are shifted so $t = 0$ at the time point where the animals are exposed to the tone. From this graph some initial tendencies are seen. First, there seems to be a pronounced degree of variability when the movement pattern of the different animals is compared. Some of the animals have a low level of movement maintained throughout the experiment whereas other animals averagely have a high movement level. The high degree of variability is also valid with respect to the movement of the individual animals at different time points and this seems to be more pronounced for the relatively high

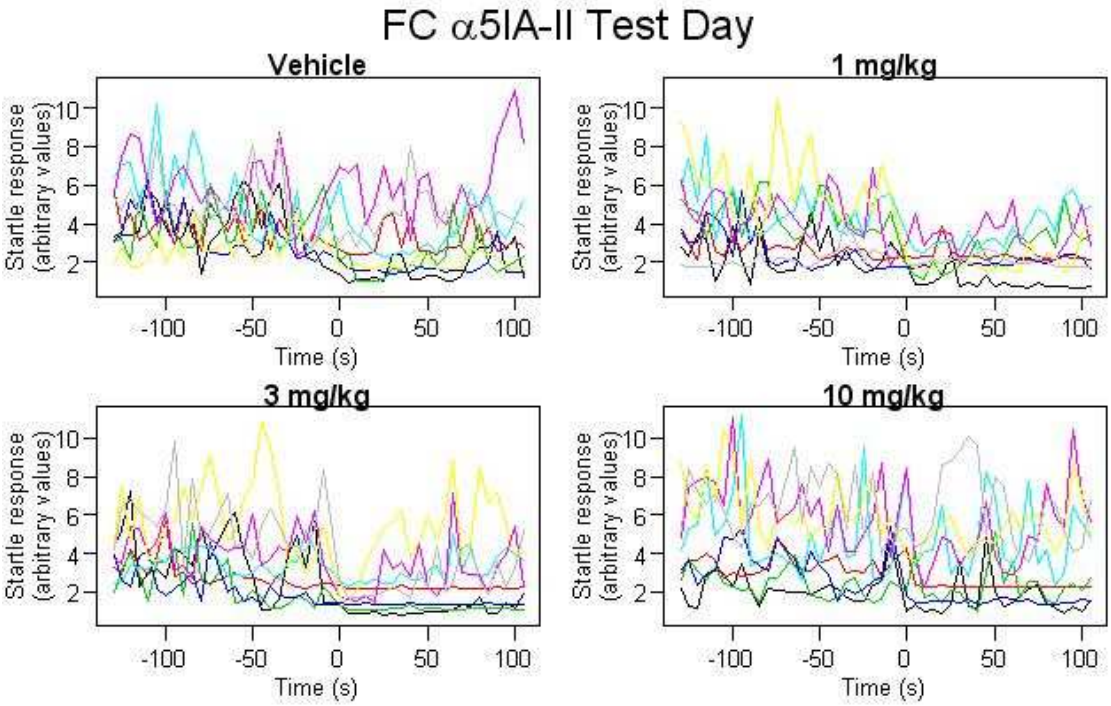


Figure 4.2. The response values of the animal vs. time for each dose

response values. Moreover, it is possible to see a change in the response level for some of the animals when the tone occurs at $t = 0$ which is consistent with the expected.

Due to the relative higher variability seen for the high response values, it is considered if the assumptions of the model used later are violated in any way. The

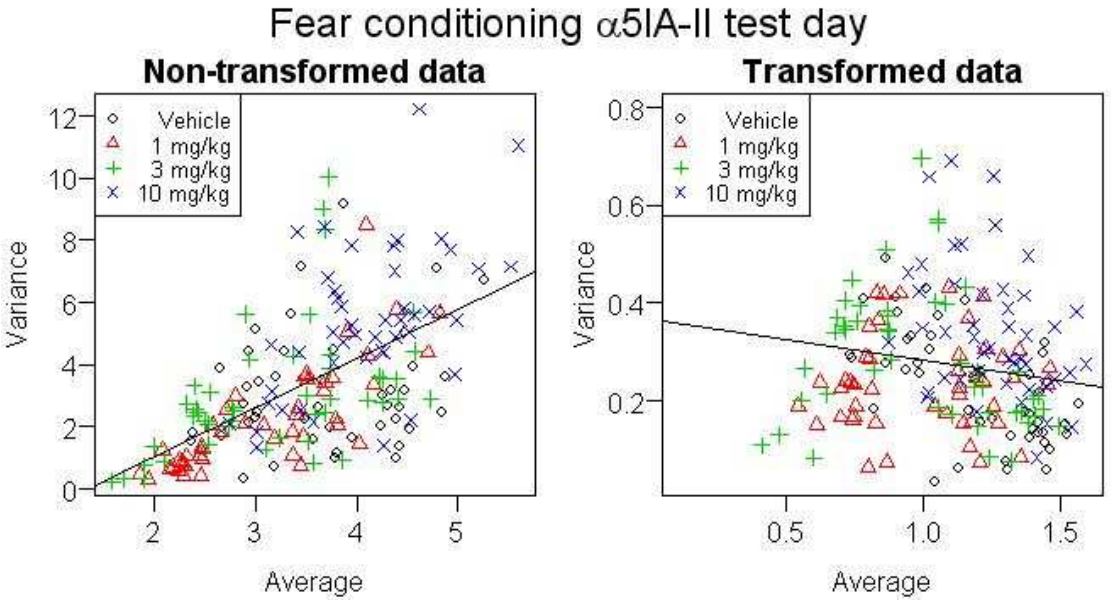


Figure 4.3. (a) The variance vs. the average for the non-transformed values. The magnitude of the variance depends to some extent at the average (b) Reduced coherence between the average and the variance is seen for the ln-transformed values

residuals of the model shall be independent and normally distributed and in order to clarify if this is satisfied the variance for each dose and 5 second period is depicted against the average in figure 4.3.a. It is seen that the variances generally increase with the average. For this reason the values are ln-transformed and the corresponding graph is seen in figure 4.3.b. Here it is seen that the variances are randomly scattered and that the size of the variance is independent of the average. Consequently, the ln-transformed response values will be used henceforth.

In figure 4.4 the two systems are depicted against the non-transformed and the ln-transformed values, respectively. Additionally, a plot with the progression in time for each of the two systems is shown. It was expected that 'system' would not contribute significantly with variation or bias, but in these figures it looks as both is rendered pronouncedly. The raw values reflect affection at both the average and the variation, though, again it seems like the higher variability is associated with the relative high level of the average. This is supported by the ln-transformed values, where the variation in system 2 is reduced substantially and has become comparable to system 1. Yet, as clearly seen in the last figure there is still an unexpected difference in the average level for the two systems.

In order to examine if the system effect is due to some specific boxes the ln-

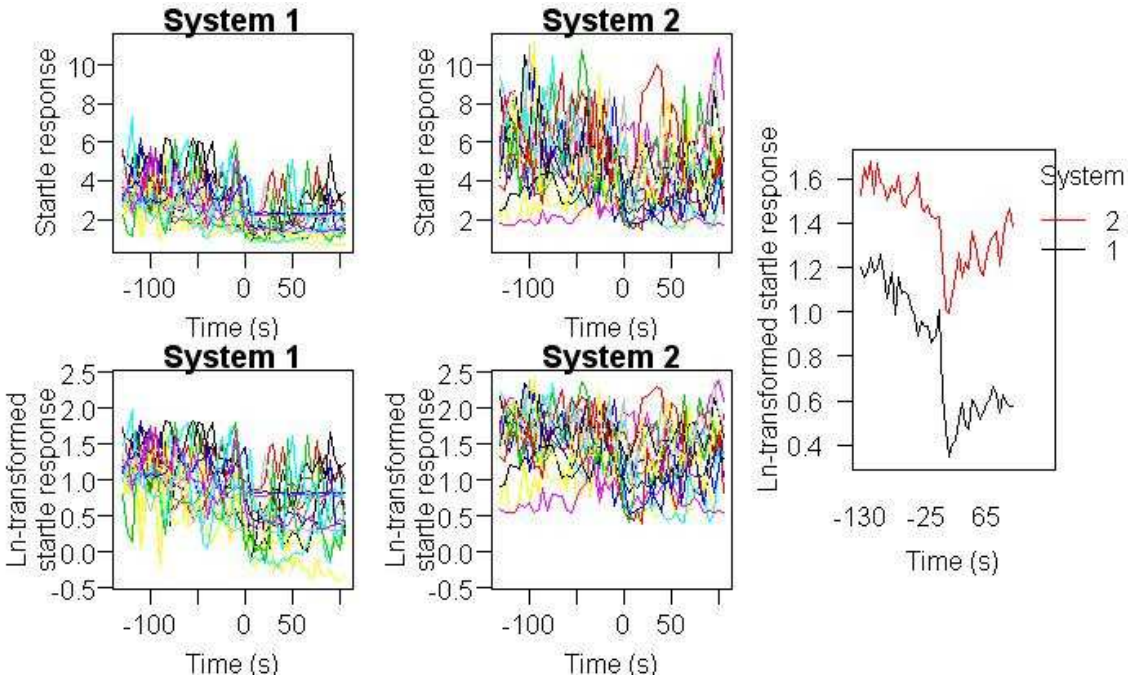


Figure 4.4. (a) In the first row the non-transformed startle response is depicted against time (b) In the second row the ln-transformed startle response vs. time is seen (c) To the right the overall average for each system is depicted against time.

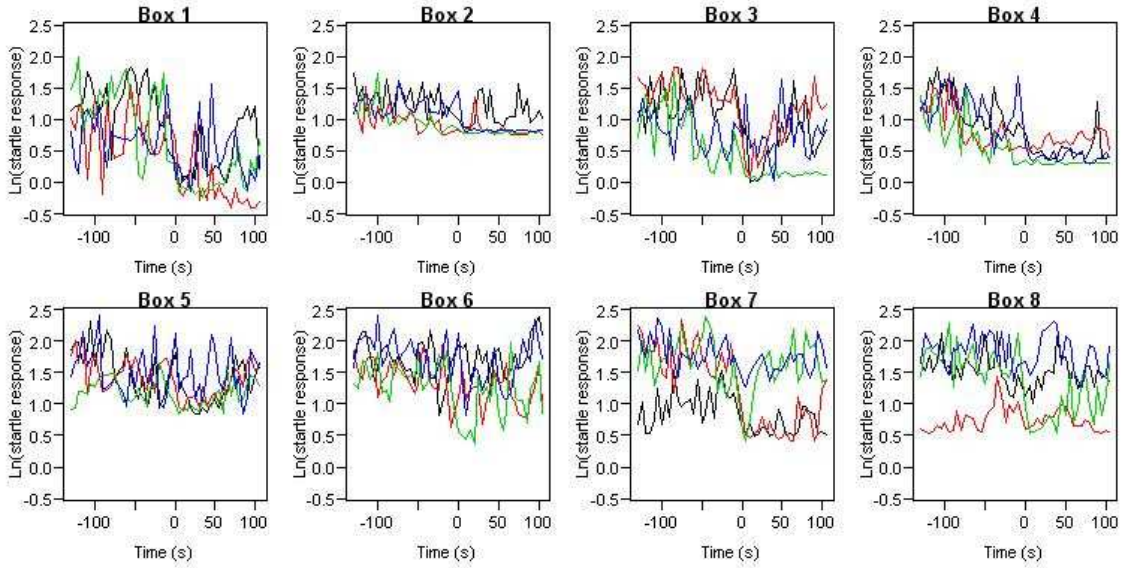


Figure 4.5. The ln-transformed startle response depicted against time for each of the eight boxes. Box 1-4 belong to system 1 whereas box 5-8 belong to system 2.

transformed values are depicted vs. the different boxes (figure 4.5) and a box plot for the two systems is shown in figure 4.6. It is again seen that the bias owing to the two systems is present but the average value for all boxes within each system is maintained at a fairly constant level. However, box 1 has an unusually high variation compared to the other boxes, especially opposed to box 2 for which a very low variation is seen.

The bias induced by the two systems should be equalised during the experiment since the experiment is balanced with respect to boxes and doses. Unfortunately, additional variation is added to the residual component which may cause a difficulty in detecting significance if it is present and the systems are not accounted for.

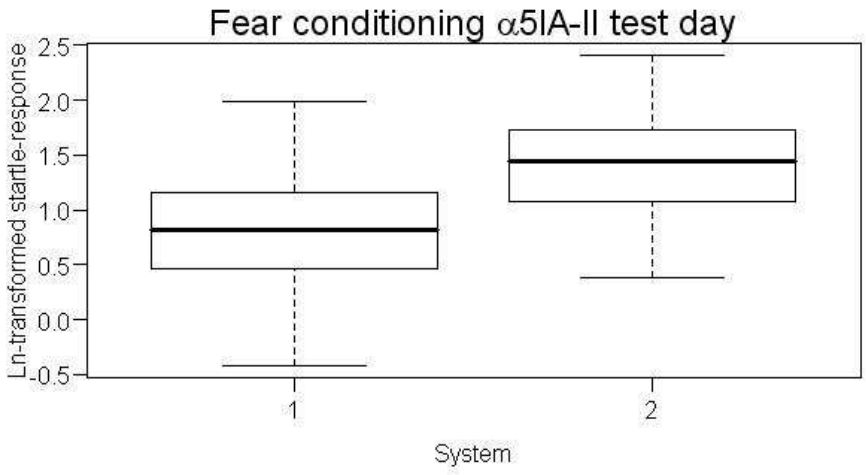


Figure 4.6. A box plot showing the bias induced by the different systems.

Furthermore, it seems as box 1 and 2 may inflate and shrink the response values, respectively. Both cases are suspicious and may yield unreliable response values in which case it can be worth considering excluding boxes from the analysis. Thus, before fear conditioning data is analysed the effect from the individual systems and/or boxes are further explored.

4.1.2. Examination of variability induced by experimental equipment

It is possible that the heterogeneity between box 1 and 2, respectively, is due to randomness. In that case the variance within each box is expected to be chi-square distributed. It is also a possibility that a disparity in the variation of the different boxes is due to some atypical groups of animals and, thus, is purely fortuitous.

The following model is fitted the data for each box

$$\ln(Y_{ijk}) = \mu + p_i + \beta_1 \cdot t_j + \beta_{i2} \cdot pt_{ij} + A_k + \varepsilon_{ijk},$$

where

$$i = 1,\ldots,2, \quad j = -130,\ldots,105, \quad k = 1,\ldots,32,$$
$$A_k \sim N(0,\sigma_A^2), \quad \varepsilon_{ijk} \sim N(0,\sigma_\varepsilon^2),$$

μ, p_i and t_j are the fixed effects for the intercept, period and slope concerning time, respectively. A_k is the random effect of the kth animal and ε_{ijk} is the within-group error. The residual components are then depicted against the chi-square distribution (figure 4.7.a). Furthermore, for each animal the following model is fitted the data

$$\ln(Y_{ij}) = \mu + p_i + \beta_1 \cdot t_j + \beta_{i2} \cdot pt_{ij} + \varepsilon_{ij}$$

where

$$i = 1,\ldots,2, \quad j = -130,\ldots,105, \quad \varepsilon_{ij} \sim N(0,\sigma_\varepsilon^2),$$

μ, p_i and t_j are the fixed effects for the intercept, period and slope concerning time, respectively and ε_{ij} is the within-group error. These residual components are similarly showed against the chi-square distribution (figure 4.7.b). It is seen that the largest variance is more than twice the size of the smallest variance which is a remarkable difference. In the first plot, box 2 is only slightly deviant from the remainder but box 1

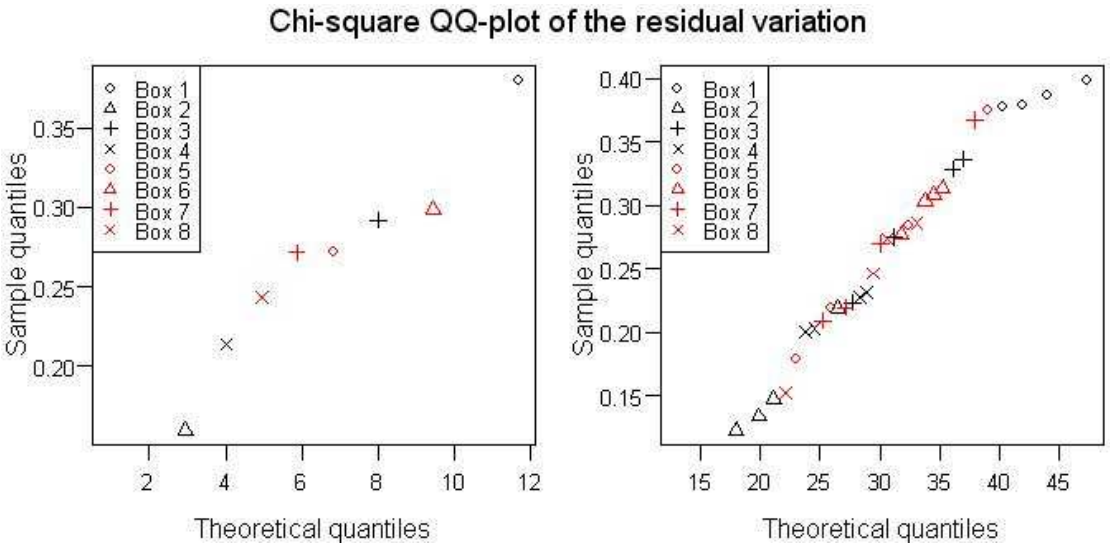


Figure 4.7. (a) Residual components from model 'MB' depicted in a chi-square QQ-plot (b) Residual components from model 'MA' in a chi-square QQ-plot. In both cases the residual components from box 1 and box 2 have the highest and lowest values, respectively.

seems to be at a higher level than expected. In the second plot it is seen that box 1 and 2 is almost consistently positioned at the top and bottom of the plot, respectively. This pattern indicates that the variance heterogeneity may be on account of the boxes per se and not because of atypical animals in the different groups.

Based on these findings it was decided to do an additional experiment in order to find out how the boxes affect the response. Alternatively to the usual rats an 'artificial rat' was used, which is a squared box with a spring inside that is triggered in response to an acoustic cue above a certain threshold. The movement of the artificial rat is assumed relatively constant for the different activations.

The experiment was designed with the attempt to resemble the fear conditioning experiment in order make this experiment as supportive as possible for the evaluation of fear conditioning and to optimise the comparability of the results from the two experiments. All boxes were calibrated and the artificial rat was placed in a box and a tone occurred whereupon the response was measured for one second. This constitutes one trial and it was replicated successively 40 times. This was done for each box and the measurements of all boxes constituted a round. It was repeated five times in order to examine the progression of the measurements for a time period resembling the approximate length of a standard experiment that have five rounds. This procedure was iterated four days with no calibration of the boxes meanwhile in order to access the

development of the response values throughout the period of a fear conditioning experiment.

The raw data from the first trial of the first round and box is shown in figure 4.8. The waves of the spring are detectable but the sampling frequency is inadequate in order to make a proper description of the response curve. In order to compare the different boxes it is convenient to calculate a summary measure from each trial but as seen from the graph neither the maximum nor the average value seems to be completely reliable since the response values are sensitive to the time of measurement. Still, some tendencies might be possible to see from the experiment, although, ideally a new experiment should be carried out with a sampling interval at e.g. 1 millisecond.

The average value was calculated for the first 400 ms of each trial. The 40 average values from each round and each day were depicted vs. the ln-transformed response values with different colours assigned to each box in figure 4.9. Some outliers are seen but in the cases where the response is at a constantly low level throughout a round it is probably due to a loose connection to the artificial rat. For the individual low response points it may be due to a measurement error, but it might also be caused by noise in the laboratory that triggered the spring and due to a lag-phase rendered it

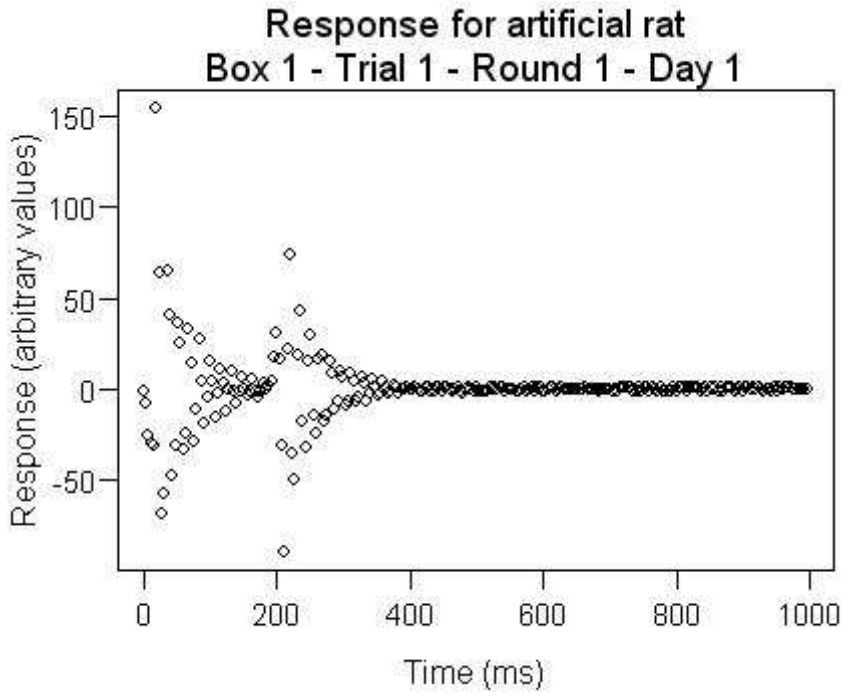


Figure 4.8. The response of the artificial rat from the system test. The wave is subsided after approximately 400 ms. The sampling interval is too large to detect a reliable and repeatable summary measure.

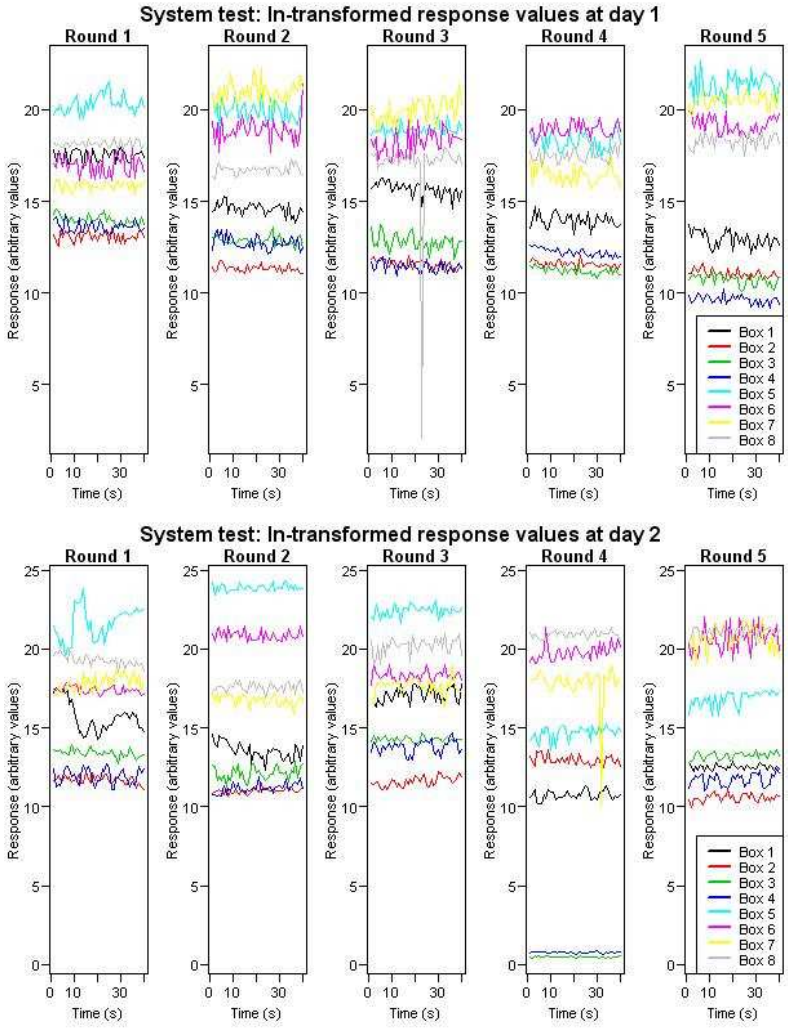


Figure 4.9.a The response from the system test. A considerable variability is seen for the different rounds. In general, the boxes from system 1 yields lower values than the boxes from system 2

insensitive to the acoustic cue furnished by the box. Because of this uncertainty the low values are removed in the future calculations.

At day 1 the response values of system 1 consistently drift downwards whereas the opposite is the case for system 2. Moreover, it is seen that some of the boxes are kept at a fairly stabile level, e.g. box 6 and 8, whereas box 1, 4 and 7, have a more fluctuating response pattern. Interestingly, over the days the variability of the response is decreasing and the smallest deviation is seen at day 4.

In order to take account for the shifting values it may be advantageous to include the interaction between box and round in a model. However, this is exactly what is done when ‘animal’ is included and, thus, ‘animal’ and the ‘box’-‘round’ interaction will be completely confounded. This imply that the estimated variance component for ‘animal’

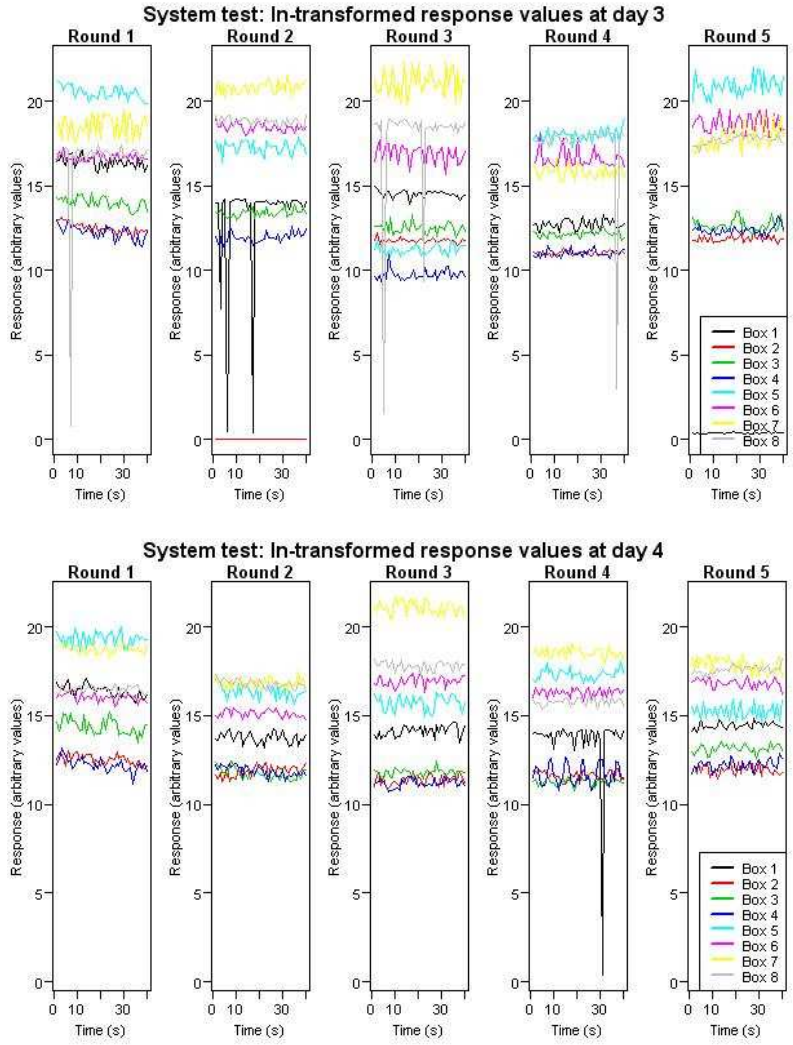


Figure 4.9.b. The response pattern for the system test for day 3 and 4. The response pattern is stabilized slightly at the end of the experiment.

contains some variability caused by the heterogeneity of the boxes in different rounds and it is not possible with the current design of fear conditioning to separate the effect of these two terms.

In order to compare variation for each box throughout a day the model

$$\ln(Y_i) = \mu + \varepsilon_i, \quad i = 1,\ldots,200, \quad \varepsilon_i \sim N(0,\sigma_\varepsilon^2),$$

is fitted each box and day and the residual components are depicted against the chi-square distribution (figure 4.10). It is seen that no box has a variation at a consistently higher or lower level than the others and this also holds true for box 1 and 2 that were different from the other boxes in the fear conditioning α5IA-II experiment. Because of

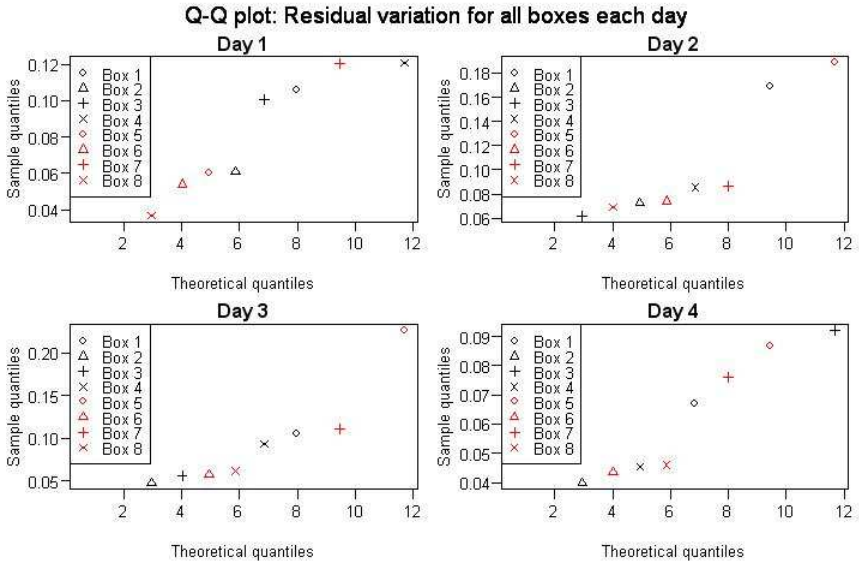


Figure 4.10. The residual variation depicted in a chi-square QQ-plot. It is seen that no box have a consistently higher or lower variability than the remainder.

the inconsistency in the graphs as well as the uncertainty in the response values it is decided not to take any further action regarding the individual boxes at present. Still, at some point it could be interesting to redo the experiment with a more appropriate setting in order to evaluate the experimental equipment.

Regarding the system effect it apparently induces a strong bias and this factor will therefore be included in a model and tested for statistical significance.

4.1.3. Variogram illustrating residual structure

After the above analysis of the effect from the individual systems and/or boxes, the approach of the fear conditioning data started in section ‘4.1.1 Graphical exploration of fear conditioning data’ will be continued.

The magnitude of the random effect ‘animal’ is in the following examined by inspection of the variogram. A variogram is a useful tool for illustrating the structure of residuals. In R, the empirical variogram is determined by the classical estimator

$$\hat{\gamma}(s) = \frac{1}{2N(s)} \sum_{i=1}^{M} \sum_{d(p_{ij}, p_{ij'})=s} (r_{ij} - r_{ij'})^2,$$

where r_{ij} optionally is untreated, standardised or normalised residuals and $N(s)$ is the number of residual pairs at distance s (**Pinheiro and Bates, 2000**).

For a stationary function this is similar to

$$\hat{\gamma}(s) = \sigma^2(1-\rho(s)),$$

where σ^2 is the variance of $Y(t)$ (**Diggle et al., 2002**). From this expression it is seen that the correlation parameter is determinative for the appearance of the variogram. In figure 4.11 a hypothetical variogram is seen. For $s \downarrow 0$, $\gamma(s) \rightarrow \tau$ (in R designated the nugget effect) and for $s \rightarrow \infty$, $\gamma \rightarrow \tau + \varepsilon$. In cases with replicates τ can be interpreted as the variance for $s = 0$, i.e. the measurement error (*Ibid.*). There are no replicates in the present experiment which also is seen in the graph where no points are present at lag 0. ε represents the variance due to the autocorrelation and v reflects the variance due to the random factors (*Ibid.*) The variability caused by the fixed effects is seen in all three areas.

Since the factor of primary interest is the main effect 'treatment' and possible interactions with it, a starting point of the model building could be to inspect the residuals of the simple model

$$\ln(Y_{ij}) = \mu + t_i + \varepsilon_{ij},$$
$$i = 1,\ldots,4, \quad j = 1,\ldots,368, \quad \varepsilon_{ij} \sim N(0,\sigma_\varepsilon^2),$$

where t_i is treatment. The corresponding variogram is seen in figure 4.12.a. Due to the

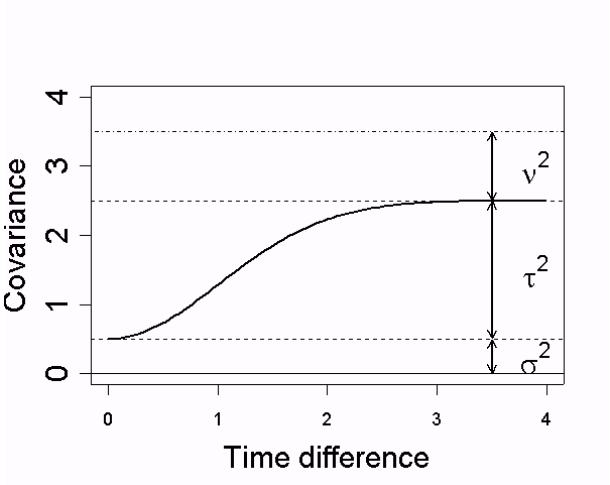


Figure 4.11. A hypothetical variogram showing the different sources of variation. ν^2, σ^2 and τ^2 represent the random intercept, the serial correlation and the measurement error, respectively.

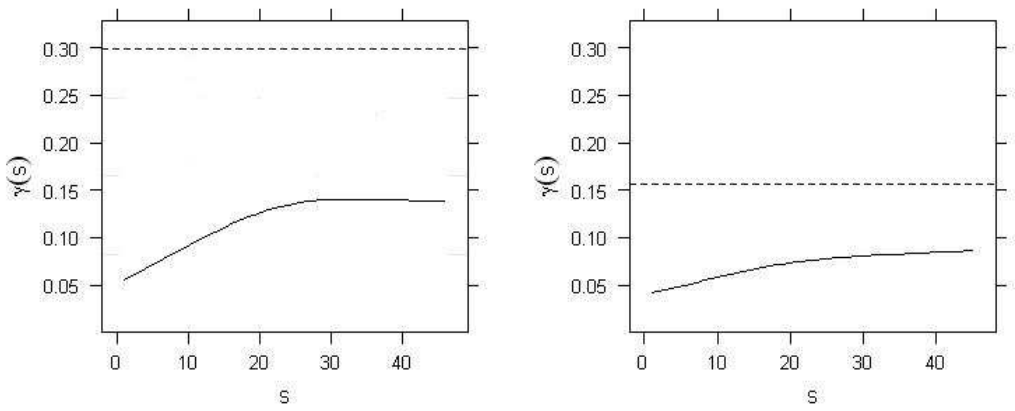


Figure 4.12. The variogram of the residuals (a) when only the 'dose' is fitted as a fixed effects and (b) when an individual intercept is included for each animal. Due to the number of points they are not shown.

considerable curvature of the smoother a significant degree of autocorrelation appears to be present in the data. Moreover, the upper area is of a considerable magnitude compared to the rest of the graph indicating a variation due to the random effects that cannot be ignored.

A new model is fitted data including an individual intercept for each animal as seen in the variogram in figure 4.12.b. There is a considerable decrease in the variation and in the curvature of the smoother which points toward that the inclusion of a random effects term is imperative for a proper fit of the data.

4.1.4. Definition and development of statistical model

Based on the diagnostic graphs it is attempted to build a model that fits the data appropriately. As a starting point the fixed effects are selected by assessment of the graphs. Then the random effects term are incorporated. Henceforth, an optimisation of the model is attempted by allowing for heteroscedasticity (**Pinheiro and Bates, 2000**). Furthermore, it is examined if the assumption regarding independence of the residuals seems reasonable to accept (*Ibid.*) If the assumption appears to be reprehensible a suitable autocorrelation structure is employed to model the dependency among the residuals (*Ibid.*) Finally, the plausibility of the initial assessment of the fixed effects structure is evaluated. The model is afterwards subjected to a residual analysis to confirm the validity of the model (**Conradsen, 2002; Pinheiro and Bates, 2000**).

For each defined model the log-likelihood, log(L), is estimated based on maximum likelihood estimation (ML) or restricted maximum likelihood estimation

(REML) and in general, the log-likelihood value is preferred to be as high as possible. Since the ML estimates of the variance components may be underestimated the REML estimates are often preferred (**Pinheiro and Bates, 2000**).

The likelihood ratio can be used to test models nested under each other, i.e. one model is a special case of the other. For models fitted with REML estimates this only holds true if the fixed effects structure is retained for the two models. The likelihood ratio test (LRT) statistic

$$\text{LRT} = 2\log(L_2/L_1) = 2[\log(L_2)-\log(L_1)]$$

is chi-squared distributed with degrees of freedom (df) corresponding to df(general model) – df(restricted model) under the null hypothesis that the restricted model is adequate (*Ibid.*) Below, only nested models differing by one term are tested to facilitate the interpretation. Furthermore, in this framework the level of significance is $\alpha = 0.05$ and consequently, for $p > 0.05$ the restricted model is retained whereas it is rejected for $p < 0.05$. The test carried out in this way is often conservative, that is, p-values observed from the chi-square(k_2-k_1) distribution are greater than they should be (*Ibid.*)

In cases where the models cannot be regarded as nested under each other the likelihood ratio test will not be suitable for test of the models (*Ibid.*) Instead the judgment can be based on the Akaike Information Criterion (AIC) and the Bayesian Information Criteria (BIC). These are evaluated as

$$\text{AIC} = -2\log L + 2\, n_{par}$$
$$\text{BIC} = -2\log L + n_{par}\log(N)$$

where n_{par} is the number of parameters in the model and N is the total number of observations used to fit the model (*Ibid.*) As seen from the expressions an increasing penalty is imposed with the number of parameters in the model. As opposed to the log likelihood value the AIC and BIC value should be as low as possible (*Ibid.*)

Regarding the fixed effects the likelihood ratio test is not appropriate for test of the fixed effects terms. As mentioned it is meaningless to compare two REML fitted models and a likelihood ratio test of two ML fitted models can be somewhat

anticonservative, i.e. the *p*-values are smaller than they should be. Instead, the fixed effects are conditioned at the random effects variance-covariance parameters and the fixed effects parameters are standard least-squares estimates. They are tested by means of a usual *F*-test where the coefficients for each level are tested jointly (*Ibid.*) If any of the fixed effects including 'treatment' appears to be significant the individual coefficients are subsequently tested by means of a *t*-test.

In the following an initial model is defined and subsequently refined. Regarding the fixed effects structure in the initial model definition it appears reasonable from figure 4.4.c to include 'time' and 'period' since there seems to be a progression concerning 'time' that moreover has a very diverse shape for the two different periods. From the same figure a considerable system effect is seen and included in the model as well. The effect of 'treatment' is apparently not very pronounced but due to the specific interest of this term it is initially included in the model.

Regarding the random effects terms it is relevant to include 'animal' and 'round' since these terms are representative for a general population of other animals and rounds. An initial model with these two random effects included is defined. Since it is not possible to include two or more crossed random effects in a model using 'lme' in R it is defined using a revised version 'lmer' (**Bates and Sarkar, 2006**)

$$\ln(y_{ijklmn}) = \mu + d_i + p_j + d \cdot p_{ij} + \beta_1 \cdot \text{time}_k + \beta_{2i} \cdot d \cdot \text{time}_{ik} + \beta_{3j} \cdot p \cdot \text{time}_{jk} + \beta_{4ij} \cdot d \cdot p \cdot \text{time}_{ijk} + s_l + A_m + R_n + \varepsilon_{ijklmn}$$

where

$$i = 1,\ldots,4,\ j = 1,\ldots,2,\ k = -130,\ldots,105,\ l = 1,\ldots,2,\ m = 1,\ldots,32,\ n = 1,\ldots,8,$$
$$A_m \sim N(0,\sigma_A^2), \quad R_n \sim N(0,\sigma_R^2), \quad \varepsilon_{ijklmn} \sim N(0,\sigma_\varepsilon^2),$$

μ, t_i, p_j, time_k and s_l are the fixed effects for the intercept, treatment, period, slope concerning time and system, respectively. A_m is the random effect of the *m*th animal and they are assumed independent for different *m*. R_n is the random effect of the *n*th round and they are likewise assumed independent for different *n*. ε_{ijklmn} is the within-group error assumed independent of each other and of the random effects.

To test if ‘animal’ is a significant contributing factor it is withdrawn from the model but the restricted model did not fit the data as well ($p < 0.0001$) and the term was included again. Equivalently, ‘round’ was withdrawn from the model and the restricted model was approved to describe the data adequately ($p = 0.45$). As mentioned previously ‘animal’ is confounded with the ‘box’-‘round’ interaction and therefore it is expected that the presence of ‘animal’ accounts for some of the variability caused by ‘round’. Therefore, it is not surprising that the term is insignificant and it is not necessarily possible to interpret it as the response being unaffected of ‘rounds’. Due to the superiority of ‘lme’ to ‘lmer’ when only a single random effect is to be integrated the model is redefined using this function.

An assumption of the linear mixed effects model is that the residuals are normally distributed with constant variance and consequently the variances of the residuals in each group should be of equal size (**Pinheiro and Bates, 2000**). In order to inspect if this assumption is fulfilled some of the factors are depicted vs. the ln-transformed values (figure 4.13). For ‘system’ and ‘box’ a potential heteroscedasticity is seen and this can be modelled with a variance function model. Different variances for each level of a stratification variable *s* is characterised by the variance model

$$\mathrm{Var}(\varepsilon_{ij}) = \sigma^2 \delta^2_{s_{ij}}$$

where $s = 1,\ldots,S$ and δ is a vector of variance parameters with $\delta_1 = 1$ and δ_l, $l = 2,\ldots,S$, equal to the ratio between the standard deviation of the lth and the first stratum (*Ibid.*) When a heteroscedasticity is defined with respect to ‘system’ the model is not improved significantly ($p = 0.72$). However, for boxes a significantly better fit of the data is

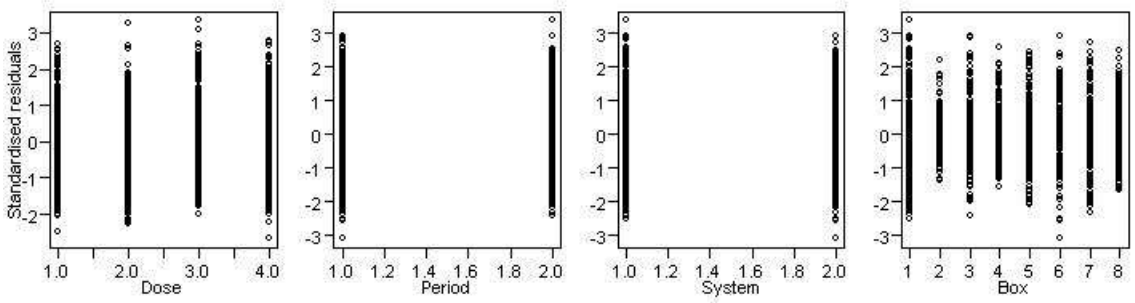


Figure 4.13. The standardized residuals for each level of the different factors. For ‘dose’ and ‘period’ the variances of the different levels look uniform whereas for ‘system’ and ‘box’ a heteroscedasticity might be present.

gained (L-ratio = 144.49, $p < 0.0001$) and the variance function model is comprised in the model. The variance parameters for the different strata is estimated

$$\boldsymbol{\delta} = (1.00, 0.42, 0.76, 0.56, 0.71, 0.78, 0.71, 0.64).$$

As described the variance parameter of box 1 is 1 and the variance parameters of the remaining boxes expresses the size of the variance compared to the variance of box 1. Large differences are seen between the variance parameters, and this is particularly are the case for box 1 and 2, where the variance of box 1 is more than twice the size of box 2. This is consistent with the observations described in section 4.1.2 and the quantification of the heteroscedasticity thus appears reasonable.

Several values are measured at the same animal and these values are not necessarily independent of each other. Due to the inclusion of a random effect in the model a correlation structure is defined with equal correlation $\sigma_A^2/(\sigma_A^2+\sigma^2)$ for all lags of each animal (*Ibid.*). It is in the following examined if a modification of this correlation structure is favourable. In figure 4.14.a the degree of the residual autocorrelation is depicted and a significant but decreasing dependency is seen for the first 5-7 lags. An alternative illustration is the variogram in figure 4.14.b where the residual autocorrelation for the same lags are evident. The correlation structure $\gamma(s,\rho)$ can be modelled by several different variogram models (**Pinheiro and Bates, 2000**). Due to the continuous decrease in correlation with increasing time lags the autoregressive model with one parameter is applied. The correlation function is

$$h(s,\varphi) = \varphi^s, \quad s = 0,1,... \quad (\textit{Ibid.})$$

The model improved the fit significantly (L-ratio = 294.15, $p < 0.0001$). In figure 4.14.a it is seen that the autocorrelation at lag 2 does not equal the square of the autocorrelation at lag 1 and therefore the autoregressive model is combined with a moving average correlation model with one parameter

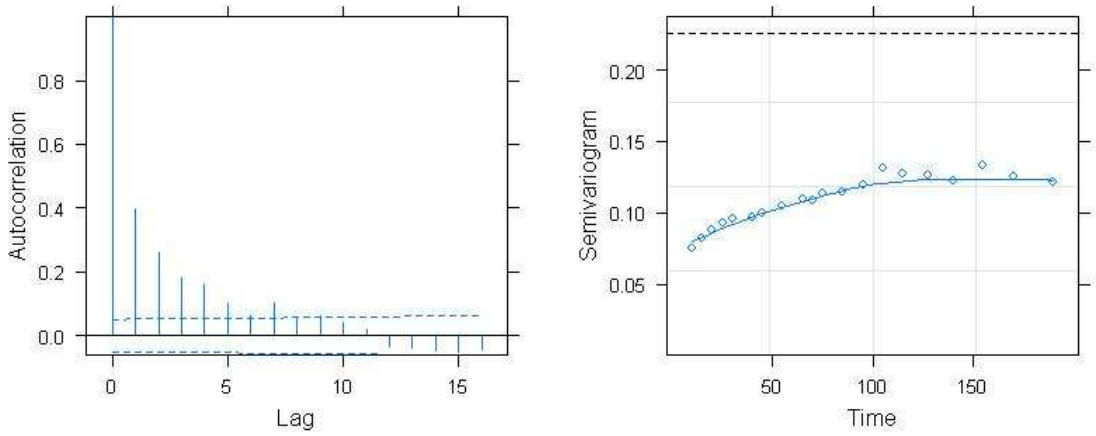


Figure 4.14. (a) The autocorrelation of the data where a positive autocorrelation is seen for the first few lags (b) The autocorrelation is also evident in the corresponding variogram.

$$h(s,\theta) = \begin{cases} \frac{\theta}{1+\theta^2}, & s = 1 \\ 0, & s > 1 \end{cases} \quad (\textit{Ibid.})$$

and a significant better fit is obtained (L-ratio = 343.36, $p < 0.0001$). The parameters estimated in the model are $\varphi = 0.82$ and $\theta = -0.46$. Due to the shape of the smoother at the variogram two of the variogram models traditionally used for spatial correlation structures is applied as well. The exponential variogram model is defined

$$\gamma(s,\rho) = 1\text{-}\exp(\text{-}s/\rho) \quad (\textit{Ibid.})$$

and the rational quadratic variogram model

$$\gamma(s,\rho) = (s/\rho)^2 / [1+(s/\rho)^2] \quad (\textit{Ibid.})$$

Both of the variogram models increase the fit (L-ratio = 311.00, $p < 0.0001$ and, L-ratio = 303.86, $p < 0.0001$, respectively). When the model is defined as above $\gamma(s,\rho) \rightarrow 0$ for $s \downarrow 0$. If a nugget effect is included a discontinuity in γ at 0 is allowed so $\gamma(s,\rho) \rightarrow c_0$ for $s \downarrow 0$ (*Ibid.*), and this inclusion improved the fit significantly (L-ratio = 356.97, $p < 0.0001$). Since the test of the exponential model with a nugget effect yields the highest log likelihood the residual correlation structure is modelled with this function (figure 4.15.a). The correlations for the first four lags become

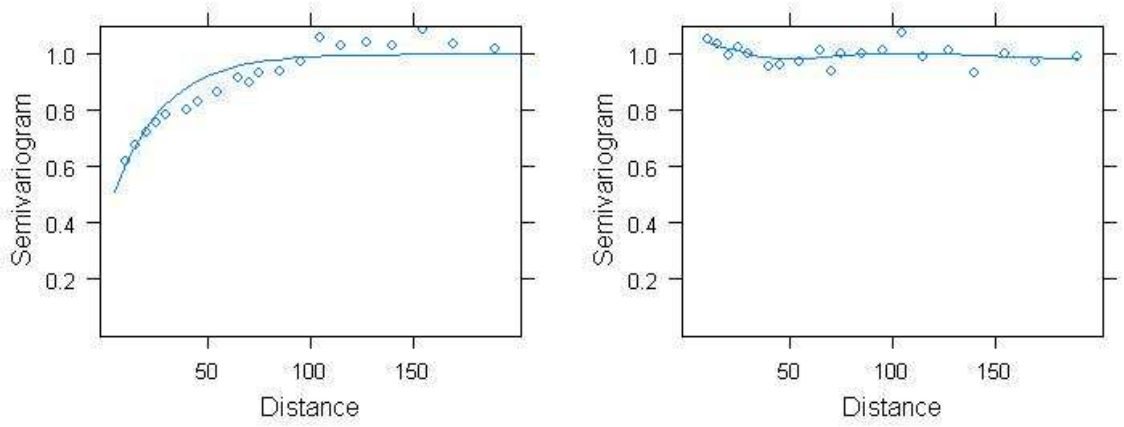


Figure 4.15. (a) The variogram showing the standardized residuals fitted with the exponential variogram model (b) The normalized residuals after fitting with the exponential variogram model.

$$\begin{bmatrix} 1.00 & 0.49 & 0.40 & 0.33 & 0.27 \\ 0.49 & 1.00 & 0.49 & 0.40 & 0.33 \\ 0.40 & 0.49 & 1.00 & 0.49 & 0.40 \\ 0.33 & 0..40 & 0.49 & 1.00 & 0.49 \\ 0.27 & 0.33 & 0.40 & 0.49 & 1.00 \end{bmatrix}$$

After modelling of the residual correlation structure no additional autocorrelation is retained in the model as seen in the variogram in figure 4.15.b. The residual are standardised and normalised, respectively, in the two graphs for which reason the abscissa is scaled compared to the former shown variograms.

The random effects and the autocorrelation are modelled and it is now assumed that the fixed effects can be properly tested. The assessed *p*-values are seen in table 4.1. It is seen that most of the terms are significant whereas the possible interactions for 'treatment' with both 'time' and 'period' is not. The terms are removed one at a time starting with the 'treatment'-'time'-'period' interaction due to the hierarchical principal (**Montgomery, 2005**) and thereupon the term with the highest *p*-value. Since the experiment is balanced with respect to the different factors the remaining *p*-values are only changed slightly and are not shown. It is noticed that all the terms completely or partly composed of 'treatment' are insignificant.

Table 4.1. The *p*-values from the analysis of traditional fear conditioning experiment with α5IA-II treated rats on test day.

	p-value
Intercept	<0.0001
Treatment	0.1973
Time	<0.0001
Period	<0.0001
System	<0.0001
Treatment:Time	0.7851
Treatment:Period	0.2462
Time:Period	0.0001
Treatment:Time:Period	0.4325

The final model can be formulated

$$\ln(y_{ijkl}) = \mu + p_i + \beta_1 \cdot \text{time}_j + \beta_{2i} \cdot p \cdot \text{time}_{ij} + s_k + A_l + \varepsilon_{ijkl}$$

where

$$i = 1,\ldots,2,\ j = \text{-}130,\text{-}125,\ldots,100,105,\ k = 1,\ldots,2,\ l = 1,\ldots,32,$$
$$A_m \sim N(0,\sigma_A^2),\quad \varepsilon_{ijklm} \sim N(0,\sigma_\varepsilon^2),$$

μ, p_j, t_k and s_l are the fixed effects for the intercept, period, slope concerning time and system, respectively. A_m is the random effect of the mth animal and they are assumed independent for different m. ε_{ijklm} is the within-group error assumed independent of each other and of the random effects.

4.1.5. Validity checking of statistical assumptions

In order to verify that the model describes the data appropriately a residual analysis is performed (**Conradsen, 2002; Pinheiro and Bates, 2000**). The rational for doing so is to see if all systematic variation and effects are removed from the data after the application of the model. If this is the case the residuals are randomly and independently distributed and consequently at most of the residual plots the points are to be scattered randomly. First, the residuals are depicted against the fitted values conditioned at the two systems (figure 4.16). The effect of the system is clearly seen as it induces a shift in the expected value. A slight tendency is seen towards increasing residuals with increasing fitted values. This tendency does not seem alarming, though, and it is not considered to be a serious

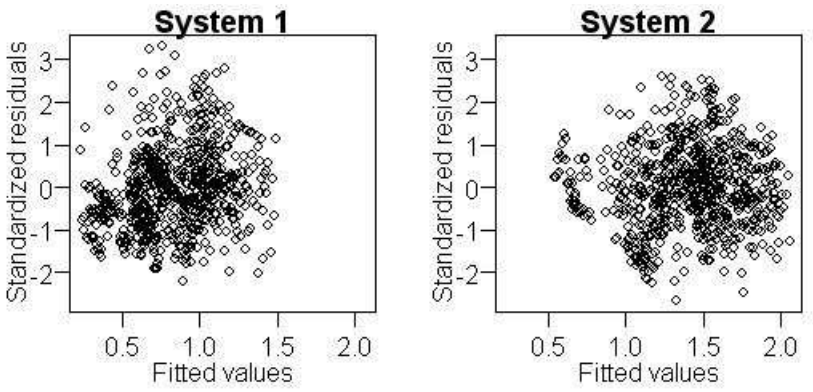


Figure 4.16. The standardized residuals depicted against the fitted values for each system. The bias due to the system is evident in the plot. The residuals seem to be slightly dependent of the fitted values but it is not assessed to be a marked effect.

violation of the assumptions. In figure 4.17 the standardised residuals are depicted against the significant terms in the model. For all the effects the residuals are very similar in their structure across the groups, which is in consistency with the expected. Due to the irregularities regarding the boxes the standardised residuals are depicted against them (figure 4.18.a). The magnitudes of the variability are very similar for all the boxes, which also was expected. In figure 4.18.b the observed values vs. the fitted values are seen. Some variability is still retained in the data but this is expected due to the character of the experiment with the inherited variability due to the heterogeneous movement pattern of the animals. A clear linear connection is seen between the fitted and the observed values and the model is considered reasonable to explain the data. Finally, in order to inspect if the residuals and the different parameters are normally distributed the normal plots for these quantities are shown in figure 4.19. From these plots no violation of the assumptions are seen.

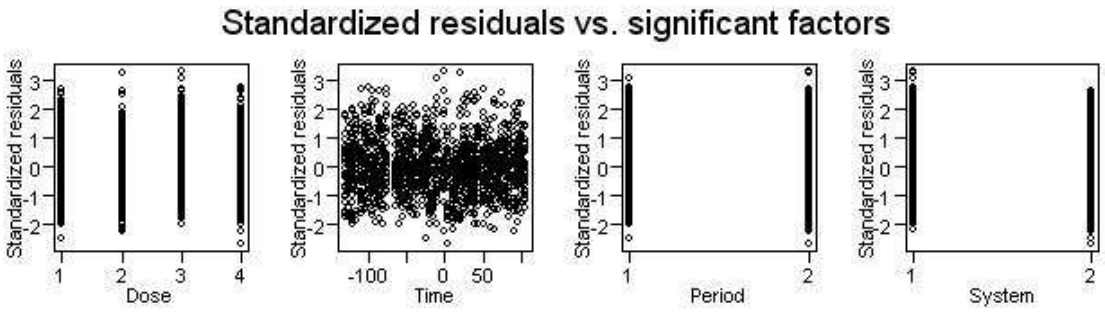


Figure 4.17. The standardized residuals depicted against the significant terms in the model. The residuals are randomly structured in the different plots

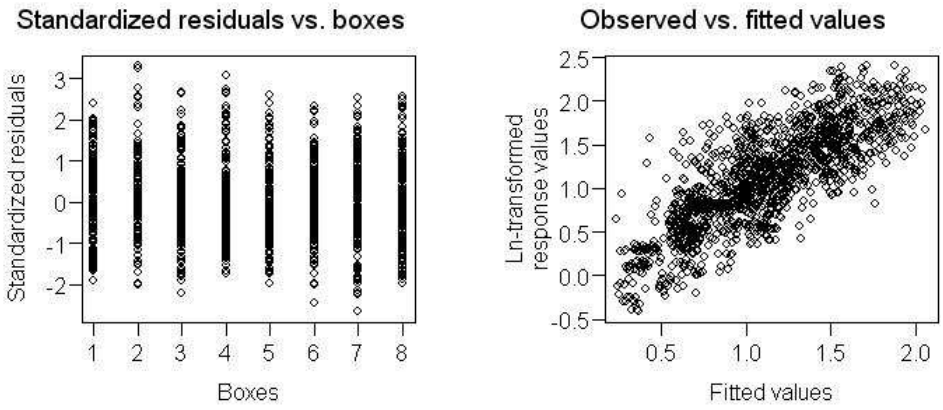


Figure 4.18. (a) The standardized residuals depicted against the different boxes. As opposed to figure 4.13 the size of the variances are now equal for all groups (b) The ln-transformed observed values vs. the fitted values estimated from the model.

4.1.6. Interpretational aspects of the fear conditioning analysis

In this section the interpretation of the results obtained in the analysis of the fear conditioning experiment will be considered. Furthermore, to exemplify the meaning of the biologically important terms alternative variants of the results will be presented and discussed in relation to the relevant terms.

Due to the ln-transformation of the response values some issues are to be considered when the results are interpreted. Normally the null-hypothesis in a *t*-test is H_0: $\alpha_1 - \alpha_2 = 0$, where α_1 is the parameter estimate of the reference level and α_2 is the

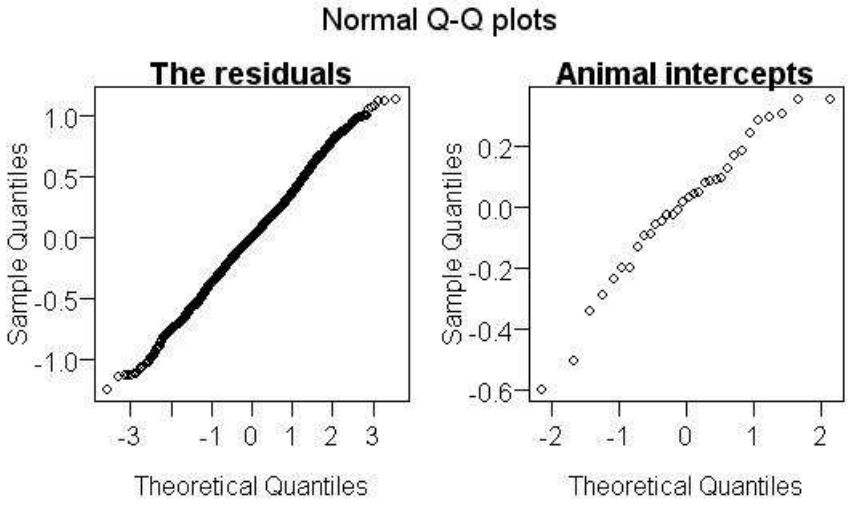


Figure 4.19. A normal plot of all the residuals and the individual intercepts for each animal estimated in the model. No serious deviation from a straight line is seen in the plots and thus, the residuals and intercepts are considered to be randomly distributed.

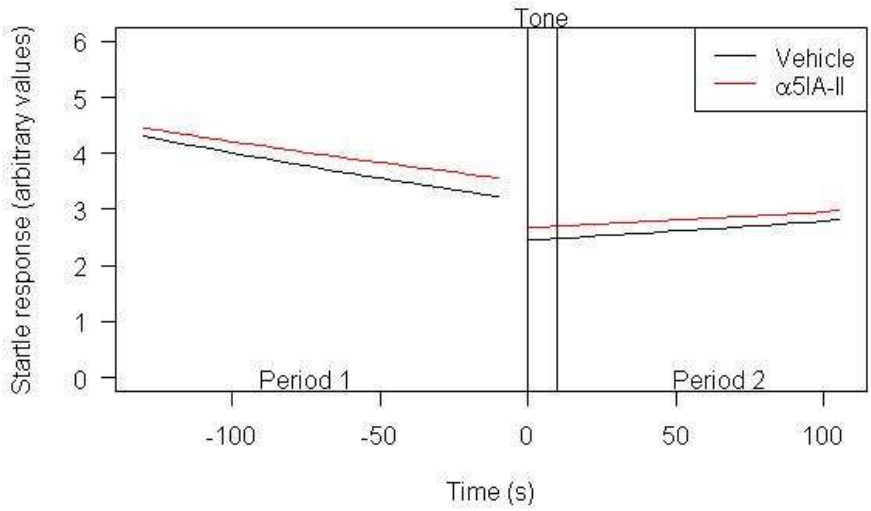


Figure 4.20. The predicted values from the model describing the data in the 5IA-II fear conditioning experiment. Neither 'dose' nor 'dose'-'treatment' interaction is significant.

parameter estimate of the level concerned. However, due to the ln-transformation and since $\ln(\alpha_1) - \ln(\alpha_2) = \ln(\alpha_1/\alpha_2)$ the test now concerns if $\ln(\alpha_1/\alpha_2) = 0$ which is equivalent to $\alpha_1/\alpha_2 = 1$. Thus, owing to the ln-transformation the ratio between the two levels is tested rather than the difference. In both cases the objective is to test if the two levels differ significantly. It should thus be kept in mind that when a significant difference is tested it concerns whether the ratio differs from one opposed to if the subtracted terms differ from zero.

The denotation of the relevant tested terms from the *F*-test will be described and can be compared to a simplified graph (figure 4.20). The ‘intercept’ is the intersection with the ordinate and it is tested if this value is different from zero. This test does usually not have any relevance. In the test of ‘treatment’ it is evaluated if there is a difference between at least two of the different dose groups including the vehicle group. ‘Time’ is a continuous variable and is thus a slope and it is tested if it is differs from zero. When ‘period’ is tested it is seen if there is a difference in the movement level between the two different periods. The different interactions concern whether the result obtained for the main effects applies to all different combinations of doses and periods. If the ‘treatment’-‘time’ interaction is significant the time slope is not equal for all the different doses. The ‘treatment’-‘period’ interaction expresses if the possibly difference between the different doses are equal before and after the tone. The interaction between ‘time’ and ‘period’ is significant if the slope is different before and after the tone.

Finally, if the three-way interaction ‘treatment’-‘time’-‘period’ is significant the difference in the slopes for each of the doses is not the same before and after the tone.

When the data obtained from the α5IA-II animals on the test day were analysed no terms concerning ‘treatment’ were significant. If this had been the case a *t*-test would have been performed in order to compare each of the different levels of a factor to a reference group. Some of these terms are described here in order to relate to the results from other fear conditioning experiments shown in section ‘5. Results’. Not all terms are considered being of equal biological importance and therefore only the terms relevant in the evaluation of ‘treatment’ is considered. The remaining terms are only included in the analysis to eliminate variance from the residual component and hence, to increase the precision of the estimates in order to facilitate detection of significance.

The effect of the individual levels of ‘treatment’ concerns if there is a difference between any of the doses compared to the vehicle before the tone. From the different levels of the ‘treatment’-‘time’ interaction it is possible to see if the slopes of each of the doses differ from the slope of the vehicle group before the tone. The ‘treatment’-‘period’ interaction is informative about whether the difference between each of the doses and the vehicle after the tone is different from the corresponding difference obtained before the tone, i.e. it is tested if the inference regarding the main effect ‘treatment’ also is valid after the tone. Finally, the three-way interaction between ‘treatment’, ‘time’ and ‘period’ is relevant for evaluating if the slope differentiation of the individual doses compared to the vehicle after the tone is different from before the tone. That is, it is tested if the significance obtained for the ‘treatment’-‘time’ interaction is preserved across the two periods.

As mentioned ‘treatment’ is declarative about the period before the tone, where the behaviour of the animals is influenced by their ability to associate the context with the shock which is a hippocampus dependent task. The ‘treatment’-‘period’ interaction is informative about the period after the tone compared to the period before the tone. In the analysis of the α5IA-II test day data the ‘treatment’ levels were insignificant before the tone, i.e. the main effect ‘treatment’ was insignificant for all levels. The ‘treatment’-‘period’ interaction was also insignificant for all levels and hence it is possible to draw the inference that each of the ‘treatment’ levels is not significantly different from the vehicle after the tone. The ‘treatment’-‘time’ and ‘treatment’-‘time’-‘period’ interaction

are not considered to be of major interest since it only concerns differences in slopes and not differences in the response level and they will not be discussed further.

In the following some examples of other outcomes are shown regarding the significance level of ‘treatment’ and the ‘treatment’-‘period’ interaction. In the graphs seen in figure 4.21 a simplification of some later results are illustrated. In the case seen in figure 4.21.a the main effect ‘treatment’ is not significant, which is evident from the small distance between the drug and vehicle before the tone. The distance between the drug and the vehicle changes considerably across the occurrence of the tone and the ‘treatment’-‘period’ interaction is assumed to be significant. Since the difference between the drug and vehicle after the tone is significantly differently from the insignificant difference before the tone, it is inferred that the drug and vehicle is significantly different from each other after the tone. Another case is seen in figure 4.21.b where there is a considerable distance between the drug and vehicle before the tone and thus it is assumed that ‘treatment’ is significant. The size of the distance after the tone is comparable to the distance before the tone and the ‘treatment’-‘period’ is therefore considered to be insignificant. Since the distance after the tone does not differ significantly from the significant difference seen before the tone, the difference between the drug and the vehicle after the tone is significantly different. A final example is seen in figure 4.21.c. As before ‘treatment’ is significant due to the considerable distance between the drug and vehicle. The distance between the drug and vehicle is enlarged across the tone and the ‘treatment’-‘period’ interaction is also significant. The distance between the drug and the vehicle after the tone is consequently significantly different

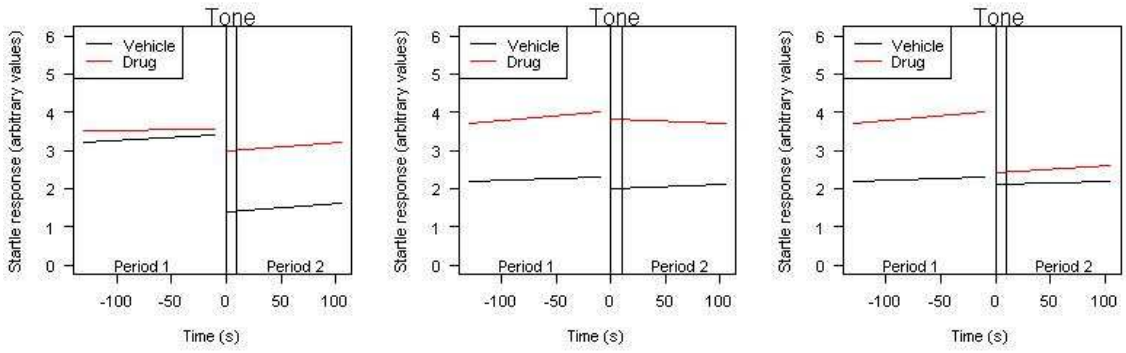


Figure 4.21. Examples of different results in the analyses (a) ’dose’ is insignificant and the ’dose’-’period is significant (b) ’dose’ is significant and the ’dose’-’period’ interaction is insignificant (c) both ’dose’ and the ’dose’-’period’ interaction are significant.

from the significance obtained before the tone and it is reasonable to infer that the difference between the drug and the vehicle after the tone is significant.

4.1.7. Considerations regarding remaining fear conditioning analyses

In the analysis of the fear conditioning experiments the above developed model is used as a starting point for each of the datasets. As fixed effects are ‘treatment’, ‘time’, ‘period’ and the interactions between them since these factors are dependent of the type of drug and thus, might be significant in some of the fear conditioning experiment. As in the former analysis the insignificant fixed effects were removed once at a time until the remaining effects were significant. However, the *p*-values of the full model are shown justified by the nearly invariable estimates and in order to obtain a general view of the size of the *p*-values regardless of their significance level. The *p*-values from the modified model are only reported if the alteration becomes of practical importance. The *p*-values are reported in section ‘5. Results’.

A residual analysis was performed for each of the experiments and outliers were withdrawn from the data if requisite. The residual analyses are not shown.

For the animals in the FC α5IA-II experiment not experiencing a shock during the experiment ‘period’ is not expected to affect the response and consequently, any term where ‘period’ is included will be eliminated from the model.

In the trace fear conditioning, two different programs are used at the conditioning day in order to differentiate regarding the time gap between the tone and the shock. Only one program can be employed for each system and round and thus, the animals ascribed to equal combinations of these two factors must all be exposed to the same time gab, i.e. ‘trace’ or ‘no trace’. Due to this restriction the experiment can no longer be balanced regarding the doses and systems, which unfortunately is due to the earlier detected system effect that might be present. Therefore, there may be a partial confounding between ‘treatment’ and ‘system’ effect and, thus, an irretrievable bias might be present. ‘Treatment’ was balanced out as much as possible.

Owing to priority the data obtained at the conditioning day are not analysed except for in the α5IA-II traditional fear conditioning. Hence, almost all the statistical analyses has been subjected to data obtained at the test and retest day. Any possible

inferences drawn from the non-analysed data will be based at graphs belonging to the particular experiment.

4.2. Analysis of pre-pulse inhibition experiments

As in the analysis of the fear conditioning the data from one of the pre-pulse inhibition experiments will be utilised in the development of a statistical analysis and afterwards the statistical model will be applied to the remaining data sets.

The output from the experiment was modified slightly to adjust it to the statistical analysis. In the experiment each animal was subjected to five different pre-pulses each replicated 12 times in a semi-randomised order. An average was calculated for each of these 60 trials.

The factors conceivable to affect the response values are the two drugs administered prior to the experiment, the size of the pre-pulse and the system effect that was found to induce a bias in the analysis of fear conditioning. The drug intended to induce schizophrenic symptoms is called ‘treatment’ and the drug anticipated to reverse the induced deficits is called ‘pre-treatment’.

4.2.1. Graphical exploration of data

Some graphs are inspected in order to attain an impression of the variation in the data. First, it is examined if the response values should be transformed before the application of the statistical method. This is done analogous to the fear conditioning experiment where the variances for each trial are depicted against the corresponding averages (not shown) and it is decided that ln-transformed values should be employed in the analysis.

A bar plot of the response values is seen in figure 4.22 with individual bars for each combination of ‘treatment’ and ‘pre-treatment’. It is seen that the response values decrease with increasing size of the pre-pulse. Furthermore, a clear distinction between the two treatment levels is seen. For the three highest pre-pulses the animals given vehicle are moving considerable less than the animals given PCP. An effect due to different doses of NS.A is also seen, although it is less pronounced than for the administration of PCP.

In figure 4.23 the percentage change in movement for the different pre-pulses compared to the pulse alone is depicted. The same information is essentially seen as in

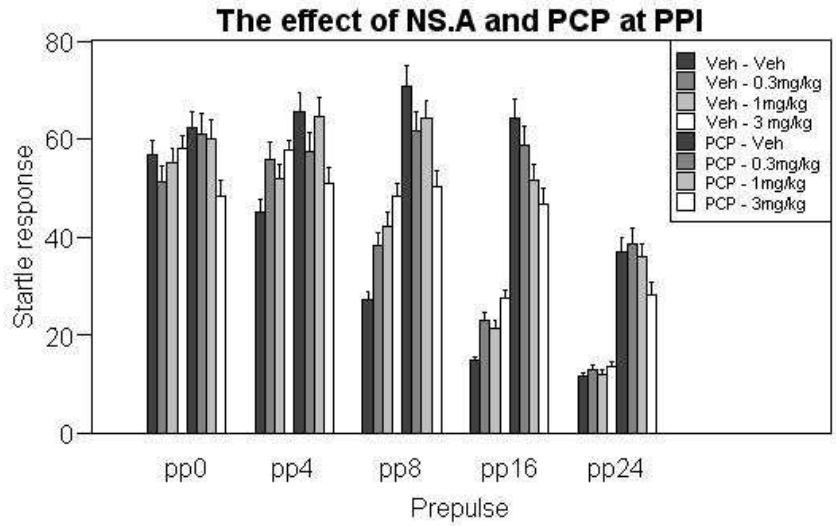


Figure 4.22 The startle response is shown when a startling pulse is presented alone (pp0), and when different pre-pulses are presented prior to the startling pulse. The different groups refer to different treatment of the rats, which have been injected with either NS.A per se, PCP per se or with both PCP and NS.A

the former figure but the accessibility is improved since it is closer connected to the biological objective, i.e. to assess the percent inhibition due to the size of the pre-pulse.

4.2.2. Definition and development of a statistical model

The model was selected based at the principles described in the analysis of fear conditioning. The model was a linear mixed effects model with an individual intercept assigned to each animal.

$$\ln(y_{ijklm}) = \mu + pt_i + t_j + pt \cdot t_{ij} + pp_k + \cdot pt \cdot pp_{ik} + t \cdot pp_{jk} + pt \cdot t \cdot pp_{ijk} + s_l + A_m + \varepsilon_{ijklm}$$

where

$$i = 1,\ldots,4, \quad j = 1,\ldots,2, \quad k = 1,\ldots,5 \quad l = 1,\ldots,2, \quad m = 1,\ldots,64$$

$$A_m \sim N(0,\sigma_A^2), \quad \varepsilon_{ijklm} \sim N(0,\sigma_\varepsilon^2),$$

μ, pt_i, t_j, pp_k and s_l are the fixed effects for the intercept, pre-treatment, treatment, pre-pulse and system, respectively. A_m is the random effect of the mth animal and they are

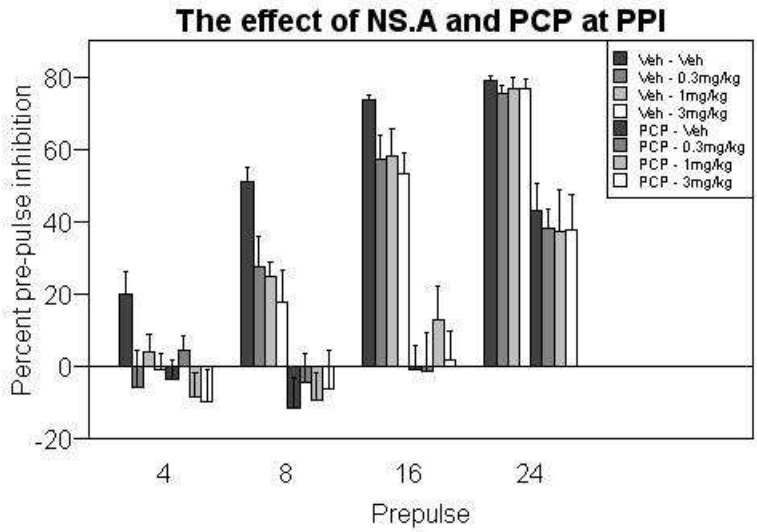


Figure 4.23 The percentage inhibition when different pre-pulses have been presented prior to the startling pulse. The different groups refer to different treatments of the rats, which have been injected with either NS.A per se, PCP per se or with both PCP and NS.A

assumed independent for different *m*. ε_{ijklm} is the within-group error assumed independent of each other and of the random effects.

Due to the unequal variances for the different boxes discovered in the previous analysis it is tested if a heteroscedasticity is present in the current case. The residuals are depicted against the boxes (figure 4.24) and it is seen that the size of the variance is approximately equal for all of them except box 2 where the variance is increased. When the heteroscedasticity is implemented in the model a significant improvement is obtained ($p < 0.0001$).

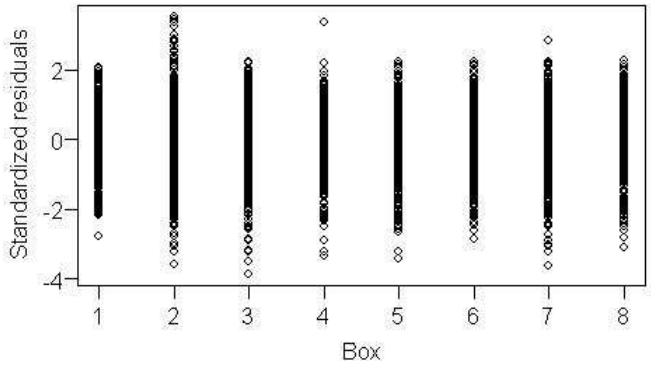


Figure 4.24. The standardized residuals depicted against the different boxes. The variance of box 2 has a high variability compared to the other boxes. Compared to figure 4.13 it is noticeable that box 2 had the smallest variance in the α5IA-II fear conditioning experiment.

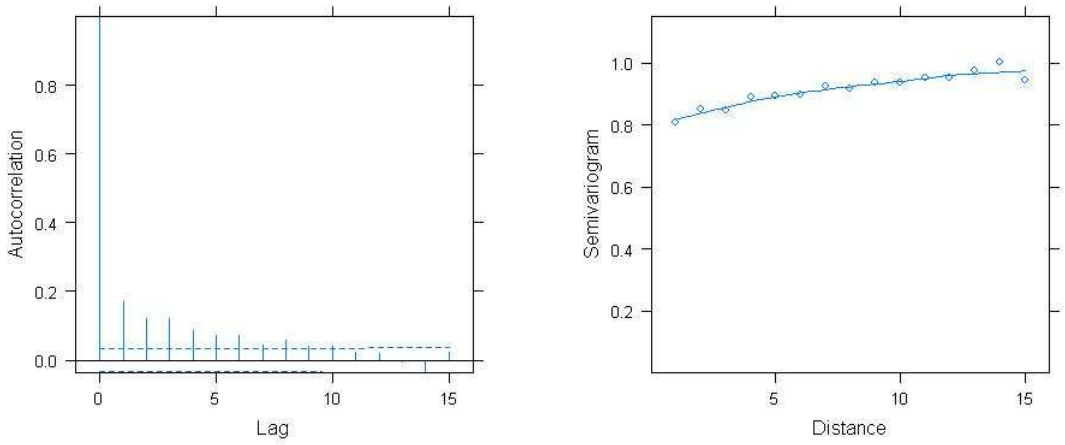


Figure 4.25. Depiction of the residual autocorrelation. (a) Positive autocorrelation is seen for the first lags. (b) The corresponding variogram is showing the same residual autocorrelation.

It is moreover examined if any autocorrelation is present in the residuals. The empirical autocorrelation and the related variogram are seen in figure 4.25. A significant amount of autocorrelation is seen and the correlation models explicated previously are employed. The autoregressive model combined with the moving average model with one parameter each yields a significant better fit (L-ratio = 291.50, $p < 0.0001$) as is the case for the exponential variogram model (L-ratio = 131.77, $p < 0.0001$) and the rational quadratic variogram model (L-ratio = 162.48, $p < 0.0001$). The rational quadratic variogram model had the highest log-likelihood ratio of the two variogram models and a nugget effect is included which proved to be advantageous (L-ratio = 281.18, $p < 0.0001$). Altogether, the highest log-likelihood was found when the autocorrelation structure was fitted with the combined autoregressive moving average model.

The fixed effects are tested and the *p*-values are seen in table 4.2. The ‘pre-treatment’ and ‘treatment’ both form part of terms found to be significant. ‘System’ is insignificant and is excluded from the model. The result from the analysis should be compared to a graph in order to be meaningful. A describtion of how to interpret the results is seen in section ‘4.2.5. Interpretational aspects of pre-pulse inhibition experiment’ and this will be exploited in the presentation of the results seen in ‘5. Results’. In this section results from the analyses of the different experiments will be presented together with figures.

Table 4.2. The *p*-values from the analysis of pre-pulse inhibition experiment with NS.A treated rats.

	p-value
Intercept	<0.0001
Pre-treatment	0.9909
Treatment	<0.0001
Pre-pulse	<0.0001
System	0.3153
Pre-treatment:Treatment	0.2330
Pre-treatment:Treatment	0.0027
Treatment:Pre-pulse	<0.0001
Pre-treatment:Treatment:Pre-pulse	0.0017

4.2.3. Validity checking of statistical assumptions

A residual analysis was performed in order to see if any of the model assumptions are violated. Specifically, it was examined if the intercepts of the animals were grouped with respect to the different systems they were tested in. If the intercepts of the animals tested in system 2 tend to be higher than of the animals tested in system 1 it may account for some bias not immediately seen in the analysis. The normal plot of the intercepts seen in figure 4.26 does not show any grouping. Additionally a box-plot was made with the individual animal intercepts depicted against the boxes (figure 4.27). Both the average and the variance of the random effects are affected by the box it is tested in, but in a different way from what was seen in the analysis of the fear conditioning since there is no clear distinction between the boxes from system 1 and 2. The boxes should thus be included in the model instead of the system but due to time considerations it is not done here. On the contrary to fear conditioning the pre-pulse

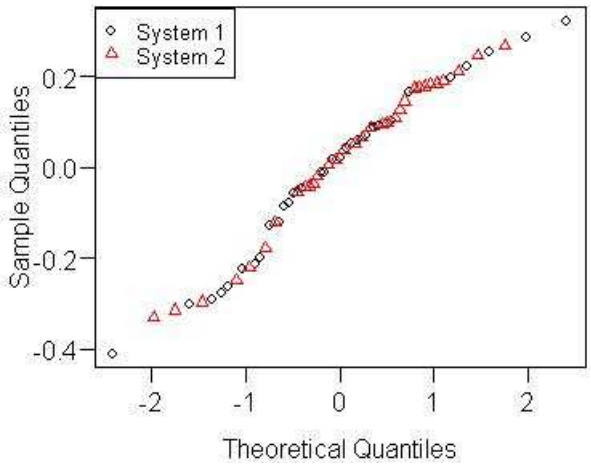


Figure 4.26. A normal plot of the individual intercepts for each animal estimated in the model. The black and red points indicate that the animals were measured in system 1 and 2, respectively. No grouping due to the systems is evident in the plot.

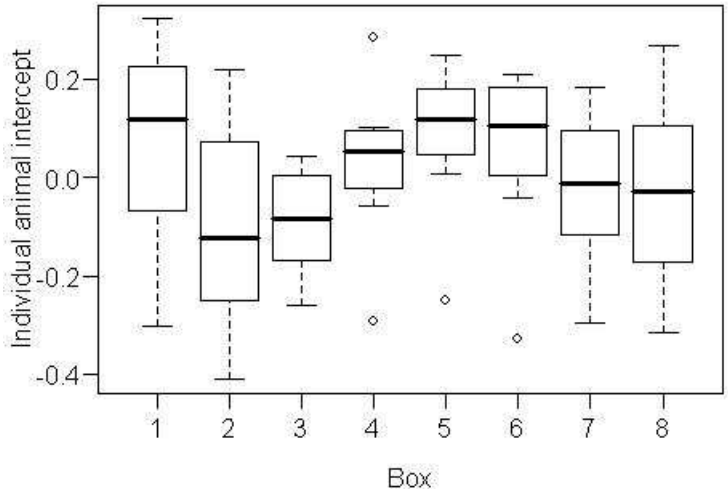


Figure 4.27. A box plot of the individual intercepts depicted against each box. Both the average and variance differ noticeably and it would have been reasonable to include 'box' as a factor in the model.

inhibition experiment is carried out as the first experiment after calibration and this may be the reason why the altered structure is seen.

4.2.4. Development of separate models for different treatment groups

In order to facilitate the interpretation, separate models were made for the two levels of 'treatment', i.e. for the administration of vehicle and PCP. By this means the terms including 'treatment' is eliminated from the model. The *p*-values for the vehicle group are seen in table 4.3. The interaction between 'pre-treatment' and 'pre-pulse' is significant and the *p*-values from the *t*-test are seen in table 4.4.

Table 4.3. The *p*-values from the analysis of pre-pulse inhibition experiment with NS.A co-administered with vehicle.

	p-value
Intercept	<0.0001
Pre-treatment	0.4618
Pre-pulse	<0.0001
System	0.5017
Pre-treatment:Pre-pulse	<0.0001

Table 4.4. The significant *p*-values from the *t*-test comparison of the individual levels to a reference level for animals administered NS.A and vehicle in pre-pulse inhibition experiment.

	p-value
Pre-treatment(0.3mg/kg):Pre-pulse(pp4)	0.0002
Pre-treatment(1mg/kg):Pre-pulse(pp4)	0.0013
Pre-treatment(3mg/kg):Pre-pulse(pp4)	0.0002
Pre-treatment(0.3mg/kg):Pre-pulse(pp8)	<0.0001
Pre-treatment(1mg/kg):Pre-pulse(pp8)	<0.0001
Pre-treatment3mg/kg):Pre-pulse(pp8)	<0.0001
Pre-treatment(0.3mg/kg):Pre-pulse(pp16)	<0.0001
Pre-treatment(1mg/kg):Pre-pulse(pp16)	0.0001
Pre-treatment(3mg/kg):Pre-pulse(pp16)	<0.0001

Table 4.5. The *p*-values from the analysis of pre-pulse inhibition experiment with NS.A co-administered with PCP.

	p-value
Intercept	<0.0001
Pre-treatment	0.6234
Pre-pulse	<0.0001
System	0.5915
Pre-treatment:Pre-pulse	0.5454

The same analysis was performed for the PCP administered animals and the *p*-values are seen in table 4.5. Neither 'pre-treatment' nor the 'pre-treatment'-'pre-pulse' interaction was significant.

A residual analysis was performed for each of the analyses. No violation of the model assumptions were seen in either case (not shown).

4.2.5. Interpretational aspects of pre-pulse inhibition experiment

As mentioned in section '4.1.6. Interpretational aspects of fear conditioning experiment' the test of the different terms concerns the relative difference rather than the absolute difference. That is advantageous in this context since the biological objective of the experiment is to assess the inhibition of a movement response for pre-pulses of different dB relative to the reference movement level where no pre-pulse is given. Thus, it is possible to assess the degree of inhibition without manual calculation of the relative values, which is favourable in two respects. First, when the ratio between two variables is computed the variability of the original variables is discarded and the test is based at the variability of the calculated measure and accordingly, the precision of the estimate will be affected. In addition, since the variance of the response values are found to depend on the average it is likely also to be valid for the ratios, thus the variance might decrease with increasing averages. It should therefore be examined if the ratios have to be transformed in order not to violate the model assumptions.

When the results from the statistical analysis are interpreted it is useful to compare it to the graph shown in figure 4.22. The interpretation is exemplified by the results obtained from the analysis of the data where vehicle was given as 'treatment'. The reference level for 'pre-treatment' is vehicle and for 'pre-pulse' it is pp0. If 'pre-treatment' proves to be significant in the *F*-test it means that the at least two of the doses are different at the pp0 level. In the analysis p_{dose} = 0.4618 and is thus not

significantly different which also seems reasonable based at the graph. Similarly, if ‘pre-pulse’ is significant at least two of the pre-pulses are different for the vehicle group. The *p*-value is in this case $p_{pre\text{-}pulse}$ is less than 0.0001 and is highly significant. This is consistent with the graphs where there is a profound difference at the vehicle response values for e.g. pp0 and pp24. If the ‘pre-treatment’-‘pre-pulse’ interaction is significant it means that the ratio between at least two doses for the same pre-pulse is different from the ratio between the same doses for another pre-pulse. This implies that if the interaction is significant the significance level of the main effects is not valid at all levels and vice versa if the interaction is found to be insignificant the inference drawn from the main effects applies to all levels.

In order to further examine the effects the *p*-values from the *t*-test are evaluated. For pre-pulse 4, 8 and 16 all ‘pre-treatment’-’pre-pulse’ interactions are significant. In case of dose 0.3 mg/kg and pre-pulse 4 it means that the ratio between pre-pulse 4 and pre-pulse 0 for 0.3 mg/kg is significantly different from the ratio between pre-pulse 4 and pre-pulse 0 for vehicle. The remaining significant terms are interpreted correspondingly.

With the interpretational perspectives in mind it is appealing to evaluate the relevance of the different terms. If ‘pre-treatment’ is found to be significant it means that there is a difference at the different doses for pre-pulse 0. This may be interesting in some connections but in this framework it does not capture the essence of the experimental objective. Regarding the significance of ‘pre-pulse’ it is expected to be significant since it means that the response of vehicle is divergent for different pre-pulses. If this is not the case the experiment might have failed in some way and thus, this factor functions as a kind of control but like ‘pre-treatment’ it is not a factor of main interest. The interaction between ‘pre-treatment’ and ‘pre-pulse’ is informative about the differentiability of the different doses for each pre-pulse. Since the thought of the experiment is to evaluate the effect of the different doses at the pre-pulse inhibition this term exactly reflects the object of interest.

As mentioned figure 4.22 and 4.23 almost illustrate the same information. From the latter it is not possible to see the difference due to doses at pre-pulse 0 but as stated above this is of minor importance. The statistical outcomes of the ‘pre-treatment’-‘pre-pulse’ interactions are closely connected to this graph and consequently, an overview of

these tendencies is attained much more conveniently than from the other graph. If for instance the interaction between 0.3 mg/kg and pre-pulse 4 is assessed, it is directly seen as the difference between the two bars at pre-pulse 4 illustrating this dose and the vehicle, respectively, as opposed to the other figure where two ratios, i.e. four bars, are to be compared. For that reason, the figure showing the relative inhibition is generally used to illustrate the statistical outcomes in the results section.

In order to attain an overall view of the results the predicted values from the full model were depicted against the size of the pre-pulse with one line for each of the different drug doses (figure 4.28). Here it is possible to see how the interaction between 'pre-treatment', 'treatment' and 'pre-pulse' is shaped. A clear distinctive progression is seen for the vehicle and PCP groups which is consistent with the significant 'pre-treatment'-'treatment'-'pre-pulse' interaction obtained in the model. For low pre-pulses NS.A reverses the PCP induced impairment to some extent but for pre-pulse 24 almost no effect is seen. It is seen that the difference between the vehicle and PCP treated animals is diminished for pre-pulse 24 and it should be considered if a floor effect is present for the PCP groups at pre-pulse 24 and consequently if the effect of this level is

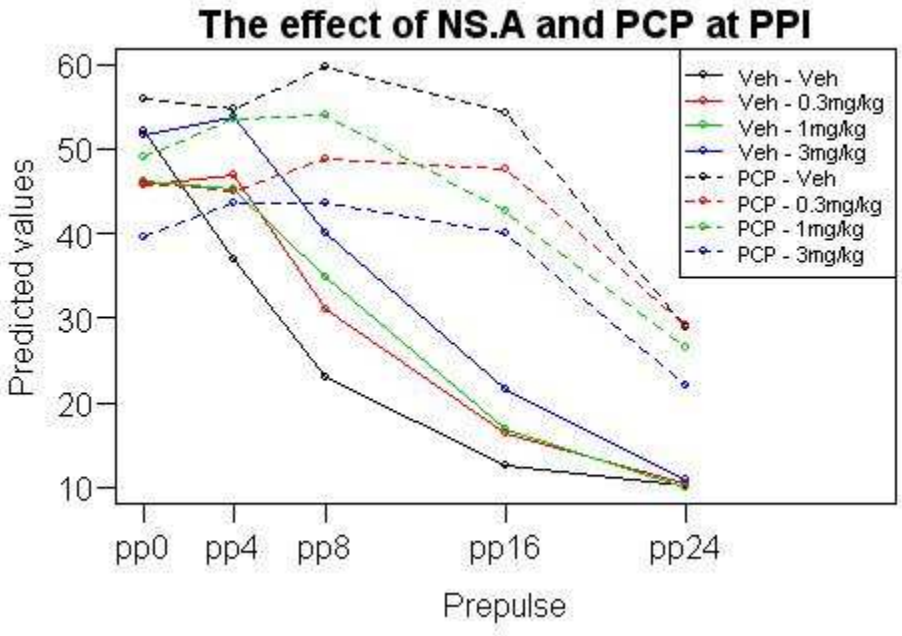


Figure 4.28. Predicted values when animals are administered NS.A and PCP. The predicted values from the model are depicted against pre-pulse with separate lines for each 'pre-treatment'-'treatment' combination. A different progression is seen for the two treatment levels vehicle and PCP. The 'pre-treatment' NS.A did not reverse the induced impairment

underestimated. In this case the reduced difference for the two levels of ‘treatment’ at pre-pulse 24 may in fact be a misconception. Anyhow, none of the drug doses seems to reverse the PCP induced pre-pulse inhibition deficits of a particular importance. This graph is similarly enclosed in the results section to support the conception.

4.3. Statistical perspectives

In this project, statistical models for fear conditioning and pre-pulse inhibition experiment have been acquired. With the models it is possible to analyse data obtained in the experiments and to achieve biologically approachable results. Nevertheless, several interesting things remain to be examined.

First, the main drawback of the present model is the lack of ability to distinguish the periods during and after the tone. Biologically the two periods involves different neural processes and therefore it is not meaningful to process the two periods jointly. A way to approach this issue should be considered.

Another important thing is to do a further investigation of the behaviour of the experimental equipment especially with respect to the systems and boxes the animals are tested in. As shown, the boxes consistently induce a significant amount of bias and variation which makes the estimates unreliable. Thus, additional studies could be performed with the artificial rat in order to reveal the pattern of measurement irregularity and to examine if it is possible to eradicate the source of fluctuation.

A simulation study would be beneficial in order to examine if a simplification of the analysis affects the sensitivity of the model, and the simulation could be based at estimates obtained in the current analysis. It is possible to modify the model at several levels and one opportunity is to exclude the modelling of box heteroscedasticity and residual autocorrelation. This procedure will interfere with the model assumptions but it is interesting to examine the extensiveness of this and to see if it still is possible to extract reliable estimates. It is also possible to analyse the data similar to the standard method performed at NeuroSearch, i.e. application of ANOVA at summary measures computed for different periods in the experiment. However, some modifications are still needed as for instance an ln-transformation of the response values and an inclusion of boxes/systems in the model. The main advantage of the method is the transparency when it comes to the interpretation of the results. The drawback is the reduced amount

of information utilised in the analysis which affects the significance level as well as a lack of comparison across the different periods.

A practical implementation of the statistical protocol at NeuroSearch could be to employ one of the simpler models for daily use and to utilise the more advanced model when important trends are to be detected with enhanced sensitivity, to be verified and to be published.

5. Results

In the following parts the results from the fear conditioning and pre-pulse inhibition experiments will be presented descriptively as well as with respect to statistical analysis. A discussion of the correspondence between descriptive and statistical analysis will be given.

5.1. Effects of $GABA_A$ receptor modulators on fear conditioning memory

In the following discussion of the effects of α5IA-II, NS.A and alprazolam on fear conditioning and extended-tone fear conditioning (eFC), the results are organised such that the data of interest as can be discerned from the graphs generated is described and then statistical analysis to confirm or refute these descriptions is applied. The actual order of data takes the form of first confirming that the behavioural training procedures do alter the behaviour of the rat so that it can be believed that a memory process is in operation (i.e., an increase in freezing behaviour on test/re-test days of animals receiving tone stimulus/context-shock training compared to animals exposed to context and tone without having shock during conditioning). Secondly, the conditioning data is shown for all treatment groups to illustrate the basal movement levels of the animals in a new environment and their response to delivery of a mild foot-shock. This data is relevant to ascertain any drug effect on baseline movement per se or alteration in an animals response to the shock stimulus, since such data might, (i) give insight to actions of the drug per se given the knowledge on the pharmacology of the drugs as outlined in the theoretical part; and (ii) help in the interpretation of test/re-test day behaviour. As mentioned in section ‘4.1.7. Considerations regarding remaining fear conditioning analyses’ a statistical analysis have only been applied to the conditioning data where α5IA-II was administrated. Thirdly relevant illustrations with specific doses of a compound is shown on the test day and, where applicable, on re-test day. Of course there are some variants on this general layout of the effects in fear conditioning and extended-tone fear conditioning between the three drugs described.

In the trace fear conditioning (tFC) model only the effects of α5IA-II were assessed. Nonetheless, the layout is similar to that described above, with the important distinction that in the first graph it is seen if the animals trained on a trace fear conditioning protocol show movement levels somewhat intermediate between a fear conditioning shock group of animals and animals receiving no shock but still being exposed to a new context and tone. The graph from the conditioning day illustrates the behaviour of all groups in order to outline the differences in the training of the different groups. Graphs of test day only show one treatment group (3 mg/kg α5IA-II) together with the vehicle group that undergo the trace fear conditioning training protocol.

Finally, α5IA-II was also tested in animals that never received a foot-shock but were still 'conditioned' to context and tone and thereafter re-introduced to the context and tone on a test day 24 hours after 'conditioning'. The rational for analysing these data was in order to evaluate if the administered drug affected the baseline movement response of animals placed in a novel context with exposure to a tone stimulus compared to control animals.

5.1.1. Effects of α5IA-II on fear conditioning memory

In figure 5.1 the effects of α5IA-II on traditional fear conditioning is summarised. The observed tendencies will be described in the next sections and compared to the findings of the statistical analysis.

5.1.1.1. Validation of model based on vehicle shock vs. no shock on test and re-test day

Figure 5.1.a shows the behaviour of the two different vehicle groups on the test day. It is seen that the group which on training day did not receive a foot shock move more than the other group before, during and after the tone. The other group which on conditioning day received a foot shock during the occurrence of the tone freeze both during and after the tone. The *p*-values from the statistical analysis of vehicle 'shock' vs. 'no shock' groups are $p_{shock:period} = 0.0347$, and consequently the freezing behaviour seen for the 'shock' group after the tone is significant. A similar test was executed on re-test data where the *p*-value was $p_{shock:period} = 0.0295$, and, thus, a significant freezing behaviour after the tone are detected on re-test day for the 'shock' group compared to the 'no shock' group.

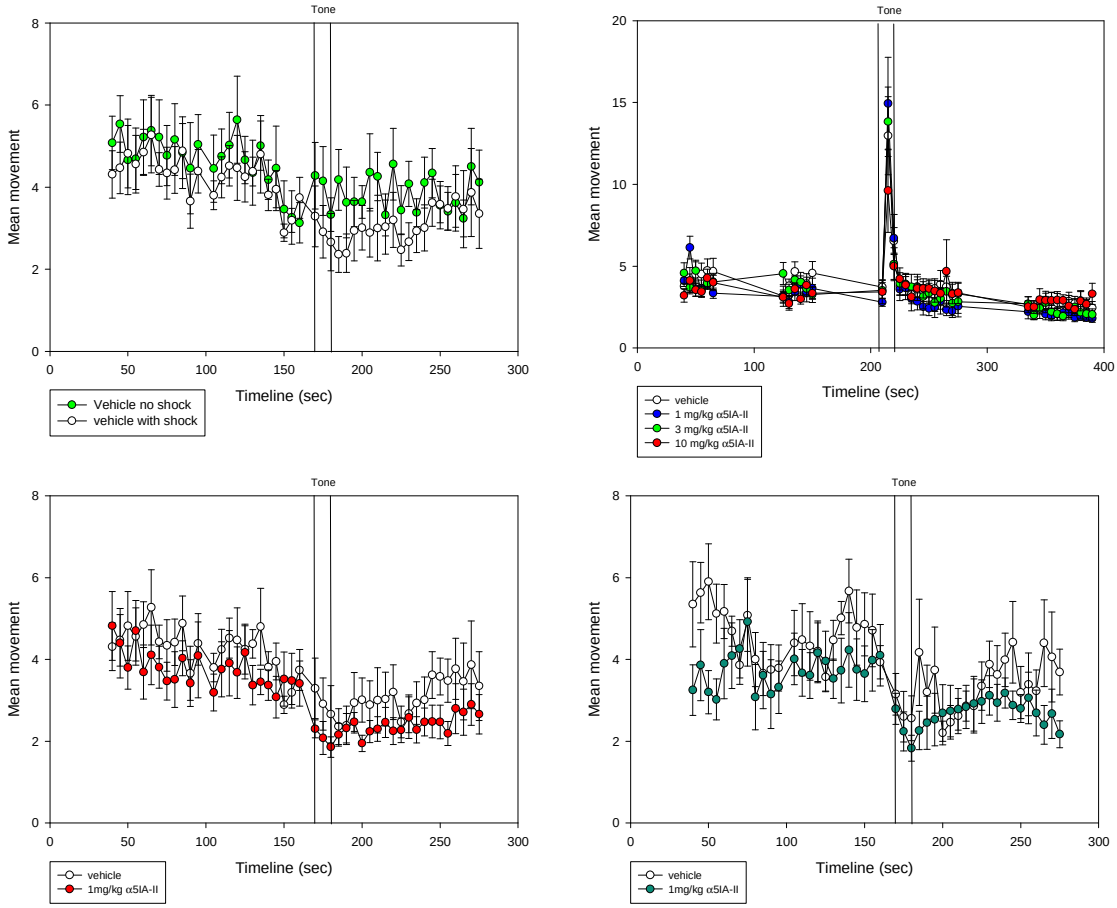


Figure 5.1. Traditional fear conditioning. (a) Test day: Behaviour of the vehicle groups. The white dots refer to the group which received a shock on training day, whereas the green dot group did not receive a shock on training day. (b) Conditioning day: Behaviour of the vehicle and the α5IA-II treated groups (1, 3 and 10 mg/kg) (c) Test day: Behaviour of the vehicle group and the 1 mg/kg α5IA-II group measured one day after training (d) Re-test day: Behaviour of the vehicle group and the 1 mg/kg α5IA-II group measured 6 days after test day.

It can be concluded that the animals freeze on the test and the re-test day due to an association between the tone and the shock, which is as expected. In the remaining experiments the same result was obtained and consequently these data will not be shown.

5.1.1.2. Behaviour of α5IA-II treated rats on conditioning day

The 1 mg/kg α5IA-II treated rats have the highest movement level during the tone-shock period as seen in figure 5.1.b. The 3mg/kg groups also have a higher response level than the vehicle during this period whereas the 10 mg/kg α5IA-II treated rats have a lower movement level compared to the vehicle.

Table 5.1. The *p*-values from the analysis of traditional fear conditioning experiment with α5IA-II treated rats on conditioning day.

	p-value
Intercept	<0.0001
Treatment	0.1059
Time	<0.0001
Period	<0.0001
System	<0.0001
Treatment:Time	0.0082
Treatment:Period	0.0001
Time:Period	<0.0001
Treatment:Time:Period	0.0006

Table 5.2. The *p*-values from the *t*-test comparison of the individual levels to a reference level for the α5IA-II treated rats on conditioning day in traditional fear conditioning experiment.

	p-value
Treatment(1mg/kg)	0.0391
Treatment(1mg/kg):Period(Shock)	0.0008
Treatment(3mg/kg):Period(Shock)	0.0045
Treatment(1mg/kg):Time:Period(Shock)	0.0168
Treatment(3mg/kg):Time:Period(Shock)	0.0033

As seen in table 5.1 and 5.2 it is statistically confirmed that the 1 and 3 mg/kg groups have a higher movement response than the vehicle group during the tone, whereas no difference of the 10 mg/kg treated rats from the vehicle group is demonstrated. It is furthermore found that the 1 mg/kg treated rats have a lower movement level before and after the tone.

5.1.1.3. Behaviour of α5IA-II rats on test day

As seen in figure 5.1.c the 1 mg/kg α5IA-II group on the test day freezes more than the vehicle group before, during and after the tone, indicating that α5IA-II improved the rats' ability to associate the shock with both the context and the tone. The graph for the 3 mg/kg α5IA-II group shows the same tendencies. The 10 mg/kg group is similar to the vehicle unless after the tone where the groups differentiate with an increased movement level for the 10 mg/kg group (see appendix 5.1). The ability to remember the context is hippocampus dependent, tone related anxiety is primarily amygdala dependent, whereas the behaviour after the tone is dependent of both hippocampal and non-hippocampal brain areas. Since the 10 mg/kg group do not differentiate from the vehicle group before the onset of the tone it could be speculated that α5IA-II when administered at 10 mg/kg primarily influences non-hippocampal dependent memory.

Table 5.3. The *p*-values from the analysis of α5IA-II treated rats on test day in traditional fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.1973
Time	<0.0001
Period	<0.0001
System	<0.0001
Treatment:Time	0.7851
Treatment:Period	0.2462
Time:Period	0.0001
Treatment:Time:Period	0.4325

None of the terms is significant as seen in table 5.3 and the differences observed between the vehicle and the other doses are hence not confirmed statistically. It is possible that a floor effect is present which makes it impossible to detect a significant difference between the freezing of the α5IA-II group compared to the vehicle group.

5.1.1.4. Behaviour of α5IA-II rats on re-test day

Figure 5.1.d shows that the 1 mg/kg α5IA-II group on the re-test day freezes more than the vehicle group before, during and after the tone, indicating that α5IA-II has improved the rats' ability to associate the shock with both the context and the tone. A similar tendency is seen for the 3 mg/kg group before the tone, whereas the 10 mg/kg did not differentiate from the vehicle group (see appendix 5.1).

This is not verified statistically since none of the terms including 'treatment' are significant (see table 5.4). None of the α5IAII groups are thus significantly different from vehicle.

Table 5.4. The *p*-values from the analysis of α5IA-II treated rats on re-test day in traditional fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.2107
Time	<0.0001
Period	<0.0001
System	<0.0001
Treatment:Time	0.6374
Treatment:Period	0.4836
Time:Period	0.0404
Treatment:Time:Period	0.1377

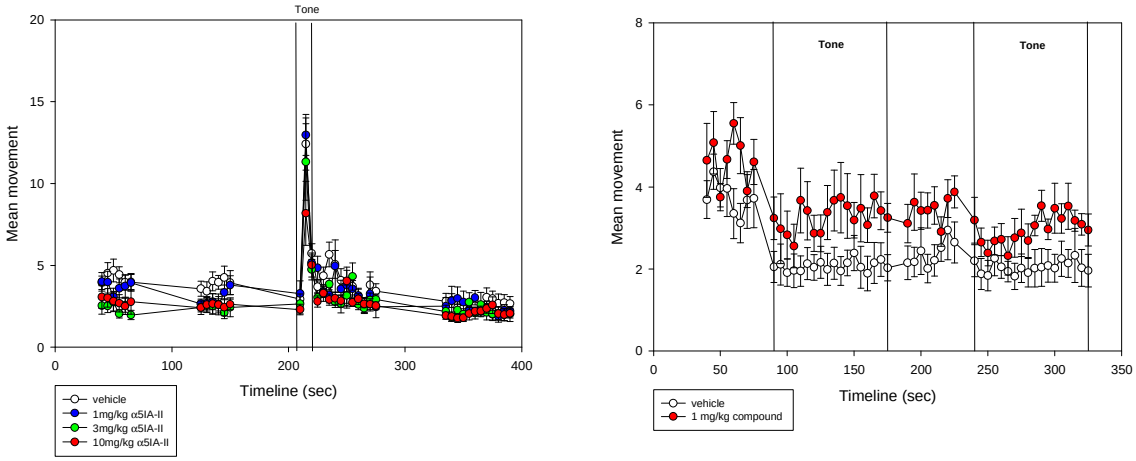


Figure 5.2. Extended-tone fear conditioning (a) Conditioning day: Behaviour of the vehicle and the α5IA-II treated groups (1, 3 and 10 mg/kg) (b) Test day: Behaviour of the vehicle group and the 1 mg/kg α5IA-II group measured one day after training.

5.1.2. Effects of α5IA-II on extended-tone fear conditioning memory

The graphs in figure 5.2 show the behavioural effects of α5IA-II tested in extended-tone fear conditioning.

5.1.2.1. Behaviour of α5IA-II treated rats on conditioning day

Figure 5.2.a shows the same behavioural tendencies during the occurrence of the tone and shock as seen in the traditional fear conditioning experiment, with the highest movement level seen for the 1 mg/kg α5IA-II treated rats and the lowest movement level expressed by the 10 mg/kg α5IA-II treated rats.

5.1.2.2. Behaviour of α5IA-II rats on extended-tone test day

The 1 mg/kg α5IA-II group on the test day moves more than the vehicle group before and during the tones (see figure 5.2.b), indicating that α5IA-II has impaired the rats' ability to associate the shock with both the context and the tone. The graph for the 3 mg/kg α5IA-II group shows the same tendencies, whereas the 10 mg/kg group only moves more than vehicle during the occurrence of the tones (see appendix 5.2). Only the 10 mg/kg thus seems to influence the periods in different ways compared to the vehicle, which is similar to the tendencies observed for this dose on traditional fear conditioning test day.

Similar to the traditional α5IA-II fear conditioning experiment none of the observed differences from the vehicle were detected in the statistical analysis, although

Table 5.5. The *p*-values from the analysis of α5IA-II treated rats on conditioning day in extended-tone fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.1104
Time	<0.0001
Period	<0.0001
System	0.0198
Treatment:Time	0.1499
Treatment:Period	0.6688
Time:Period	0.3446
Treatment:Time:Period	0.8007

a tendency might be present for the 'treatment' effect with $p_{treatment} = 0.1104$ (see table 5.5). Some peculiarities should be noticed regarding the results from this experiment. Although it is insignificant the response values of the vehicle group tend to be lower than the two lowest drug groups (see figure 5.2.b and appendix 5.2), which is opposite to the anticipated. Hence, the lack of significant difference seen in the traditional fear conditioning, which was speculated to be due to a floor effect, is thus not supported by this experiment.

5.1.3. Effects of α5IA-II on trace fear conditioning memory

The behavioural effects of α5IA-II on trace fear conditioning memory are seen in figure 5.3.

5.1.3.1. Behaviour of vehicle treated rats: effect of gap inserted between tone and foot-shock

Figure 5.3.a shows three different vehicle groups. The vehicle group that did not receive a foot shock at the conditioning day had a higher movement level throughout the whole period compared to the vehicle groups that received foot shock at the conditioning day. When the noshock group was compared to the trace group the *p*-value was $p = 0.0027$. This is as expected, since only the animals which associate the context or the tone with a foot shock is expected to express freezing behaviour. From the graphs the expected differentiation between the trace group and the no-trace group can not be seen (and supported by $p = 0.7101$), and it seems as the inserted time gap between the occurrence of the tone and the shock does not impede the rats' ability to associate the tone with the shock. Clearly, the lack of a trace-induced retardation in test day memory makes any

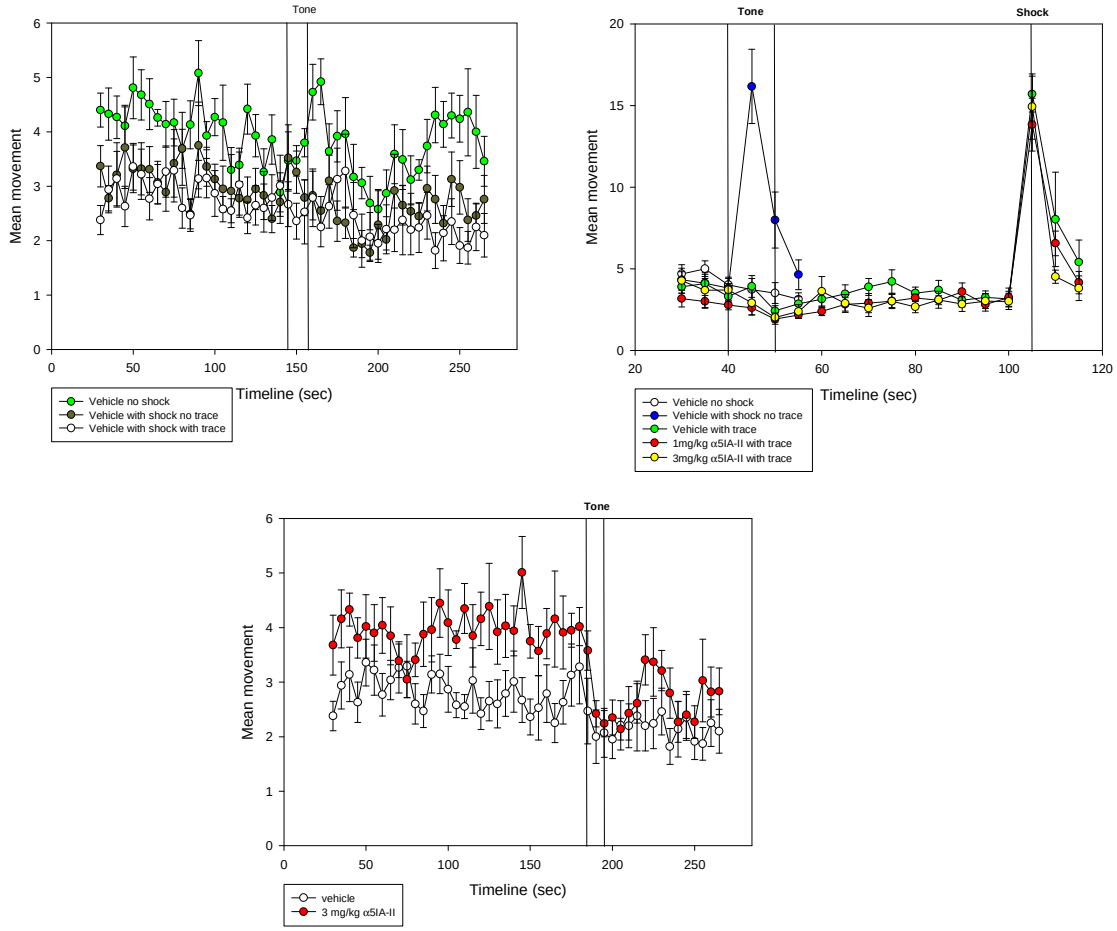


Figure 5.3. Trace fear conditioning (a) Test day: Behaviour of the three different vehicle groups: the green group did not receive a shock at the training day, the grey group received a shock during the occurrence of the tone at the training day, the white group also received a shock at the training day, but a time gap at 60 s. was inserted between the tone and the shock (b) Conditioning day: Behaviour of vehicle treated groups and α5IA-II treated groups. The three vehicle groups: the white group did not receive a shock, the blue group received a shock during the occurrence of the tone, the green group also received a shock, but a time gap at 60 s. was inserted between the occurrence of the tone and the shock. The same protocol was used on the two α5IA-II (1 and 3 mg/kg) treated groups, which received the shock 60 s. after occurrence of the tone. (c) Test day: Behaviour of the 3 mg/kg α5IA-II measured one day after training.

interpretation attained with the drug tested equivocal and therefore only brief statements are made below describing the effect of α5IA-II in trace fear conditioning.

5.1.3.2. Behaviour of α5IA-II treated rats on conditioning day

The behaviour of the rats on conditioning day is illustrated in figure 5.3.b. It is clearly seen that the α5IA-II treated rats receives the foot shock 60 seconds after the occurrence of the tone. Association of events that do not overlap is hippocampus dependent, and consequently trace fear conditioning primarily examine hippocampus dependent memory.

Table 5.6. The *p*-values from the analysis of α5IA-II treated rats on test day in trace fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.0128
Time	0.0006
Period	<0.0001
System	0.0871
Treatment:Time	0.4287
Treatment:Period	0.0573
Time:Period	0.1700
Treatment:Time:Period	0.8986

Table 5.7. The *p*-values from the *t*-test comparison of the individual levels to a reference level for the α5IA-II treated rats on test day in trace fear conditioning experiment.

	p-value
Treatment(3mg/kg)	0.0044
Treatment(1mg/kg):Period	0.0492

5.1.3.3. Behaviour of α5IA-II rats on test day

In figure 5.3.c the 3 mg/kg α5IA-II group moves more than the trace vehicle group before and after, but not during the tone. For the 1 mg/kg α5IA-II rats no difference compared to the vehicle is seen except from the last few time points where the response values of the 1 mg/kg α5IA-II rats suddenly increases (see appendix 5.3).

The increased movement of the 3 mg/kg α5IA-II group compared to vehicle is significant (table 5.6 and 5.7), and consequently α5IA-II may have impaired the hippocampus dependent ability of the rats to associate the context with the shock, which is opposite to expected. The dose 1 mg/kg α5IA-II is significantly different from the vehicle after the tone, but as mentioned it is owing to a few time points not nearby from the occurrence of the tone and hence it is not considered to be of biological interest.

5.1.4. Summary of effects of α5IA-II on memory

It was not possible to show that α5IA-II significantly influenced the rats' ability to associate the tone with the shock, which was consistent in both the test and re-test of the traditional fear conditioning and in the test of the extended-tone fear conditioning. The lack of significance regarding a possibly improved learning on test day of the traditional fear conditioning experiment can be due to the above mentioned floor effect. As mentioned already, any interpretation of the effects of α5IA-II (3 mg/kg) in the trace fear conditioning experiment is difficult.

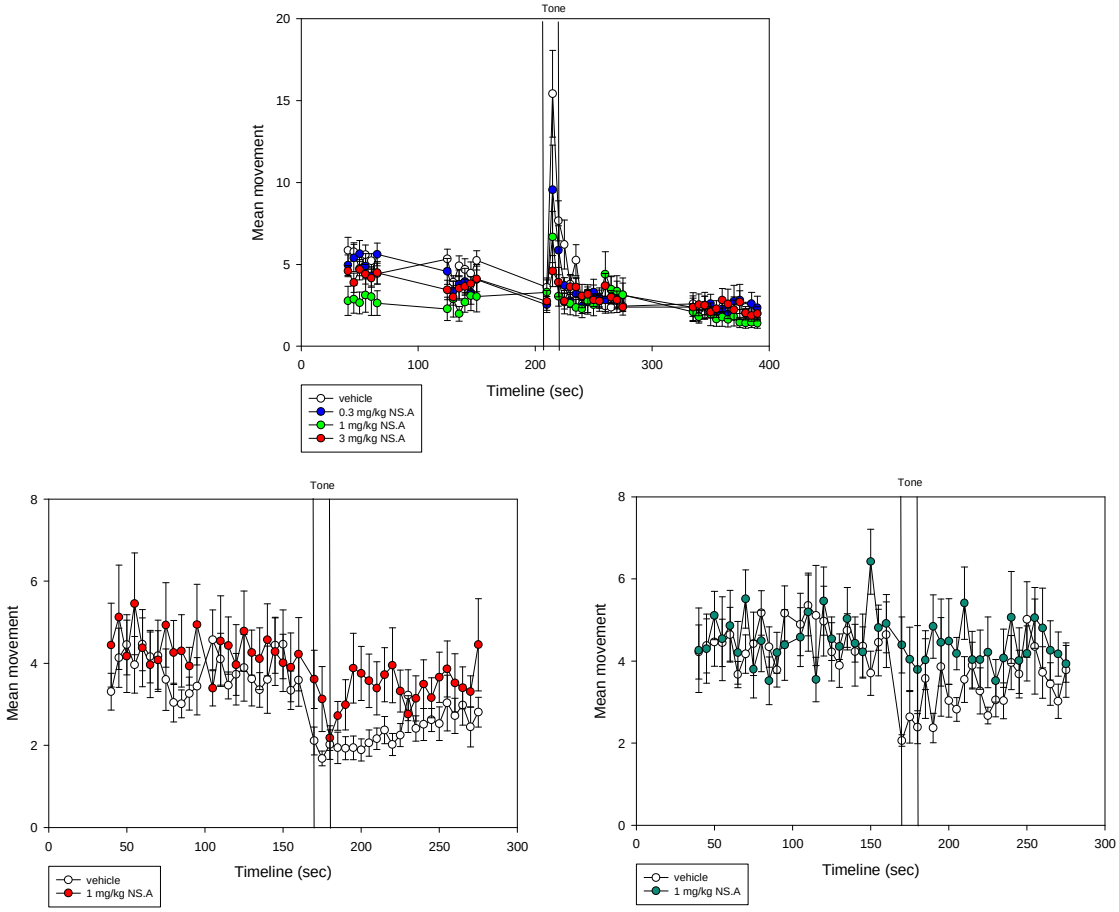


Figure 5.4. Traditional fear conditioning (a) Conditioning day: Behaviour of the vehicle and the NS.A treated groups (0.3, 1 and 3 mg/kg) (b) Test day: Behaviour of the vehicle group and the 1 mg/kg NS.A group measured one day after training (c) Re-test day: Behaviour of the vehicle group and the 1 mg/kg NS.A group measured 6 days after test day

5.1.5. Effects of NS.A on traditional fear conditioning memory

Figure 5.4 shows the behavioural effects of NS.A tested in traditional fear conditioning.

5.1.5.1. Behaviour of NS.A treated rats on conditioning day

It is seen in figure 5.4.a that the 1 mg/kg NS.A treated group in the period before the tone-shock period have the lowest movement level. During the tone-shock period all NS.A treated groups respond less than the vehicle group and a clear NS.A dose dependent decrease in movement is seen.

Table 5.8. The *p*-values from the analysis of NS.A treated rats on test day in traditional fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.0590
Time	0.0001
Period	<0.0001
System	0.0004
Treatment:Time	0.3665
Treatment:Period	0.1052
Time:Period	0.0047
Treatment:Time:Period	0.7139

Table 5.9. The *p*-values from the *t*-test comparison of the individual levels to a reference level for the NS.A treated rats on test day in traditional fear conditioning experiment.

	p-value
Treatment(1mg/kg):Period	0.0356
Treatment(3mg/kg):Period	0.0104

5.1.5.2. Behaviour of NS.A rats on test day

Figure 5.4.b shows that the 1 mg/kg NS.A group moves more than the vehicle group during and after the tone, although they freeze transiently during the tone. A lack of freezing behaviour is seen throughout the whole experimental period for the 0.3 and 3 mg/kg NS.A groups (see appendix 5.4).

The interaction between 'treatment' and 'period' was not significant in the full model (table 5.8), but after removal of the other insignificant terms the *p*-value changed to $p_{treatment:period} = 0.0316$.

The observations regarding the 1 mg/kg NS.A group are supported by the statistical findings that the main effect 'treatment' is nonsignificant and the 'treatment'-'period' interaction is significant (table 5.9). This implies that the movement level of the 1 mg/kg NS.A rats is significantly different from the vehicle during and after the tone. Furthermore, for the 3 mg/kg NS.A group the 'treatment'-'period' interaction is significant while the main effect 'treatment' is borderline significant with $p_{3mg/kg} = 0.0540$. This supports the observed lack of freezing throughout the experiment and consequently, there does not seem to be any association between the context or tone and the shock. The lack of freezing observed for the 0.3 mg/kg NS.A group was not verified in the analysis.

Table 5.10. The *p*-values from the analysis of NS.A treated rats on re-test day in traditional fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.0571
Time	0.0025
Period	<0.0001
System	0.0036
Treatment:Time	0.0307
Treatment:Period	0.0430
Time:Period	0.0196
Treatment:Time:Period	0.1032

Table 5.11. The *p*-values from the *t*-test comparison of the individual levels to a reference level for the NS.A treated rats on re-test day in traditional fear conditioning experiment.

	p-value
Treatment(1mg/kg):Period	0.0254

5.1.5.3. Behaviour of NS.A rats on re-test day

The 1 and 3 mg/kg NS.A groups do not show any freezing behaviour in the experiment and due to the freezing of the vehicle at the onset of the tone the movement level of the two groups differed during and after the tone (figure 5.4.c). No difference was seen for the 0.3 mg/kg and the vehicle groups (see appendix 5.4).

The 'treatment'-'period' interaction was significant for the 1 mg/kg group and borderline significant for the 3 mg/kg group ($p_{3mg/kg:period}$ = 0.0512). Due to the insignificance of the main effect 'treatment' it implies that the two groups differ from the vehicle during and after the tone and thus the observations were confirmed statistically, table 5.10/5.11.

5.1.6. Effects of NS.A on extended-tone fear conditioning memory

The behavioural effects of NS.A tested in extended-tone fear conditioning are summarised in figure 5.5.

5.1.6.1. Behaviour of NS.A treated rats on conditioning day

Figure 5.5.a shows that all NS.A treated groups move less than the vehicle group during the tone-shock period. However, the clear NS.A dose dependent decrease in movement level seen in the traditional fear conditioning is not seen, since the 3 mg/kg NS.A treated group moves more than the 1 mg/kg treated group.

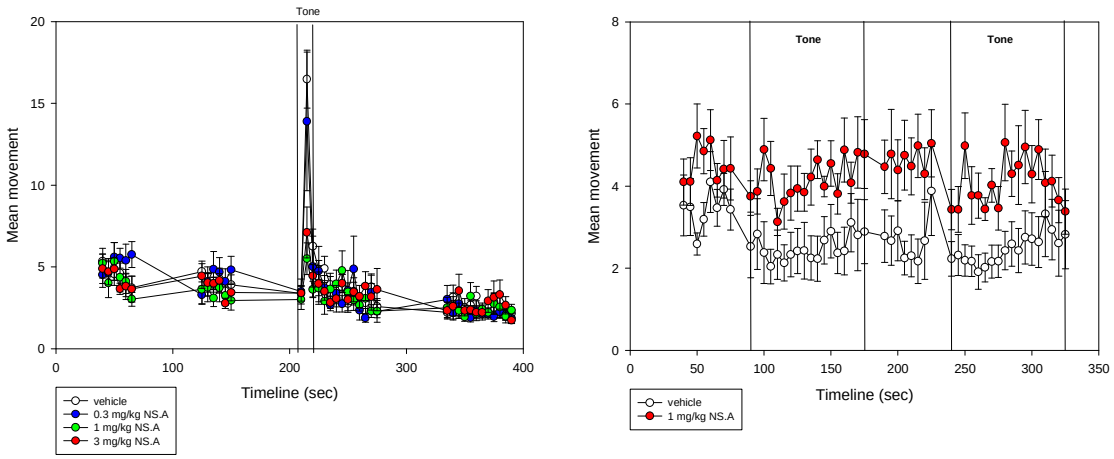


Figure 5.5. Extended-tone fear conditioning (a) Conditioning day: Behaviour of the vehicle and the NS.A treated groups (0.3, 1, and 3 mg/kg) (b) Test day: Behaviour of the vehicle group and the 1 mg/kg NS.A group measured one day after training

5.1.6.2. Behaviour of NS.A rats on extended-tone test day

The 1 mg/kg NS.A group moves more than the vehicle group before and during the tones as seen in figure 5.5.b. It is noticed that the animals maintain their relatively high movement level throughout the experiment including the time gap between the two tones. The same behaviour is seen for 0.3 and 3 mg/kg NS.A groups (see appendix 5.5).

In table 5.12 and 5.13 it is seen that 'treatment' is significant for both 1 and 3 mg/kg and since no 'treatment'-'period' interaction is significant, the movement level of the 1 and 3 mg/kg NS.A groups are significantly different from the vehicle throughout the experimental period. The observations for 0.3 mg/kg were not verified statistically.

Table 5.12. The *p*-values from the analysis of NS.A treated rats on test day in tone-extended fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.0001
Time	0.1959
Period	0.3511
System	0.6440
Treatment:Time	0.1573
Treatment:Period	0.0778
Time:Period	0.9758
Treatment:Time:Period	0.7801

Table 5.13. The *p*-values from the *t*-test comparison of the individual levels to a reference level for the NS.A treated rats on test day in extended-tone fear conditioning experiment.

	p-value
Treatment(1mg/kg)	0.0004
Treatment(3mg/kg)	<0.0001

5.1.7. Summary of effects of NS.A on memory

In fear conditioning, animals administered 1 and 3 mg/kg NS.A had impaired memory for the tone/context-shock association on both the test and re-test day compared to the vehicle group. In the extended tone experiment, NS.A treated rats move more than vehicle treated rats on the test day, again indication an impairment in memory for the tone/shock-context association.

5.1.8. Effects of alprazolam on traditional fear conditioning memory

The graphs in figure 5.6 summarise the behavioural effects of alprazolam tested in the traditional fear conditioning.

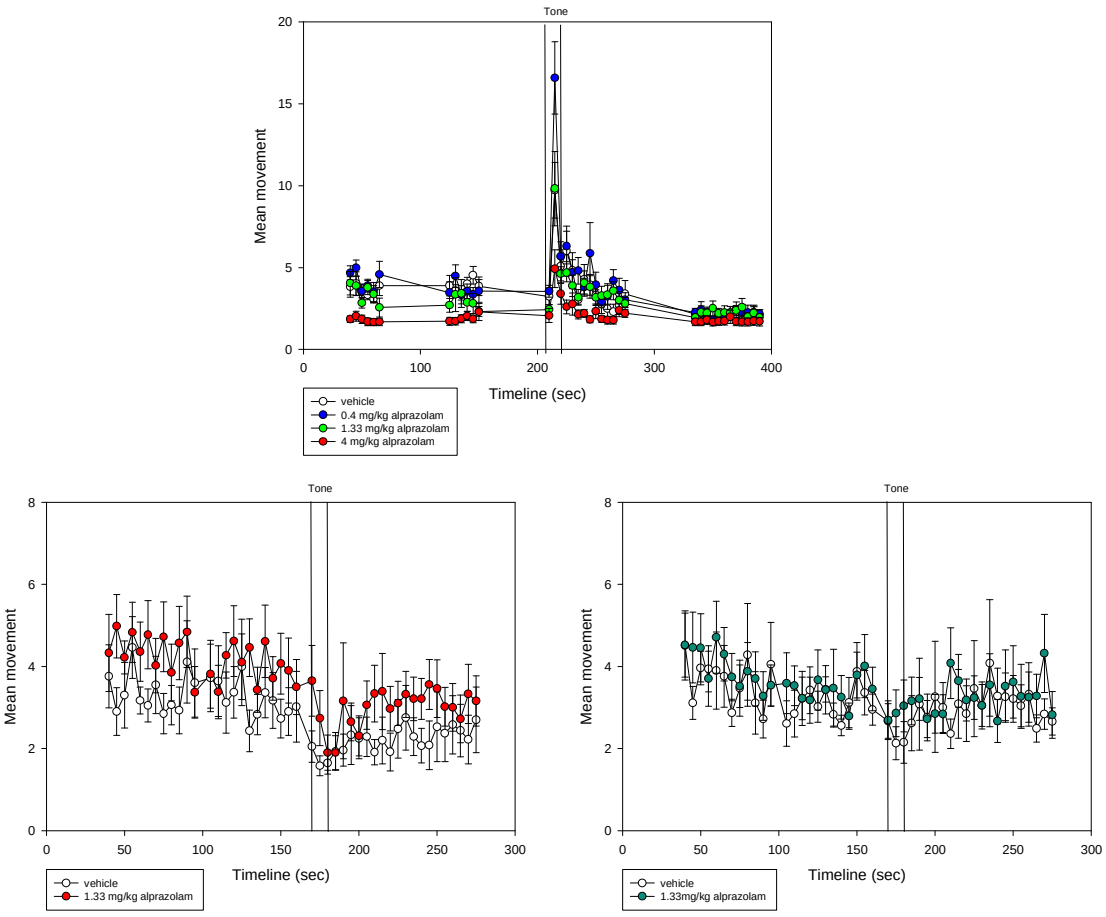


Figure 5.6. Traditional fear conditioning (a) Conditioning day: Behaviour of the vehicle and the alprazolam treated groups (0.4, 1.33 and 4 mg/kg) (b) Test day: Behaviour of the vehicle group and the 1.33 mg/kg alprazolam group measured one day after training (c) Re-test day: Behaviour of the vehicle group and the 1.33 mg/kg alprazolam group measured 6 days after test day

5.1.8.1. Behaviour of alprazolam treated rats on conditioning day

Figure 5.6.a shows that the 0.4 mg/kg alprazolam treated group compared to the other groups move extensively during the tone-shock period. The movement level of the 4 mg/kg alprazolam treated group is lower than the other groups before the tone-shock period. In addition, the movement level is hardly altered during the experiment and only a slightly change in response is seen during the shock. The movement level of the vehicle group and the 1.33 mg/kg alprazolam treated group are equivalent and are in between the movement level of the two other groups.

5.1.8.2. Behaviour of alprazolam rats on test day

It is seen in figure 5.6.b that the movement of 1.33 mg/kg alprazolam group is increased in all three periods compared to the vehicle. A more pronounced increase in movement is seen for the 4 mg/kg alprazolam group throughout the experiment whereas no difference is seen for the 0.4 mg/kg group compared to the vehicle (see appendix 5.6).

None of the terms including 'treatment' proved to be significant (table 5.14) but due to the relatively low *p*-value of 'treatment' a *t*-test was performed and the *p*-value of the highest dose was $p_{4mg/kg} = 0.0504$. The observations regarding the 0.4 mg/kg and 1.33 mg/kg are not verified statistically but for the 4 mg/kg group the statistical finding tend to support the observations owing to the borderline significance obtained for this group.

5.1.8.3. Behaviour of alprazolam rats on re-test day

It is see in figure 5.6.c that the 1.33 mg/kg alprazolam group moves more than the vehicle group during the occurrence of the tone, whereas the movement levels of the two groups are similar before and after the tone. The 4 mg/kg alprazolam group move

Table 5.14. The *p*-values from the analysis of alprazolam treated rats on test day in traditional fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.0926
Time	<0.0001
Period	<0.0001
System	0.1642
Treatment:Time	0.7944
Treatment:Period	0.2580
Time:Period	<0.0001
Treatment:Time:Period	0.9745

Table 5.15. The *p*-values from the analysis of alprazolam treated rats on re-test day in traditional fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.1616
Time	<0.0001
Period	0.0186
System	0.0361
Treatment:Time	0.6942
Treatment:Period	0.8730
Time:Period	0.0001
Treatment:Time:Period	0.5249

more than the vehicle group throughout the experiment and the movement level of the 0.4 mg/kg group is similar to the vehicle before and during the tone whereas it is lowered after the tone (see appendix 5.6).

All 'treatment'-related terms were insignificant (table 5.15) which was expected due to the insignificance obtained on test day. Thus, none of the described observations were supported statistically.

5.1.9. Effects of alprazolam on extended-tone fear conditioning memory

The behavioural effects of alprazolam tested in extended-tone fear conditioning are summarised in figure 5.7.

5.1.9.1. Behaviour of alprazolam treated rats on conditioning day

As seen in figure 5.7.a the 0.4 mg/kg alprazolam treated group jerks extensively during

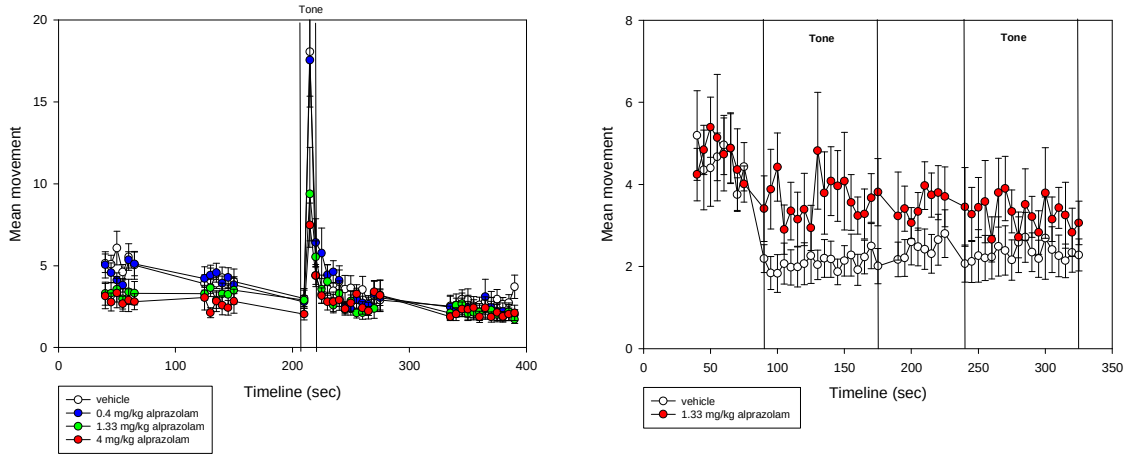


Figure 5.7. Extended-tone fear conditioning (a) Conditioning day: Behaviour of the vehicle and the alprazolam treated groups (0.4, 1.33 and 4 mg/kg) (b) Test day: Behaviour of the vehicle group and the 1.33 mg/kg alprazolam group measured one day after training

the tone-shock period compared to the other alprazolam groups and nearly reach the movement level of the vehicle treated group. The movement level of the 4 mg/kg alprazolam treated group only increases slightly during this period and a clear alprazolam induced dose dependent decrease in the movement level compared to the vehicle group is observed. Except from the movement level of the vehicle treated group, the behaviour reflects the observations seen on the conditioning day in the traditional fear conditioning experiment.

5.1.9.2. Behaviour of alprazolam rats on extended-tone test day

A lack of freezing during the occurrence of both tones for the 1.33 mg/kg alprazolam group is seen in figure 5.7.b which also applies to the 4 mg/kg alprazolam group, although the movement of this group during the second tone tend to be higher than the 1.33 mg/kg group (see appendix 5.7). For the 0.4 mg/kg alprazolam group the movement were decreased before the tones and was similar to the vehicle throughout the rest of the experiment, although the response values tend to be lowered during the last tone.

The statistical output seen in table 5.16 did not support any of the differences described above. Thus, no significant effect of alprazolam is detected in this experiment.

5.1.10. Summary of effects of alprazolam on memory

The test day graphs all showed an identical dose-related impairment of memory. However it was not possible to show a significant reduced freezing behaviour of the rats administered alprazolam neither at the test, re-test or extended-tone test day.

Table 5.16. The *p*-values from the analysis of alprazolam treated rats on test day in extended-tone fear conditioning experiment.

	p-value
Intercept	<0.0001
Treatment	0.0434
Time	0.6863
Period	0.3279
System	0.8576
Treatment:Time	0.6759
Treatment:Period	0.6613
Time:Period	0.3476
Treatment:Time:Period	0.9424

5.1.11. Effect of α5IA-II per se evaluated by omitting the unconditioned stimulus

Figure 5.1.b and figure 5.2.a both show that 1 mg/kg α5IA-II treated animals during the tone-shock period at the conditioning day have an elevated movement level compared to the vehicle, whereas the movement level of the 10 mg/kg α5IA-II treated animals were lower than vehicle treated animals. The elevated movement level of the 1 mg/kg α5IA-II animals was significant. Thus, it was speculated if the acute drug effect seen on the conditioning day during the tone-shock period could affect the test day results independent of the shock effect. Consequently an experiment with an experimental protocol identical to the traditional fear conditioning was performed, but with omission of the foot-shock on training day. The graphs in figure 5.8 summarises the effects of α5IA-II on test day and re-test day in the experiment without any unconditioned stimulus on 'conditioning' day.

5.1.11.1. Behaviour of α5IA-II rats on 'conditioning' day without any unconditioned stimulus

A significant movement level on conditioning day throughout the whole period was observed for the 10 mg/kg α5IA-II treated animals ($p < 0.0001$) compared to vehicle animals. The 1 and 3 mg/kg groups did not differ from the movement level of the vehicle group (appendix 5.8).

5.1.11.2. Behaviour of α5IA-II rats on test day without previous unconditioned stimulus

In figure 5.8.a no noticeable alteration is seen in the movement level in any of the

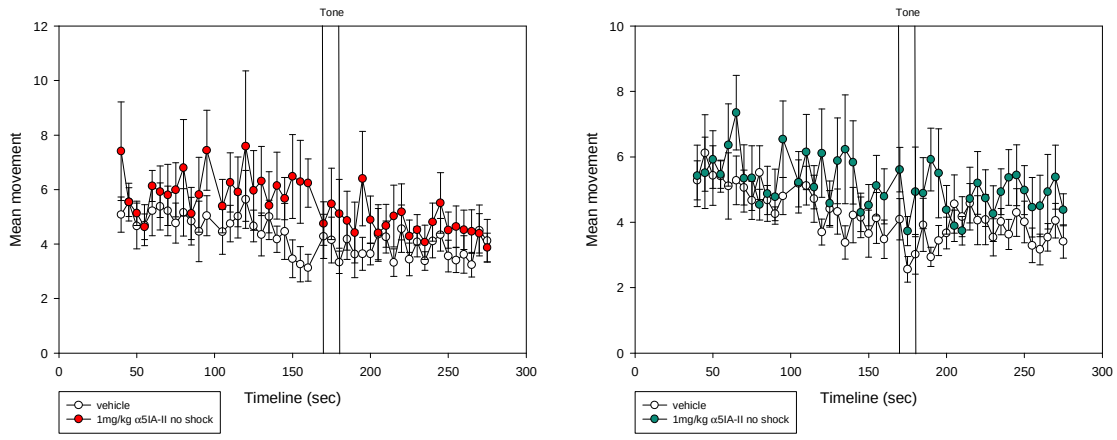


Figure 5.8. No foot shock data (a) Test day: Behaviour of the vehicle group and the 1 mg/kg α5IA-II group measured one day after 'conditioning'. (b) Re-test day: Behaviour of the vehicle group and the 1 mg/kg α5IA-II group measured 6 days after test day.

groups during or after the tone compared to the period before the tone (see appendix 5.9). The different α5IA-II groups might have a slightly increased movement level compared to the vehicle group before the tone.

This was not confirmed in the statistical analysis, since neither the 'treatment' nor the 'treatment'-'time' interaction was significant (table 5.17). Thus, it is concluded that the behaviour of the α5IA-II groups did not differ from the vehicle group on test day.

5.1.11.3. Behaviour of α5IA-II rats on re-test day without previous unconditioned stimulus

No noticeable alteration was seen in the movement level of either the vehicle group or the α5IA-II groups in the period after the tone compared to the behaviour before the tone (figure 5.8.b). During the tone a minor tendencies of freezing may be seen for both of the groups.

Since no significance regarding the 'treatment'-related terms was seen on test day it is not expected that the terms in question are significant on re-test day and as seen in table 5.18 this proved to be the case.

It is summarised that the acute drug effects observed at the conditioning day were not present on test day or re-test day. It is thus concluded that differences in movement level on test or re-test day caused by α5IA-II are dependent of the conditioned stimuli given on conditioning day and associated to learning processes.

Table 5.17. The *p*-values from the analysis of α5IA-II treated rats on test day in traditional fear conditioning experiment with no previous conditioned stimuli.

	p-value
Intercept	<0.0001
Treatment	0.3621
Time	<0.0001
System	<0.0001
Treatment:Time	0.1924

Table 5.18. The *p*-values from the analysis of α5IA-II treated rats on re-test day in traditional fear conditioning experiment with no previous conditioned stimuli.

	p-value
Intercept	<0.0001
Treatment	0.3468
Time	<0.0001
System	<0.0001
Treatment:Time	0.1634

5.2. Pre-pulse inhibition

In this section the effects of NS.A, α5IA-II and alprazolam on impaired pre-pulse inhibition will be discussed. The results are presented in the same manner as for the experiments with fear conditioning, with both observational trends in the graphs described followed by statistical analysis and final interpretation. As mentioned in section ‘4.2.5. Interpretational aspects of pre-pulse inhibition experiment‘ the term of interest is the ‘pre-treatment’-‘pre-pulse’ interaction and the significance level will be reported for each experiment. ‘Treatment’ is not of primary interest and the *p*-value is only reported if any departure from the expected is seen. In the present studies, impairment in pre-pulse inhibition was defined as reduction in startle response of the rats when there prior to the startle eliciting acoustic pulse has been presented a pre-pulse at either 4, 8, 16 or 24 dB above background noise.

Initially a study of pre-pulse inhibition impairment properties of PCP was executed, where PCP was administered to the rats at different doses and with different pre-treatment times. This was done in order to determine the optimal dose and pre-treatment time for PCP induced pre-pulse inhibition impairment. Hereafter the effects of NS.A, α5IA-II and alprazolam on PCP induced impaired pre-pulse inhibition and the effects of the $GABA_A$ receptor modulators per se were tested in pre-pulse inhibition experiments. The effects of NS.A were also tested in amphetamine induced impaired pre-pulse inhibition.

In order to analyse the data a model was developed as described in section ’4.2 Analysis of pre-pulse inhibition experiments’. For all experiments with administration of ‘pre-treatment’ and ‘treatment’ the three way interaction ‘pre-treatment’-‘treatment’-‘pre-pulse’ was significant and the groups administeded different ‘treatment’ doses was found to have a different progression in regard to the pre-pulse size. Separate models were made for the two groups to facilitate the interpretation. Interaction plots (see section ‘4.2.5. Interpretational aspects of pre-pulse inhibition experiment’) derived from the full statistical model are shown for each NS.A, α5IA-II and alprazolam experiments. These are presented in order to elucidate the compounds’ effects on pre-pulse inhibition per se, on impaired pre-pulse inhibition, the influence of the different pre-pulse levels and the interaction between these factors.

Additionally a study of pre-pulse inhibition impairment properties of methylphenidate was performed in mice and rats, respectively. The results obtained from this study are presented in appendix 5.10.

5.2.1. Influence of PCP on pre-pulse inhibition behaviour in rats

The results from the experiment investigating the ability of PCP to cause impairment in pre-pulse inhibition are outlined in this section.

5.2.1.1. Effect of PCP on pre-pulse inhibition 10 min post-administration

Initially it was determined if it was possible to replicate the general findings in the literature that an acute injection of PCP will engender a deficit in pre-pulse inhibition approximately within one hour post PCP administration as demonstrated in numerous publications previously (see section '2.3.2.1.1. Pre-pulse inhibition and acute PCP treatment').

In figure 5.9 a clear dose dependent impairment in pre-pulse inhibition is seen in animals administered PCP (1.25, 2.5, 5 mg/kg). The *p*-value of the 'treatment'-'pre-pulse' interaction was highly significant ($p < 0.0001$) and table 5.19 clearly shows that the relative impairment of the doses was significant compared to the relative impairment of vehicle for all pre-pulses. By looking at the parameterestimates it is seen that the impairment is most noticeable for 2.5 mg/kg, which also in evident in figure 5.9.

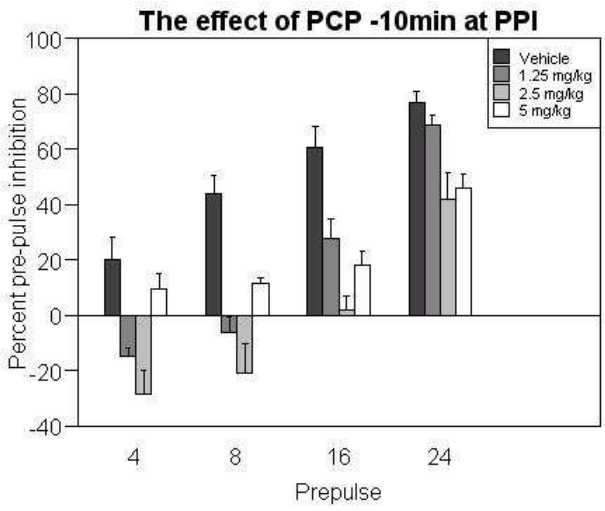


Figure 5.9. Pre-pulse inhibition. The percentage inhibition compared to naive startle response caused by the different pre-pulses 4, 8, 16 and 24 dB above background noise. 10 minutes prior to the experiments the rats have been treated with either vehicle or PCP (1.25, 2.5 or 5 mg/kg)

Table 5.19. The *p*-values from the *t*-test comparison of the individual levels to a reference level when animals were administered PCP 10 minutes prior to pre-pulse inhibition experiment.

	p-value
Treatment(1.25mg/kg):Pre-pulse(pp4)	0.0024
Treatment(2.5mg/kg):Pre-pulse(pp4)	<0.0001
Treatment(1.25mg/kg):Pre-pulse(pp8)	<0.0001
Treatment(2.5mg/kg):Pre-pulse(pp8)	<0.0001
Treatment(5mg/kg):Pre-pulse(pp8)	<0.0001
Treatment(1.25mg/kg):Pre-pulse(pp16)	<0.0001
Treatment(2.5mg/kg):Pre-pulse(pp16)	<0.0001
Treatment(5mg/kg):Pre-pulse(pp16)	<0.0001
Treatment(1.25mg/kg):Pre-pulse(pp24)	0.0005
Treatment(2.5mg/kg):Pre-pulse(pp24)	<0.0001
Treatment(5mg/kg):Pre-pulse(pp24)	<0.0001

5.2.1.2. Effect of PCP on pre-pulse inhibition 24 or 72 hours post-administration

Contrary to the deficits induced by PCP described above when animals were tested with the same doses of PCP (1.25, 2.5, 5 mg/kg) and tested for pre-pulse inhibition behaviour there was neither 24 nor 72 hours post-administration deficit in pre-pulse inhibition as seen in figure 5.10.

This is verified statistically where the 'treatment'-'pre-pulse' interaction was insignificant in both experiments. The *p*-value for 24 hours post-test was $p_{treatment:pre\text{-}pulse} = 0.2104$ whereas it was $p_{treatment:pre\text{-}pulse} = 0.6102$ for 72 hours post-test.

Since the greatest impairment was obtained with 2.5 mg/kg PCP administered 10 minutes prior to the experiment it was consequently chosen to model impairment in pre-pulse inhibition in the following experiments.

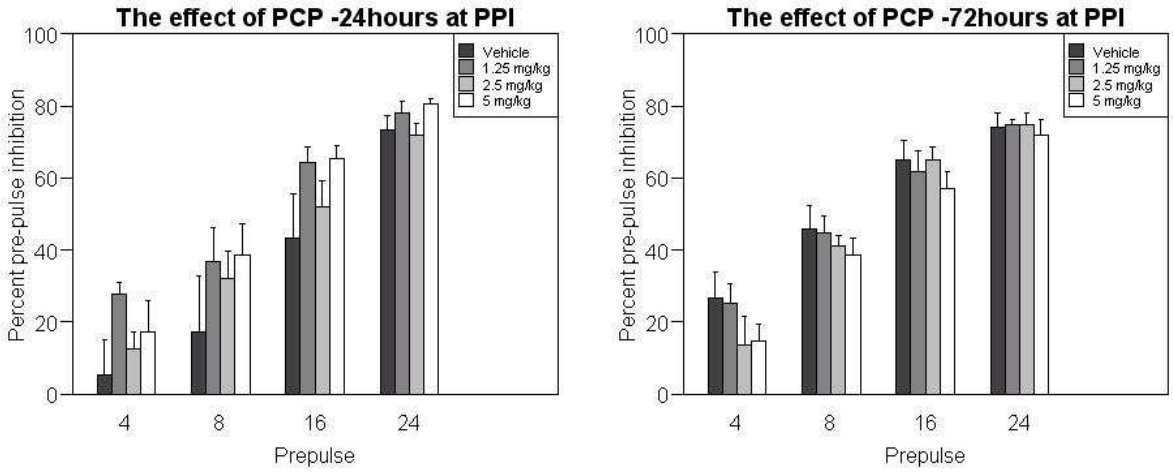


Figure 5.10. Pre-pulse inhibition. The percentage inhibition compared to naive startle response caused by the different pre-pulses 4, 8, 16 and 24 dB above background noise is shown. Prior to the experiment the rats have been treated with either vehicle or PCP (1.25, 2.5 or 5 mg/kg) with pre-treatment time at (a) 24 hours or (b) 72 hours.

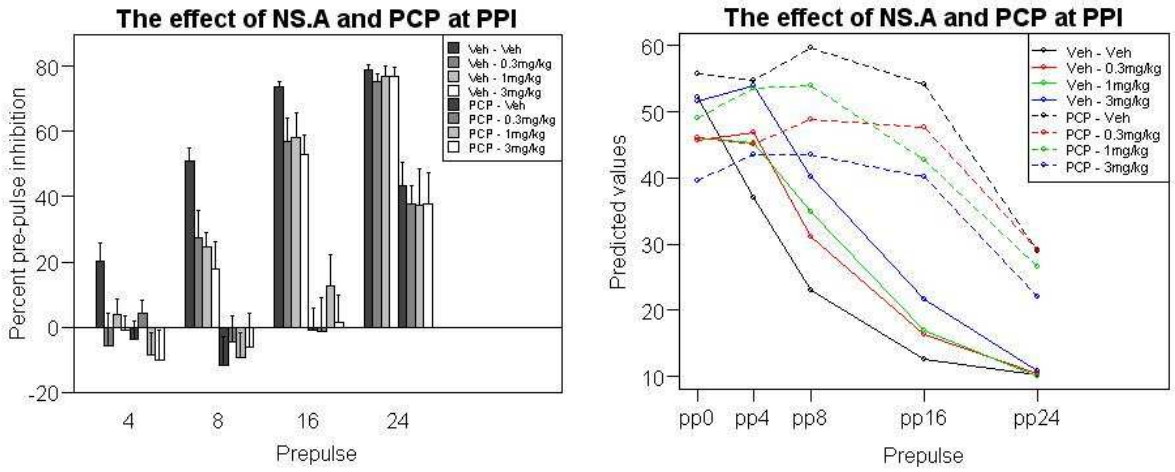


Figure 5.11. Pre-pulse inhibition. Prior to the experiment the rats have been pre-treated with either vehicle or NS.A (0.3, 1 or 3 mg/kg) and in addition treated with vehicle or PCP (2.5 mg/kg) (a) The percentage inhibition compared to naive startle response caused by the different pre-pulses 4, 8, 16 and 24 dB above background noise (b) The effect of the different pre-pulses on startle response.

5.2.2. Effect of $GABA_A$ receptor modulators on PCP induced impairment of pre-pulse inhibition

In this section the results showing the ability of NS.A, α5IA-II and alprazolam to alleviate acute PCP induced impairment in pre-pulse inhibition are presented.

5.2.2.1. Effect of NS.A on PCP impaired pre-pulse inhibition

When animals were given NS.A per se (0.3, 1, 3 mg/kg) pre-pulse inhibition impairment were induced at pre-pulse 4, 8 and 16 in a slightly dose dependent manner (see figure 5.11). For pre-pulse 24 no difference in pre-pulse inhibition was observed between the doses. NS.A did not affect the pre-pulse inhibition impairment of the PCP treated animals (2.5 mg/kg) for any of the pre-pulses.

These observations are supported by the statistical findings. The ‘pre-treatment’-’pre-pulse’ interaction was significant when NS.A was administered per se with a *p*-value less than 0.0001 and table 5.20 shows that NS.A per se significantly impair pre-pulse inhibition for pre-pulse 4, 8 and 16. When NS.A was administered together with PCP none of the ‘pre-treatment’-related terms were significant and it is concluded that NS.A did not affect PCP impaired pre-pulse inhibition.

Table 5.20. The *p*-values from the *t*-test comparison of the individual levels to a reference level for animals administered NS.A and vehicle in pre-pulse inhibition experiment.

	p-value
Pre-treatment(0.3mg/kg):Pre-pulse(pp4)	0.0002
Pre-treatment(1mg/kg):Pre-pulse(pp4)	0.0013
Pre-treatment(3mg/kg):Pre-pulse(pp4)	0.0002
Pre-treatment(0.3mg/kg):Pre-pulse(pp8)	<0.0001
Pre-treatment(1mg/kg):Pre-pulse(pp8)	<0.0001
Pre-treatment(3mg/kg):Pre-pulse(pp8)	<0.0001
Pre-treatment(0.3mg/kg):Pre-pulse(pp16)	<0.0001
Pre-treatment(1mg/kg):Pre-pulse(pp16)	0.0001
Pre-treatment(3mg/kg):Pre-pulse(pp16)	<0.0001

5.2.2.2. Effect of α5IA-II on PCP impaired pre-pulse inhibition

For animals administered α5IA-II per se (1, 3, 10 mg/kg) pre-pulse inhibition was not affected noticeably. A tendency of increased pre-pulse inhibition is seen for the 10 mg/kg dose mainly for all of the pre-pulses. In addition it should be noticed that unusual low startle response behaviour is seen for the animals administered 10 mg/kg (figure 5.12), and that this high dose in the fear conditioning experiments also caused a different behaviour compared to the other dose groups.

For the PCP treated animals the 10 mg/kg dose group is distinct from the other groups at pre-pulse 4 and 8. Unexpectedly, the remaining groups show an impaired pre-

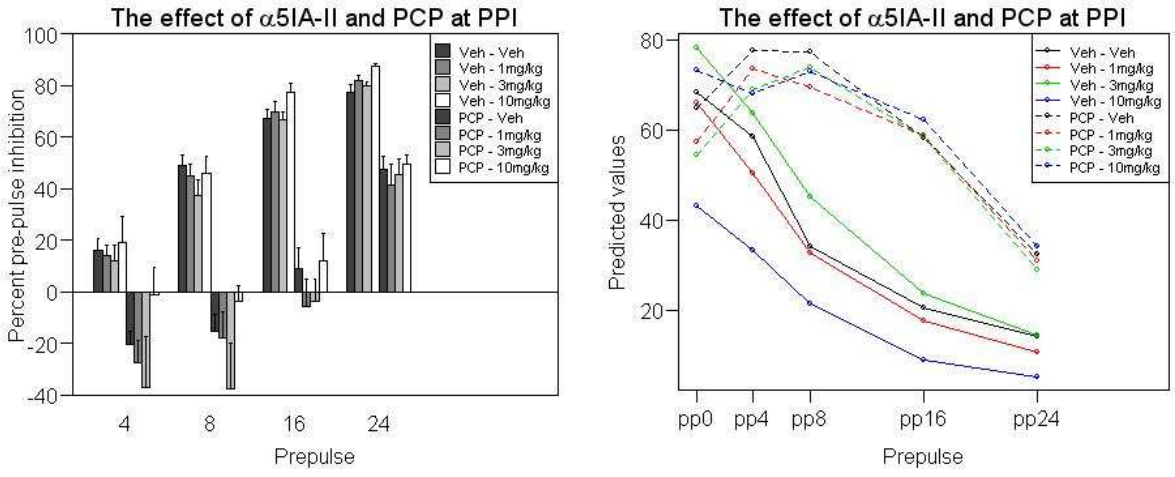


Figure 5.12. Pre-pulse inhibition. Prior to the experiment the rats have been pre-treated with either vehicle or α5IA-II (1, 3 or 10 mg/kg) and in addition treated with vehicle or PCP (2.5 mg/kg) (a) The percentage inhibition compared to naive startle response caused by the different pre-pulses 4, 8, 16 and 24 dB above background noise (b) The effect of the different pre-pulses on startle response.

Table 5.21. The *p*-values from the *t*-test comparison of the individual levels to a reference level for animals administered α5IA-II and vehicle in pre-pulse inhibition experiment.

	p-value
Pre-treatment(10mg/kg)	0.0469
Pre-treatment(10mg/kg):Pre-pulse(pp16)	0.0056
Pre-treatment(1mg/kg):Pre-pulse(pp24)	0.0305
Pre-treatment(10mg/kg):Pre-pulse(pp24)	<0.0001

pulse inhibition compared to the pulse-alone meaning that the animals startle more when a small pre-pulse is present. For pre-pulse 24 no difference is seen in the pre-pulse inhibition between the groups.

The observed behaviour of the α5IA-II per se treated animals is verified statistically. 'Pre-treatment' and the 'pre-treatment'-'pre-pulse' interaction were significant with *p*-values 0.0046 and 0.0002, respectively, and from the results of the subsequent *t*-test listed in table 5.21, it is concluded that 10 mg/kg of α5IA-II increases pre-pulse inhibition at all pre-pulses. The significant difference between 1 mg/kg and the vehicle at pre-pulse 24 does not seem to be of biologically relevance when the graph is assessed. The obtained significance might be due to the small variance seen for these groups.

For the PCP treated animals no 'pre-treatment'-related terms were significant and the described observations were thus not detected in the statistical analysis.

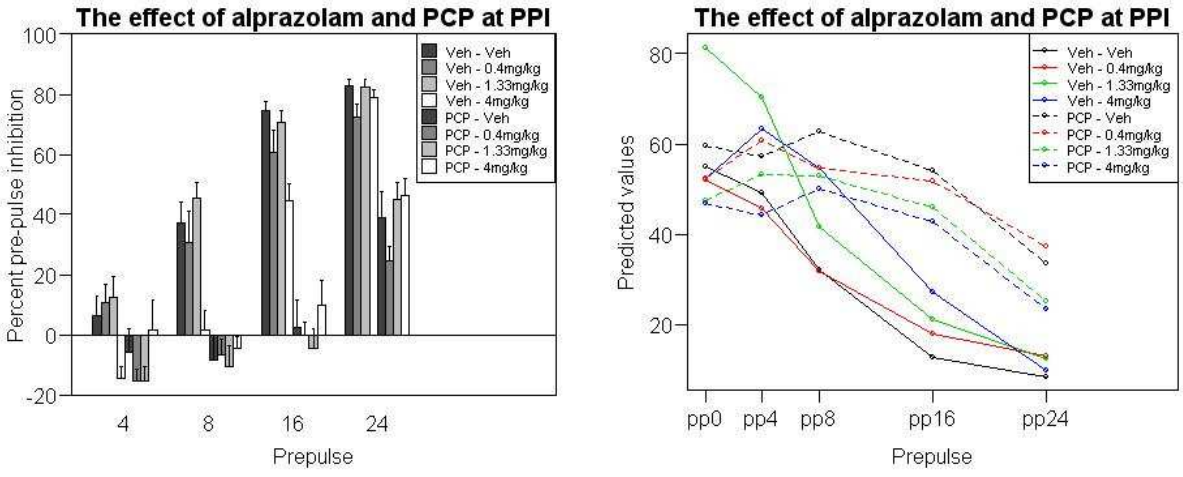


Figure 5.13. Pre-pulse inhibition. Prior to the experiment the rats have been pre-treated with either vehicle or alprazolam (0.4, 1.33 or 4 mg/kg) and in addition treated with vehicle or PCP (2.5 mg/kg) (a) The percentage inhibition compared to naive startle response caused by the different pre-pulses 4, 8, 16 and 24 dB above background noise (b) The effect of the different pre-pulses on startle response.

Table 5.22. The *p*-values from the *t*-test comparison of the individual levels to a reference level for animals administered alprazolam and vehicle in pre-pulse inhibition experiment.

	p-value
Pre-treatment(4mg/kg):Pre-pulse(pp4)	0.0028
Pre-treatment(4mg/kg):Pre-pulse(pp8)	0.0121
Pre-treatment(0.4mg/kg):Pre-pulse(pp16)	0.0048
Pre-treatment(4mg/kg):Pre-pulse(pp16)	<0.0001
Pre-treatment(0.4mg/kg):Pre-pulse(pp24)	<0.0001

5.2.2.3. Effect of alprazolam on PCP impaired pre-pulse inhibition

The animals administered 4 mg/kg alprazolam per se induced impairment in pre-pulse inhibition for pre-pulse 4, 8 and 16 and the startle response was even increased for pre-pulse 4 compared to pulse-alone (see figure 5.13). The remaining doses were similar to the vehicle. Alprazolam did not influence the PCP induced impairment for any of the pre-pulses.

In the analysis of alprazolam administered per se the 'pre-treatment'-'pre-pulse' interaction was significant with $p < 0.0001$. The *p*-values seen in table 5.22 support that alprazolam administered at 4 mg/kg impair pre-pulse inhibition when administered per se. Furthermore, the dose 0.4 mg/kg proved to be significant but this was not considered to be of biological importance.

When alprazolam was administered together with PCP no dose-related terms were significant which is consistent with the observed.

5.2.3. Effect of NS.A on amphetamine impaired pre-pulse inhibition

Contrary to our hypothesis NS.A did not reverse the PCP deficit in pre-pulse inhibition. Therefore NS.A was reassessed to determine if it would alleviate amphetamine induced pre-pulse inhibition impairment. The dose of amphetamine was chosen based at previous in-house (NeuroSearch) experiments and reliably induces deficits in pre-pulse inhibition.

When the animals were administered NS.A per se dose-dependent pre-pulse inhibition impairment was induced for pre-pulse 4, 8 and 16 (see figure 5.14). For pre-pulse 24 NS.A had no influence. For the amphetamine treated animals NS.A caused no difference for pre-pulse 4 and for pre-pulse 8. The dose 1 mg/kg differed from the remaining groups by inducing an increased startle response compared to pulse-alone.

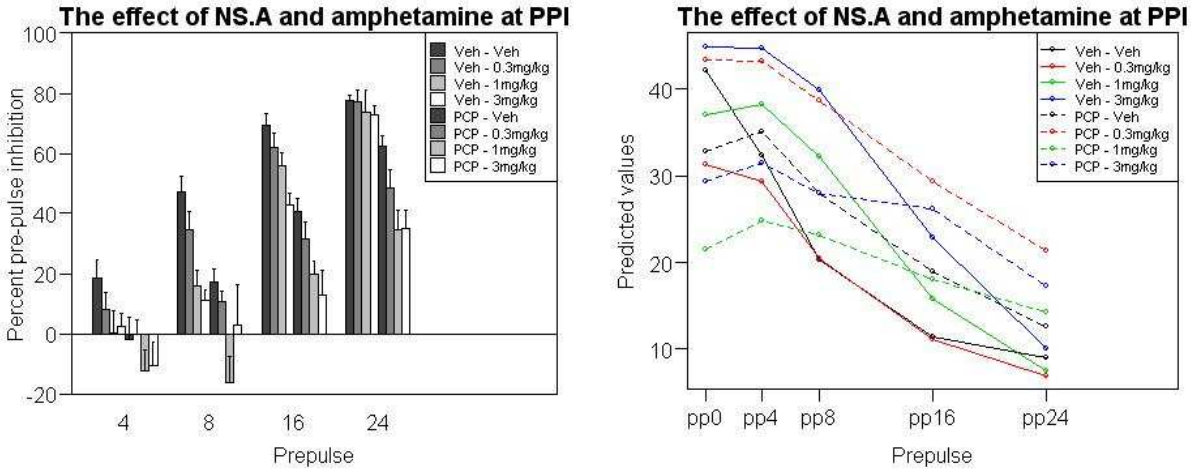


Figure 5.14. Pre-pulse inhibition. Prior to the experiment the rats have been pre-treated with either vehicle or NS.A (0.3, 1 or 3 mg/kg) and in addition treated with vehicle or amphetamine (2.5 mg/kg) (a) The percentage inhibition compared to naive startle response caused by the different pre-pulses 4, 8, 16 and 24 dB above background noise (b) The effect of the different pre-pulses on startle response.

For pre-pulse 16 and 24 NS.A worsened the amphetamine induced impairment in a dose-dependent manner.

In the analysis the 'pre-treatment'-'pre-pulse' interaction was significant when NS.A was administered per se. The *p*-values of the subsequent *t*-test are seen in table 5.23 and furthermore the dose 0.3 mg/kg was borderline significant for pre-pulse 4 ($p = 0.0605$). This verifies the observations and it can be concluded that NS.A impairs pre-pulse inhibition per se at all pre-pulses except pre-pulse 24. It also supports the results obtained when NS.A was administered with vehicle in the pre-pulse inhibition experiment described in section '5.2.2.1. Effect of NS.A on PCP impaired pre-pulse inhibition'. The similar results obtained in these parts of experiments were expected since the experimental protocols were similar.

When NS.A was administered together with amphetamine the 'pre-treatment'-'pre-pulse' interaction was significant ($p = 0.0004$). The results from the *t*-test is seen in table 5.24 and additionally dose 0.3 mg/kg was borderline significant for pre-pulse 24 ($p = 0.0589$). The statistical results support the observations for pre-pulse 16 and 24. The deviation of 1 mg/kg from the remaining doses at pre-pulse 8 was not confirmed in the analysis. From the results it is concluded that NS.A worsens the amphetamine impaired pre-pulse inhibition at the highest pre-pulses.

Table 5.23. The *p*-values from the *t*-test comparison of the individual levels to a reference level for animals administered NS.A and vehicle in pre-pulse inhibition experiment.

	p-value
Pre-treatment(1mg/kg):Pre-pulse(pp4)	0.0028
Pre-treatment(3mg/kg):Pre-pulse(pp4)	0.0121
Pre-treatment(0.3mg/kg):Pre-pulse(pp8)	0.0048
Pre-treatment(1mg/kg):Pre-pulse(pp8)	<0.0001
Pre-treatment(3mg/kg):Pre-pulse(pp8)	<0.0001
Pre-treatment(0.3mg/kg):Pre-pulse(pp16)	0.0077
Pre-treatment(1mg/kg):Pre-pulse(pp16)	<0.0001
Pre-treatment(3mg/kg):Pre-pulse(pp16)	<0.0001

Table 5.24. The *p*-values from the *t*-test comparison of the individual levels to a reference level for animals administered NS.A and amphetamine in pre-pulse inhibition experiment.

	p-value
Pre-treatment(1mg/kg):Pre-pulse(pp16)	0.0049
Pre-treatment(3mg/kg):Pre-pulse(pp16)	0.0012
Pre-treatment(1mg/kg):Pre-pulse(pp24)	<0.0001
Pre-treatment(3mg/kg):Pre-pulse(pp24)	0.0013

5.2.4. Summary of pre-pulse inhibition results

In both pre-pulse inhibition experiments executed with NS.A it was shown that NS.A administered per se impaired pre-pulse inhibition for pre-pulse 4, 8 and 16. NS.A administered together with PCP did not influence the PCP induced pre-pulse inhibition impairment. Pre-pulse inhibition impairment caused by amphetamine was exacerbated when NS.A was co-administered for pre-pulse 16 and 24.

With administration of 1 and 3 mg/kg α5IA-II per se no influence of pre-pulse inhibition was seen, whereas dose 10 mg/kg increased pre-pulse inhibition when administered per se. When α5IA-II was co-administered with PCP it did not influence pre-pulse inhibition impairment.

Impairment in pre-pulse inhibition was induced by administration of alprazolam per se at 4 mg/kg. None of the alprazolam doses affected PCP induced pre-pulse inhibition impairment.

6. Discussion

6.1. Fear Conditioning Experiments

In the following the results obtained from the present behavioural studies in rats will be discussed as direct continuation of the earlier described theory and compared to concrete experimental experience from the literature, which has also been overviewed in the earlier theoretical part of this project.

The present study did not succeed to show improved memory in rats treated with the $GABA_A$ α5 receptor selective drug α5IA-II in different versions of fear conditioning learning experiments. In the fear conditioning experiment where rats were trained to associate a context with a footshock stimulus and where the shock was delivered coincident with the offset of a tone stimulus, memory (context or conditioned stimulus) for this aversive event was not significantly greater in animals treated with α5IA-II compared to vehicle treated rats. However, the analysis of this dataset may not have been sensitive enough to verify the observation from the graphs (figure 5.1.c) that animals treated with α5IA-II at doses of 1 and 3 mg/kg showed a more rapid decline in movement on initiation of the tone stimulus compared to vehicle treated rats. Furthermore, during the 10 seconds tone the freezing level of the α5IA-II groups (1 and 3 mg/kg) was maintained at a lower level than the vehicle group, and there was some evidence that this persisted in the period immediately after tone offset. It is interesting that on re-testing these animals 6 days later this pattern of difference during/after tone between animals treated with 1 or 3 mg/kg of α5IA-II was still apparent from the graphs (figure 5.1.d). The statistical analyses applied did not distinguish the tone period from the after-tone period and therefore it is unclear if the differences between the treatment groups specifically during the tone differs significantly. It would have been interesting to analyse the tone-period alone on test and re-test days, which is possible using the linear mixed effects model, but due to time considerations this has not performed.

Nonetheless, the specific observations highlighted above were explicitly addressed when α5IA-II was re-tested at the same doses in fear conditioning on a second occasion (figure 5.2), with the same training protocol but with a change to the testing protocol. During testing the tone was presented twice with a 40 second interval

between the two tone periods on the test day. Furthermore, the tone on both occasions lasted for 90 seconds. The purpose of this protocol change was to maximise the chances of ascertaining if α5IA-II was specifically enhancing memory for the tone stimulus. However, as can be seen from figure 5.2.b in the present study, the extended-tone fear conditioning protocol, α5IA-II did not decrease freezing behaviour. By contrast, in this study it appeared to do essentially the opposite, i.e. increase movement relative to the vehicle treated animals; albeit non-significantly.

Clearly these contradictory data make it difficult to conclude on α5IA-II effects on fear conditioning. It could be questioned if the lack of significance in the present studies is due to experimental limitations meaning that a memory improving effect of α5IA-II may not be detectable in this type of model either (i) because there is a fundamental difference in the neurobiological substrate(s) mediating learning and memory in this model compared to models in which α5IA-II has been shown to have cognitive enhancing effects, such as the Morris water maze (**Collinson et al., 2006**), or (ii) in the fear conditioning model there is a floor-effect, precluding the possibility of seeing any potential enhancement. If the former is the case then additional models should be utilised in future research. On the other hand if the latter is the case then changes in the training protocol to 'retard' learning may reduce the strength of learning/memory thereby enhancing the chances of seeing an enhancing effect of a drug such as α5IA-II in this model. Some means of retarding learning, not mutually exclusive, would be to reduce the strength of the footshock, the length of the footshock or indeed the length of the tone stimulus.

The trace fear conditioning experiment showed (figure 5.3.c) a significant enhancement of the movement level of the 3 mg/kg α5IA-II group before the tone, which reflects an impaired association of the context with the electric shock, a hippocampus dependent process (reviewed by **Wallenstein et al., 1998**). This is opposite to the study where α5 knockout mice showed enhanced spatial learning in a water maze (**Collinson et al., 2002**), which like the trace fear conditioning experiment is a hippocampus dependent task.

However, care must be taken when ascribing absolute neurobiological systems to mediating the outcomes of behaviours observed in specific test situations. Thus fear conditioning and water maze are two very different tests and therefore may be dependent on diverse hippocampal and non-hippocampal structures and functions. In addition α5IA-II is not injected directly into the hippocampus but distributed

throughout the brain affecting $GABA_A$ α5 receptors in non-hippocampal areas, which could be speculated to interfere with any potential memory enhancing effect being mediated via interaction of this compound with $GABA_A$ α5 receptors in hippocampus. On the conditioning day the α5IA-II treated rats during the tone-shock period seemed to behave differently compared to the vehicle group, which might indicate that the drug influences other processes than the ones specifically involved in memory.

The examination of the effect of NS.A in the traditional fear conditioning experiment and in the fear conditioning experiment with an extended tone during test day showed that at 1 and 3 mg/kg, NS.A elevated movement levels throughout the whole test period (figure 5.4.b/c and figure 5.5.b). Since NS.A is a full α5 agonist, and given the strong evidence that the $GABA_A$ α5 receptor plays a role in memory, the deficit in memory for the aversive learning event induced by NS.A may be mediated by this receptor subtype. In any event, an identical dose-related impairment of memory was found for the aversive event after alprazolam treatment in fear conditioning (figure 5.6.b/c and figure 5.7.b), even though not significant. Alprazolam like other benzodiazepines is known to engender anterograde amnesia in man (**Enna et al., 1997**), which may be ascribed to a deficit in encoding information. The fear conditioning model of the present project would appear to be able mimicking these clinical observations since alprazolam was given prior to learning but animals were tested in the absence of alprazolam. Since NS.A and alprazolam seemed to induce similar deficits in fear conditioning it is worth speculating on the commonalities between these drugs that may account for these effects. As noted above, NS.A is a full $GABA_A$ α5 receptor agonist. Likewise alprazolam is also a full agonist of this receptor. However, in addition alprazolam is a full agonist at $GABA_A$ receptors containing either an α1, α2 or α3 subunit, whereas NS.A is essentially inactive at α1 receptors and a partial agonist at α2 and α3 receptors. Although not definitive this pharmacological comparison of alprazolam and NS.A would lead to the parsimonious conclusion that these two compounds' full agonism at $GABA_A$ α5 receptors may be responsible for mediating their memory impairing effects in the fear conditioning model.

6.2. Pre-pulse inhibition experiments

In the present study inhibition of the rats' whole-body flinch in response to a acoustic startling stimulus (110dB) where measured in trials that included pre-pulses which consisted of noise burst at 4, 8, 16 and 24 dB above background noise. Impairment of pre-pulse inhibition was induced pharmacologically by acute administration of PCP or amphetamine, which is thought to affect the neurotransmitter systems, speculated to be dysregulated in schizophrenics (reviewed by **Braff et al., 2001**).

When alprazolam was administered together with PCP no significant influence of alprazolam on the PCP induced impairment of pre-pulse inhibition was seen (figure 5.13), although some reports suggest benzodiazepines in clinic can reduce schizophrenic symptoms, although not necessarily sensorimotor impairments (reviewed by **Wolkowitz et al., 1991; Carpenter et al., 1999**). Furthermore, pre-pulse inhibition was attenuated in the rats which were administered the highest dose (4 mg/kg) of alprazolam per se. At this high dose the rats were observed to be completely flaccid, lying prostrated with their hind legs stretched behind and with partly paralyzed hind part body. This indicates as expected that alprazolam interferes with many different neural processes caused by its influence on α1, α2, α3, and α5 $GABA_A$ receptor subtypes.

Experiments with $GABA_A$ α3 receptor knockout mice, a genetic means of 'blocking' $GABA_A$ α3 receptor neurotransmission, have shown that these mice have impaired pre-pulse inhibition (**Yee et al., 2005**). In the present study alprazolam (4 mg/kg) as well as NS.A induced impairment in pre-pulse inhibition when administered per se (figure 5.11 and 5.14), which is surprising since the knockout mice (genetic 'antagonism') also have impairments. It is difficult to understand how both antagonism and agonism (i.e., NS.A and alprazolam) at the $GABA_A$ α3 receptor can lead to the same outcome, namely impaired pre-pulse inhibition. However, it should be considered that both NS.A an alprazolam are also full agonists at $GABA_A$ α5 receptors, and this may also be a factor in the results of the present study. However, again such a suggestion is not backed by research which clearly shows impaired pre-pulse inhibition after α5 $GABA_A$ receptor function is been reduced (**Bast et al., 2003; Bast et al., 2001**). Given these inconsistencies and the known issues of compensatory mechanisms in genetically modified animals it is premature to dismiss the suggestion that a drug with $GABA_A$ α2 receptor selectivity may have utility in treating schizophrenia. In the literature new strategies in the search for a drug that can

alleviate schizophrenic symptoms have hypothesised that a positive effect might be obtained by enhancing the effects of $GABA_A$ α2 receptors (**Volk et al., 2002;** reviewed by **Shulman et al., 2005;** reviewed by **Lewis et al., 2005**). Finally, it should not be overlooked that the impairment seen with NS.A of pre-pulse inhibition is in healthy rats which is not a model of schizophrenia. In this regard it is worth noting that NS.A did not exacerbate a PCP induced deficit in pre-pulse inhibition (figure 5.11), a putative model of schizophrenia.

In contrast to the lack of effect of NS.A in PCP treated rats, in amphetamine treated rats NS.A clearly exacerbated amphetamine induced pre-pulse inhibition impairment (figure 5.14). The different effects in the distinct pharmacological induced impairments might be related to the fact, that PCP blocks NMDA receptors (reviewed by **Morris et al., 2005**), whereas amphetamine is a dopamine releaser (reviewed by **Geyer et al., 2001**). Both dysregulations are as discussed in '2.1.4. Dopamine, NMDA-receptors and GABA mechanism' speculated to result in a broader dysregulation of GABAergic processes in the corticolimbothalamic systems. Although the end result is similar the impairment is initially caused by distinct neuronal dysregulations, which NS.A obviously could be thought not to influence equally.

In this discussion the α3 agonistic property of NS.A is interesting, since dopaminergic neurons express α3 containing $GABA_A$ receptors (**Fritschy et al., 1995**) and it could be speculated that NS.A facilitates inhibition of dopaminergic neurons. Improved inhibition of the dopaminergic neuron in the ventral tegmental area might induce a decrease in dopamine concentration in the nucleus accumbens and prefrontal cortex, since these regions are innervated by dopaminergic neurons projecting from the ventral tegmental area (**Sasack et al., 1992**). This scenario is opposite to the dopamine enhancing effect of amphetamine, and following NS.A and amphetamine effects could be speculated to counterbalance each other, but such a scenario was as stated above not forthcoming, and indeed the outcome was opposite to that anticipated.

As mentioned in '2.2. The role of different $GABA_A$ receptor subtypes in schizophrenia' it has been shown that selective positive allosterically modulation of α2, α3 and α5 in rodents alleviate auditory gating mechanism disorders and other symptoms that reflect those of schizophrenics (reviewed by **Guidotti et al., 2005**). In addition ELB139, a selective $GABA_A$ α2 and α3 receptor agonist, has been shown to reduce MK-801 induced psychotic activity (**Langen et al., 2006**). Even though NS.A did not reverse pre-pulse inhibition impairment in any of the present studies it might still be

interesting to investigate the effect of NS.A in other psychotic models e.g. examine the NS.A effect on pharmacological induced hyperactivity and stereotyped sniffing as it was done with ELB139, since pre-pulse inhibition impairment as emphasized in the theoretical part does not specifically model schizophrenia, but models sensorimotor gating deficits related to schizophrenia as well as other diseases (reviewed by **Geyer et al., 2001**).

In addition pre-pulse inhibition impairment in rodents only are found when PCP is administered acutely (**Myers et al., 2005; Schwabe et al., 2004; Teen et al., 2005**), whereas the neurochemical and metabolic changes that reflects those found in schizophrenic patients are observed in rodents after chronic administration of PCP (**Cochran et al., 2003;** reviewed by **Morris et al., 2005**). Even though acute administration of PCP in many studies is a very reliable model of schizophrenic symptoms, it might not be an optimal model when testing $GABA_A$ receptor selective drugs, since (i) PCP acutely influences NMDA receptors distributed throughout the whole brain, whereas the enhancing effects of $GABA_A$ α2 receptor are hypothesized to be of effect in the prefrontal cortex and hippocampus (**Volk et al., 2002**), and (ii) PCP acutely influences the NMDA receptors, which have opposite effect (evoking EPSP) compared to $GABA_A$ receptors (evoking IPSP).

Consequently, the acute PCP administration and the lack of an animal model specific for schizophrenia is complicating factors in preclinical studies concerning schizophrenia. Following it is difficult to conclude from the present study if NS.A might have any influence in regard to schizophrenia without further experiments, e.g. (i) the Extra Dimensional Shift model, or (ii) experiments where PCP are dosed chronically in order to obtain an animal model of the neurochemical changes seen in schizophrenics, and thereafter administer NS.A and evaluate the neurochemical effects of NS.A.

Knock-in hippocampal $GABA_A$ α5 receptor mutated mice have been observed to express impaired pre-pulse inhibition (**Hauser et al., 2005**). The mutation causes inhibition of the $GABA_A$ α5 receptor processes. Since α5IA-II is thought to inhibit the receptor and it was speculated that this compound would impair pre-pulse inhibition. When 1 and 3 mg/kg α5IA-II were administered per se they did not affect pre-pulse inhibition, whereas 10 mg/kg α5IA-II per se surprisingly increased or improved pre-pulse inhibition (see figure 5.12). In the traditional fear conditioning experiment the same high dose impaired learning of the rats, which was opposite to 1 and 3 mg/kg

α5IA-II, which seemed to improve learning, although not significant. The behaviour of rats treated with 10 mg/kg α5IA-II in both fear conditioning experiment and pre-pulse inhibition experiments differed from the effects of lower doses on rat behaviour in these models, with the effect of high (10 mg/kg) and low doses (1-3 mg/kg) being qualitatively opposite. It is reasonable to suggest that at high doses α5IA-II affects $GABA_A$ α5 receptors in non-hippocampal areas in addition to hippocampus (an area enriched in $GABA_A$ α5 receptors). Irrespective of the interesting effects of α5IA-II on pre-pulse inhibition per se, this compound had no influence on PCP induced pre-pulse inhibition impairment.

7. Conclusion

The different fear conditioning learning experiments did not succeed to show a significant improvement in memory in rats treated with the selective $GABA_A$ α5 receptor inverse agonist α5IA-II. In the traditional fear conditioning experiment the α5IA-II (1 and 3 mg/kg) treated rats at the test day graphs showed a more rapid decline in movement on initiation of the tone than the vehicle group. The freezing was maintained at a lower level during and after the tone. Following, 6 days later on re-test day the graphs depict the same movement pattern for the animals during and after the tone. However, the lack of significance might be due to experimental limitations such as a floor-effect or to the fact that the statistical analysis of the tone-period alone was not performed. Independent of the basis for the lack of statistically significant effects, in a related method where tone length was extended to enhance the chances and potential reliability of showing a positive effect of α5IA-II on fear conditioning memory, no improvement in memory was found.

The contradicting results make it difficult to conclude on α5IA-II effects on fear conditioning memory. Based on (i) the fact that in the literature it is widely agreed that inhibition of hippocampal $GABA_A$ α5 receptors improve memory, and (ii) that a floor-effect may have impeded the possibility of showing enhancement and (iii) as well as potential improvement in statistical analysis, it is considered interesting to continue the study of α5IA-II effect in additional models to determine if it can improve memory. It should not be overlooked that α5IA-II does improve learning/memory in a rat water maze task and that this compound has reached Phase I clinical trials, although no efficacy data is available as such trials are typically in volunteers to determine pharmacokinetics and safety. Furthermore, a related $GABA_A$ α5 selective inverse agonist, L655708 is effective in a different version of the rat trace fear conditioning model.

In traditional fear conditioning and extended-tone fear conditioning experiment NS.A (1 and 3 mg/kg) significantly impaired memory. A similar dose-related impairment of fear conditioning memory was found when alprazolam was tested,

although it was not significant. These impairments may not be surprising given the known ability of benzodiazepines to induce anterograde amnesia in humans.

In the pre-pulse inhibition experiments NS.A and alprazolam (4 mg/kg) per se induced impairment in pre-pulse inhibition. When these compounds were co-administered with PCP, neither of them affected the PCP induced pre-pulse inhibition impairment. When NS.A was administered to rats with amphetamine induced pre-pulse inhibition impairment the impairment was exacerbated.

Acute administration of PCP and use of pre-pulse inhibition impairment does not reflect the neurochemical changes of schizophrenics and is not a particularly good model of schizophrenia specificlly with regard to the $GABA_A$ system. Thus it is worth considering alternative experimental protocols before making any firm conclusions.

Administration of α5IA-II was not shown to be affective on PCP induced pre-pulse inhibition.

From a statistical approach it was found that a bias as well as variation is induced by the experimental equipment which finds expression in heterogeneity across boxes. It is useful to do a further examination of this in order to reveal if it can be rectified by any means. It proved advantageous to include the system or box effect in the model in order to diminish the residual variation and thereby to increase the precision of the estimates which enhances the sensitivity of the model. However, this is untenable in the long term since it is unknown if the response values are distorted and hence may be unreliable.

In order to assess the effect of the drugs in fear conditioning experiment a linear mixed effects model was defined with ‘treatment’, ‘time’, ‘period’ and ‘system’ as fixed effects. ‘Animal’ was defined as a random effects term with an individual intercept estimated for each of them. In order not to violate the model assumptions the heteroscedasticity obtained for the boxes was modelled by means of a variance function with the assignment of a variance parameter for each box. An exponential correlation structure was used to model residual autocorrelation. The model proved to be appropriate in describing data and no model violation was obtained.

The statistical model was modified in order to assess the effects of the drugs in pre-pulse inhibition experiment. The fixed effects were ‘pre-treatment’, ‘treatment’, ‘pre-pulse’ and ‘system’. As before ‘animal’ was defined as a random effects term.

Equivalent to the analysis of the fear conditioning experiment heteroscedasticity and residual autocorrelation was modelled. The model described the data suitably and no violations of the model assumptions were seen.

8. List of references

Adams B., Moghaddam B. (1998). Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci*. **18**: 5545-5554

Adler C. M., Malhotra A.K., Elman I., Goldberg T., Egan M., Pickar D., Breier A. (1999). Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. *Am J Psychiatry*. **156**:1646-1649

Andreasen N. C. (1982). Negative symptoms in schizophrenia: Definition and realiability. *Arch. Gen. Psychiatry*. **39**: 784-788

Andreasen N. C., Olson S. (1982). Negative versus positive schizophrenia: Definition and validation. *Arch. Gen. Psychiat*. **39**: 789-794

Antonova E., Sharma T., Morris R., Kumari V. (2004). The relationship between brain structure and neurocognition in schizophrenia: a selective review. *Schizophrenia Research*. **70**: 117-145

Baddely A. (1982). Implications of neuropsychological evidence for theories of normal memory. *Philos. Trans. R. Soc. Biol. Sci.* **298**: 59-72

Bast T., Feldon J. (2003). Hippocampal modulation of the sensorimotor processes. *Prog Neurobiol*. **70**: 319-345

Bast T., Zhang W. N., Felton J. (2001). Hyperactivity, decreased startle activity, and disrupted prepulse inhibition following disinhibition of the rat ventral hippocampus by the GABA(A) receptor antagonist picrotoxin. *Psychopharmacology (Berl).* **156**: 225-233

Bates, D., Sarkar, D. (2006). lme4: Linear mixed-effects models using S4 classes. R package version 0.9975-9.

Bear M. F., Connors B. W., Paradiso M. A. (2001). *Neuroscience Exploring the Brain.* USA: Lippincott Williams & Wilkins.

Beasley C. L., Reynolds G. P. (1997). Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophr Res.* **24**: 349-355

Benes F. .M., Kwok E. W., Vincent S. L., Todtenkopf M..S. (1998). A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressives. *Biol. Psychiatry*. **44**: 88– 97

Benes F. M., Kwok E. W., Vincent S. L., Todtenkopf M. S. (1998). A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressives. *Biol Psychiatry*. **44**: 88-97

Benes F. M., McSparren J., Bird E. D., SanGiovanni J. P., Vincent S. L. (1991). Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. *Arch Gen Psychiatry*. **48**: 996-1001

Benes F..M., Berretta S. (2000). Amygdalo-entorhinal inputs to the hippocampal formation in relation to schizophrenia. *Ann. N. Y. Acad. Sci.* **911**: 293–304

Benes F.M., Berretta S. (2001). GABAergic Interneurons: Implications for Understanding Schizophrenia and Bipolar Disorder. *Neuropsychopharmacology.* **25**: 1-27

Biggio G., Concas A., Corda M. G., Giorgi O., Sanna E., Serra M. (1990). GABAergic and dopaminergic transmission in the rat cerebral cortex: Effect of stress, anxiolytic and anxiogenic drugs. *Pharmac. Ther*. **48**: 121-142

Bliss T. V., Lomo T. (1973). Long-lasting potentialtion of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol*. **232**: 331-356

Braff D. L., Geyer M. A., Swerdlow N. R. (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology*. **156**: 234-258

Braff D. L., Geyer M.A. (1990). Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch. Gen. Psychiatry*. **47**: 181-188

Carlsson A. (1988). The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology*. **1**: 179-86

Carpenter W. T., Buchanan R. W., Kirkpatric B., Breier A. F. (1999). Diazepam treatment of early signs of exacerbation in schizophrenia. *Am J Psychiatry.* **156**: 299-303

Casacalenda N., Boulenger J. (1998). Pharmacologic Treatments Effective in Both Generalized Anxiety Disorder and Major Depressive Disorder: Clinical and Theoretical Implications. *Can J Psychiatry*. **43**: 722-730

Chizh B. A. (2002). Novel approaches to targeting glutamate receptors for the treatment of chronic pain: Review article. *Amino Acids*. **23**: 169–176

Cochran S. M., Fujimura M., Morris B. J., Pratt J. A. (2002). Acute and Delayed Effects of Phencyclidine Upon mRNA Levels of Markers of Glutamatergic and GABAergic Neurotransmitter Function in the Rat Brain. *Synapse*. **46**: 206-214

Cochran S. M., Fujimura M., Morris B.J., Pratt J.A. (2002). Acute and delayed effects of phencyclidine upon mRNA levels of markers of glutamatergic and GABAergic neurotransmitter function in the rat brain. *Synapse*. **46**: 206-214

Cochran S. M., Kennedy M., McKerchar C. E., Steward L. J., Pratt J. A., Morris B. J (2003). Induction of Metabolic Hypofunction and Neurochemical Deficits after Chronic Intermittent Exposure to Phencyclidine: Differential Modulation by Antipsychotic Drugs. *Neuropsychopharmacology*. **28**: 265-275

Collinson N., Kuenzi F..M., Jarolimek W., Maubach K. A., Cothliff R., Sur C., Smith A., Otu F. .M., Howell O., Atack J.R., McKernan R. M., Seabrook G. R., Dawson G. R., Whiting P. J., Rosahl T. W. (2002). Behavioral/Systems – Enhanced Learning and Memory and Altered GABAergic Synaptic Transmission in Mice Lacking the α5 Subunit of the GABAA Receptor. *J. Neurosci.* **22**: 5572-5580

Collinson N., Atack J. R., Laughton P., Dawson G. R., Stephens D. N. (2006). An inverse agonist selective for α5 subunit-containing $GABA_A$ receptors improves encoding and recall but not consolidation in the Morris water maze. *Psychopharmacology*. **188**: 619-628

Conradsen, K. (2002). *En introduction til statistic – Bind 2.* Danmark: IMM.

Costa E., Guidotti A. (1996). Benzodiazepines on trial: a research strategy for their rehabilitation. *TIPS*. **17**: 192-200

Crow T. J. (1980). Positive and negative schizophrenic symptoms and the role of dopamine. *Br. J. Psychiatry*. 137: 383-386

Crestani F., Keist R., Fritschy J. M., Benke D., Vogt K., Prut L., Blüthmann H., Möhler H., Rudolph U. (2002). Trace fear conditioning involves hippocampal α5 $GABA_A$ receptors. *PNAS*. **99**: 8980-8985

Davidoff S. A., Benes F. M. (1998). High-resolution scatchard analysis shows D1 receptor binding on pyramidal and nonpyramidal neurons. *Synapse*. **28**: 83-90

Davis M. (1979). Neurochemical Modulation of Sensory-Motor Reactivity: Acoustic and Tactile Startle Reflexes. *Neuroscience & Biobehavioral Reviews*. **4**: 241-263

Dawson G. R., maubach K. A., Collonson N., Cobain M., Everitt B. J., MacLeod A. M., Choudhury H. I., McDonald L. M., Pillai G., Rycroft W., Smith A. J., Sternfeld F., Tattersall F. D., Wafford K. A., Reynolds S., Seabrook G. R., Atack J. R. (2005). An Inverse Agonist Selective for α5 Subunit-Containing $GABA_A$ Receptors Enhances Cognition. *The Journal of Pharmacology and Experimental Therapeutics.* **316**: 1335-1345

DeFilipe J., Farinas I. (1992). The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic input. *Prog Neurobiol*. **39**: 563- 607

Delcomyn F. (1998). *Foundations of Neurobiology*. USA: W. H. Freeman and Company

Diggle, P.J., Heagerty, P., Liang, K.-Y., Zeger, S. (2002). *Analysis of Longitudinal Data*. Great Britain: Oxford University Press.

Dong E., Caruncho H., Liu W. S., Smalheiser N. R., Grayson D. R., Costa E., Guidotti A. (2003). The reelin-integrin interaction regulates Arc mRNA translation in synaptoneurosomes. *Proc Natl Acad Sci USA*. **100**: 5479-5484

Dorow R., Horowski R., Paschelke G., Amin M., Braestrup C. (1983). Severe anxiety induced by FG 7142, a β-carboline ligand for benzodiazepine receptors. *The Lancet*. **322**: 98-99

Enna S.J., Bowery N.G. (1997). *The GABA Receptors.* USA: Humana Press

Falkai P., Bogerts B. (1986). Cell loss in the hippocampus of schizophrenics. *Eur. Arch. Psychiatr. Neurol. Sci.* **236**: 154– 161

Freund T.F. (2003). Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. *TRENDS in Neurosciences.* **26**: 489-495

Fritschy J. M., Môhler H. (1995). $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol*. **359**: 154–194

Gabbott P. L., Bacon S. J. (1996). Local circuit neurons in the medial prefrontal cortex (areas 24a, b, c, 25 and 32) in the monkey. I. Cell morphology and morphometrics. *J Comp Neurol*. **364**: 567-608

Gao X. M., Shirakawa O., Du F., Tamminga C.A. (1993). Delayed regional metabolic actions of phencyclidine. *Eur J Pharmacol*. **241**: 7-15

Geyer M. A., Krebs-Thomson K., Braff D. L., Swerdlow N. R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology*. **156**: 117-154

Goldberg J. H., Yuste R., Tamas G. (2003). Ca2+ imaging of mouse neocortical interneurone dendrites: contribution of Ca2+- permeable AMPA and NMDA receptors to subthreshold Ca2+ dynamics. *J Physiol*. **551**:67-78

Grace A. A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. *Neurosci.* **41**: 1-24

Green M. F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *Am. J. Psychiatry*. **153**: 321-330

Grunze H. C., Rainnie D. G., Hasselmo M. E., Barkai E., Hearn E. F., McCarley R., Greene R. W. (1996). NMDA-dependent modulation of CA1 local circuit inhibition. *J Neurosci*. **16**: 2034-2043

Grunze H., Rainnie D., Hasselmo M., Barkai E., Hearn E., McCarley R., Greene R. (1996). NMDA-Dependent Modulation of CA1 Local Circuit Inhibition. The Journal of Neuroscience. 76: 2034-2043

Guidotti A., Auta J., Davis J. M., Dwivedi Y., Gerevini V. D., Impagnatiello F., Pandey G., Pesold C., Sharma R., Uzunov D. P., Costa E. (2000). Decrease in reelin and glutamic acid decarxylase67 (GAD_{67}) expression in schizophrenia and bipolar disorders: a postmortem brain study. *Arch Gen Psychiatry*. **57**: 1061-1069

Guidotti A., Auta J., Davis J.M., Dong E., Grayson D.R., Veldic M., Zhang X., Costa E. (2005). GAGAergic dysfunction in schizophrenia: new treatment strategies on the horizon. *Psychopharmacology*. **180**: 191-205

Haefely W. E., Martin J. R., Richards J. G., Schoch P. (1993). The multiplicity of actions of benzodiazepine receptor ligands. *Can J Psychiatry*. **38**: 102-108

Hamm A. O., Weike A. I., Schupp H. T. (2001). The effect of neuroleptic medication on prepulse inhibition in schizophrenia patients: current status and future issues. *Psychopharmacology*. **156**: 259-265

Hauser J., Rudolph U., Keist R., Möhler H., Feldon J., Yee B. K. (2005). Hippocampal α5 subunit-containing $GABA_A$ receptors modulate the expression of prepulse inhibition. *Molecular Psychiatry.* **10**: 201-207

Impagnatiello F., Guidotti A., Pesold C., Dwivedi Y., Caruncho H., Pisu M., Uzunov D., Smalheiser N., Davis J., Pandey G., Pappas G., Tueting P., Sharma R., Costa E. (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proc Natl Acad Sci USA.* **95**: 15718-15723

Jentsch D. J., Remond D. E., Elsworth J. D., Taylor J. R., Youngren K. D., Roth R. H. (1997). Enduring Cognitive Deficits and Cortical Dopamine Dysfunction in Monkeys After long-Term Administration of Phencyclidine. *Science*. **277**: 953-955

Jeste D.V., Lohr J.B. (1989). Hippocampal pathologic findings in schizophrenia. A morphometric study. *Arch. Gen. Psychiatry*. **46**: 1019–1024

Jones R. S., Buhl E. H. (1993). Basket-like interneurones in layer II of the entorhinal cortex exhibit powerful NMDA-mediated excitation. *Neurosci Lett*. **149**: 35-39

Kadieva M. G., Oganesyan É. T., Mutsueva S. K. (2005) Search for new drugs. Neurotoxins and medicinals for the treatment of Parkinson's disease. Part 3: Drugs indirectly influencing the dopaminergic system (a review). *Pharmaceutical Chemistry Journal*. **39**: 3-11

Keilhoff G., Becker A., Grecksch G., Wolf G., Bernstein H. G. (2004). Repeated application of ketamine to rats induces changes in the hippocampal expression of parvalbumin, neuronal nitric oxide synthase and cFOS similar to those found in human schizophrenia. *Neuroscience*. **126**: 591-598

Kim J. S., Kornhuber H.H., Schmid-Burgk W., Holzmoller B. (1980). Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neuroscience Letters*. **20**: 379-382

Laprade N., Soghomonian J. J. (1995). Differential regulation of mRNA levels encoding for the two isoforms of glutamate decarboxylase (GAD65 and GAD67) by dopamine receptors in the rat striatum. *Brain Res Mol Brain Res*. **34**: 65-74

Laroche S., Jay T. M., Thierry A. M. (1990). Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. *Neurosci lett*. **114**: 184-190

Laruelle M., Abi-Dargham A., Gil R., Kegeles L., Innis R. (1999). Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol. Psychiatry*. **46**: 56-72

Langen B., Egerland U., Bernöster K., Dost R., Unverferth K., Rundfeldt C. (2005). Characterization in Rats of the Anxiolytic Potential of ELB139 [1-(4-Chlorophenyl)-4-piperidin-1-yl-1,5-dihydro-imidazol-2-on], a New Agonist at the Benzodiazepine Binding Site of the $GABA_A$ Receptor. *The Journal of Pharmacology and Experimental Therapeutics*. **314**: 717-724

Langen B., Unverferth K., Rundfeldt C. (2006). Poster named: Effect of ELB139, a subtype selective benzodiazepine agonist, on EEG in rats. *Presented at ECNP Conference, Paris, September 2006*

Lavoie A. M., Tingey J. J., Harrison N. L., Pritchett D. B., Twyman R. E. (1997). Activation and deactivation rates of recombinant $GABA_A$ receptor channels are dependent on α-subunit isoform. *Biophys. J.* **73**: 2518-2526

Leslie C. J., Shaw D., McGabe C., Reynolds D.S., Dawson G. R. (2004). Effects of drugs that potentiate GABA on extinction of positively-reinforced operant behaviour. *Neuroscience and niobehavioral reviews*. **28**: 229-238

Levitan F. S., Schofield P. R., Burt D. R., Rhee L. M., Wisden W., Kohler M., Fujita N., Rodriguez H. F., Stephenson A., Darlinson M. G., Barnard E. A., Seeburg P. H. (1988). Structural and functional basis for $GABA_A$ receptors heterogenesity. *Nature*. **335**: 76-79

Lewis D. A., Volk D. W., Hashimoto T. (2004). Selective alterations in prefrontal cortical GABA neurotransmission in schizophrenia: a novel target fort he treatment of working memory dysfunctions. *Psychopharmacology*. **174**: 143-170

Lindefors N., Hurd Y. L., O'Connor W. T., Brene S., Persson H., Ungerstedt U. (1992). Amphetamine regulation of acetylcholine and gamma-aminobutyric acid in nucleus accumbens. *Neuroscience*. **48**: 439-48

Loup F., Weinmann O., Yonekawa Y., Aguzzi A., Weiser H. G., Fritschy J. M. (1998). A highly sensitive immunofluorescence procedure for analyzing the subcellular distribution $GABA_A$ receptor subunits in the human brain. *J Histochem Cytochem*. **46**: 1129-1139

Matsumoto M., Togashi H., Kaku A., Kanno M., Tahara K., Yoshioka M. (2005). Cortical GABAergic regulation of Dopaminergic Responses to Psychological Stress in the Rat Dorsolateral Striatum. *Synapse*. **56**: 117-121

Maubach K. (2003). $GABA_A$ Receptor Subtypes Selective Cognition Enhancers. *Current Drug Targets - CNS & Neurological Disorders*. **2**: 233-239

McGabe C., Shaw D., Atack J. R., Street L. J., Wafford K. A., Dawson G. R., Reynolds D. S., Leslie C. J. (2004). Subtype-selective GABAergic drugs facilitate extinction of mouse operant behaviour. *Neuron Pharmacology*. **46**: 171-178

Medina J. F., Repa J. C., Mauk M. D., LeDoux J. E. (2002). Parallels between cerebellum and amygdala-dependent conditioning. *Nat. Neurosci*. **3**: 122-131

Milner B., Petrides M., Smith K. L., (1985). Frontal lobes and temporal organization of memory. *Hum Neurobiol*. **4**: 137-142

Mohn A., Gainetdinov R., Caron M., Koller B. (1999). Mice with Reduced NMDA Receptor Expression Display Behaviours Related to Schizophrenia. *Cell*. **98**: 427-436

Molewijk H. E., Poel van der A. M., Mos J., Heyden van der J. A. M., Olivier B. (1995). Conditioned ultrasonic distress vocalizations in adult male rats as a behavioral paradigm for screening anti-panic drugs. *Psychopharmacology*. **117**: 32-40

Montgomery, D.C. (2005). *Design and Analysis of Experiments*. USA: John Wiley & Sons Inc.

Morris J. B., Cochran S. M., Pratt J. A. (2005). PCP: from pharmacology to modeling schizophrenia. *Current Opinion in Pharmacology*. **5**: 101-106

Morris R. G., Garrud P., Rawlins J. N. O'Keefe J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*. **297**: 681-683

Morris R. G., Pickering A., Abrahams S., Feigenbaum J.D. (1996). Space and the hippocampal formation in humans. *Brain Res Bull*. **40**: 487-490

Myers K. M., Goulet M., Rusche J., Boismenu R., Davis M. (2005). Partial Reversal of Phencyclidine-Induced Impairment of Prepulse Inhibition by Secretin. *Biol Psychiatry*. **58**: 67-73

Noga J. T., Hyde T. M., Herman M.M., Spurney C.F., Bigelow L.B., Weinberger D.R., Kleinman J.E. (1997). Glutamate receptors in the postmortem striatum of schizophrenic, suicide, and control brains. *Synapse*. **27**:168-176

Nolte J. (2002). The Human Brain. An Introduction to its Functional Anatomy. USA: Mosby, Inc.

Nusser Z., Sieghart W., Benke D., Fritschy J., Somogyi P. (1996). Differential synaptic localization of two major γ-aminobutyric acid type A receptor α subunits on hippocampal pyramidal cells. *Neurobiology*. **93**: 11939-11944

Nyíri G., Freund T. F., Somogyi P. (2001). Input-dependent synaptic targeting of α2-subunit-containing $GABA_A$ receptors in the synapses of hippocampal pyramidal cells of the rat. *Eur J Neurosci*. **13**: 428-442

Olton D., Becker J.., Handelmann G. (1979). Hippocampus, space, and memory. *Behav and Brain Sci*. **2**: 313- 365

Pierri J. N., Chaudry A. S., Woo T. U., Lewis D. A. (1999). Alterations in chandelier neuron axon terminals in the prefrontal cortex of schizophrenic subjects. *Am J Psychiatry*. **156**: 1709-1719

Pierri J. N., Volk C.., Auh S., Sampson A., Lewis D. (2003). Somal Size of Prefrontal Cortical Pyramidal Neurons in Schizophrenia: Differential Effects across Neuronal Subpopulations. *Biological Psychiatry*. **54**: 111-120

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. (2006). nlme: Linear and nonlinear mixed effects models. R package version 3.1-77.

Pinheiro, J.C., Bates, D.M. (2000). *Mixed-Effects Models in S and S-PLUS*. USA:Springer

Quirk K., Blurton P., Fletcher S., Leeson P., Tang F., Mellilo D., Ragan C. I., McKernan R. M. (1996). [3H]L-655,708, a Novel Ligand Selective for the Benzodiazepine Site of $GABA_A$ Receptors which Contain the α5 Subunit. *Neuropharmacology*. **35**: 1331–1335

R Development Core Team (2006). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rabe H., Kronbach C., Rundfeld C., Lüddens H. (2006). The novel anxiolytic ELB139 display selectivity to recombinant $GABA_A$ receptors different from diazepam. *Neuropharmacology*. XX (article in press): 1-6

Randrup A., Munkvad I. (1972). Evidence indicating an association between schizophrenia and dopaminergic hyperactivity in the brain. *Orthomolecular Psychiat*. **1**: 2-7

Rawlins J. N. P. (1985). Association across time: the hippocampus as a temporary memory store. *Behav. Brain Sci*. **8**: 479-496

Roberts E. (1972). An hypothesis suggesting that there is a defect in the GABA system in schizophrenia. *Neurosci Res Progr Bull.* **10**: 468-482

Roberts E. (1972). Prospects for research on schizophrenia: a hypothesis suggesting that there is a defect in GABA system in schizophrenia. *Neurosci . Res. Program Bull*. **10**: 468-482

Rosenkranz J. A., Grace A. A. (1999). Modulation of basolateral amygdala neuronal firing and afferent drive by dopamine receptor activation in vivo. *J. Neurosci.* **19**: 11027-39

Saint-Cyr J. A., Taylor A. E., Lang A.E. (1998). Procedural learning and neostriatal dysfunction in man. *Brain*. **111**: 941-959

Sams-Dodd F. (1997). Effect of novel antipsychotic drugs on phencyclidine-induced stereotyped behaviour and social isolation in the rat social interaction test. *Behav Pharmacol.* **8**: 196-215

Sasack S. R., Pickel V. M. (1992). Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J. Comp. Neurol.* **320**: 145-160

Schwabe K., Brosda J., Wegener N., Koch M. (2004). Clozapine enhances disruption of prepulse inhibition after sub-chronic dizocilpine- or phencyclidine-treatment in Wistar rats. *Pharmacology, Biochemistry and Behavior*. **80**: 213-219

Seeman P. (1980). Brain Dopamine Receptors. *Pharmacological Reviews.* **32**: 229-313

Seeman P., Lee T., Chau-Wong M., Wong K. (1976). Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature. **261**: 717-719

Shulman Y., Tibbo P. (2005). GABAergic Deficits in Schizophrenia: Evidence and Implications. *University of Alberta Health Sciences Journal*. **2**: 23-27

Simpson M. D., Slater P., Deakin J. F., Royston M. C., Skan W. J. (1989). Reduced GABA uptake sites in the temporal lobe in schizophrenia. Neurosci Lett. 107: 211-5.

Sokolov B.P. (1998). Expression of NMDAR1, GluR1, GluR7, and KA1 glutamate receptor mRNAs is decreased in frontal cortex of neuroleptic-free schizophrenics: evidence on reversible up-regulation by typical neuroleptics. *J Neurochem*. **71**:2454-2464

Somogyi P., Cowey A. (1981). Combined Golgi and electron microscopic study on the synapses formed by double bouquet cells in the visual cortex of the cat and monkey. *J Comp Neurol*. **195**: 547-566

Squire L. R., Zola-Morgan S. (1991). The medial temporal lobe memory system. *Science*. **253**: 1380-1386

Sternfeld F., Carling R. W., Jelley R. A., Ladduwahetty T., Merchant K. J., Moore K. W., Reeve A. J., Street L. J., O'Connor D., Sohal B., Atack J. R., Cook S., Seabrook G., Wafford K., Tattersall F. D., Collinson N., Dawson G. R., Castro J. L., MacLeod A. M. (2004). Selective, Orally Active γ-Aminobutyric $Acid_A$ α5 Receptor Inverse Agonists as Cognition Enhancers. *J. Med. Chem.* **47***:* 2176-2179

Tenn C. C., Kapur S., Fletcher P. J. (2005). Sensitization to amphetamine, but not phencyclidine, disrupts prepulse inhibition and latent inhibition. *Psychopharmacology*. **180**: 366-376

Thompson R.F., Krupa D. J. (1994). Organization of memory traces in the mammalian brain. *Annu Rev Neurosci*. **17**: 519-549

Tsai G., Coyle J. T. (2002). Glutamatergic Mechanisms in Schizophrenia. *Annu. Rev. Pharmacol. Toxicol.* **42**: 165-79

Tsai G., Passani L. A., Slusher B. S., Carter R., Baer L., Kleinman J. E., Coyle J. T. (1995). Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Arch. Gen. Psychiatry*. **52**: 829-36

Vanderschuren L., Kalivas P. (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology*. **151**: 99–120

Verney C., Alvarez C., Gerrard M., Berger B. (1990). Ultrastructural double-labelling study of dopamine terminals and GABA-containing neurons in rats anteromedial cortex. *Eur J Neurosci*. **2**: 960-972

Vincent S. L., Khan Y., Benes F. M. (1993). Cellular distribution of dopamine D1 and D2 receptors in rat medial prefrontal cortex. *J Neurosci*. **13**: 2551-2564

Vincent S. L., Khan Y., Benes F. M. (1995). Cellular colocalization of dopamine D1 and D2 receptors in rat medial prefrontal cortex. *Synapse*. **19**: 112-120

Volk D. W., Pierri J. N., Fritschy J., Auh S., Sampson A. R., Lewis D. A. (2002). Reciprocal Alterations in Pre- and Postsynaptic inhibitory Markers at Chandelier Cell Inputs to Pyramidal Neurons in Schizophrenia. *Cerebral Cortex*. **12**: 1063-1070

Volk D., Austin M., Pierri J., Sampson A., Lewis D. (2001). GABA transporter-1 mRNA in the prefrontal cortex in schizophrenia: decreased expression in a subset of neurons. *Am J Psychiatry.* **158**: 256-65

Wallenstein G. V., Eichenbaum H., Hasselmo M. E. (1998). The hippocampus as an associator of discontiguous events. *Trends Neurosci*. **21**: 317–323

Wasserman E. A., Miller R. R. (1997). What's elementary about associative learning? *Annu. Rev. Psychol*. **48**: 573–607

Weeber E. J., Beffert U., Jones C., Christian J. M., Forster E., Sweatt J. D., Herz J. (2002). Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J Biol Chem*. **277**: 39944-39952

Wenzel J., Kammerer E., Kirsche W., Matthies H., Wenzel M. (1980). Electron microscopic and morphometric studies on synaptic plasticity in the hippocampus of the rat following conditioning. *J. Hirnforsch*. **21**: 647-654

Wolkowitz D. M., Pickar D. (1991). Benzodiazepines in the treatment of schizophrenia: a review and reappraisal. *Am J Psychiatry*. **148**: 714-726

Yee B. K., Hauser J., Dolgov V. V., Keist R., Möhler H., Rudolph U., Feldon J. (2004). $GABA_A$ receptors containing the α5 subunit mediate the trace effect in aversive and appetitive conditioning and extinction of conditioned fear. *European Journal of Neuroscience*. **20**: 1928-1936

Yee B. K., Keist R., Boehmer L., Studer R., Benke D., Hagenbuch N., Dong Y., Malenka R. C., Fritschy J. M., Bluethmann H., Feldon J., Möhler H., Rudolph U. (2005). A schizophrenia-related sensorimotor deficit links α3-containing $GABA_A$ receptors to a dopamine hyperfunction. *The National Academy of Sciences of the USA.* **102**: 17154–17159

Zhang Z. J., Reynolds G. P. (2002). A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophr Res.* **55**: 1-10.

Statistical analysis of $GABA_A$ receptor modulators' effects in rats with focus on memory improvement and reversing of schizophrenic symptoms

Appendix

Line Sørensen and Merete Kjær Hansen

Appendix 3.1.

Reference nr.	Model	Strain	Drug	mg/kg	Adm. rute	Pretreatm. time	Vehicle	Arrive NS	Weight (g)	Animal nr.	Nr. pr. cages at the stable
A1	FC	SPRD male rat	Alprazolam	0.4, 1.33, 4	i.p.	30 min	Cremophor 5 %	30.08	230-266	40	5
A2	FC	SPRD male rat	α5IA-II	1, 3, 10	i.p.	30 min	Tween 5 %	09.08	225-265	64	5
A3	FC	SPRD male rat	NS.A	0.3, 1, 3	i.p.	30 min	Tween 5 %	04.10	225-255	40	4
B1	FC-tone ex.	SPRD male rat	Alprazolam	0.4, 1.33, 4	i.p.	30 min	Cremophor 5 %	08.11	225-256	40	4
B2	FC-tone ex.	SPRD male rat	α5IA-II	1, 3, 10	i.p.	30 min	Tween 5 %	18.10	210-250	40	4
B3	FC-tone ex.	SPRD male rat	NS.A	0.3, 1, 3	i.p.	30 min	Tween 5 %	01.11	230-255	40	4
C	FC-trace	SPRD male rat	α5IA-II	1, 3	i.p.	30 min	Tween 5 %	22.11	210-248	40	4
D1	DR-PPI	NMRI, female, mouse	Methylpheni date	5, 10, 20	s.c.	15 min	NaCl 0.9 %	?	25-30	32	8
D2	DR-PPI	SPRD male rat	Methylpheni date	5, 10, 20	s.c.	15 min	NaCl 0.9 %	30.08	280-330	24	5
E1	DR-PPI	SPRD male rat	PCP	1.25, 2.5, 5	s.c.	72 h	NaCl 0.9 %	13.09	210-245	32	5
E2	DR-PPI	SPRD male rat	PCP	1.25, 2.5, 5	s.c.	24 h	NaCl 0.9 %	20.09	230-265	32	5
E3	DR-PPI	SPRD male rat	PCP	1.25, 2.5, 5	s.c.	10 min	NaCl 0.9 %	20.09	(200) 220-260	32	5
F1	PPI pcp-deficit	SPRD male rat	Alprazolam	0.4, 1.33, 4	i.p.	30 min	Cremophor 5 %	25.10	220-260	64	4
F2	PPI pcp-deficit	SPRD male rat	α5IA-II	1, 3, 10	i.p.	30 min	Tween 5 %	27.09	240-290	64 (48)	5
F3	PPI pcp-deficit	SPRD male rat	NS.A	0.3, 1, 3	i.p.	30 min	Tween 5 %	18.10 & 25	210-280	14 & 50	4
G	PPI amp-deficit	SPRD male rat	NS.A	0.3, 1, 3	i.p.	30 min	Tween 5 %	22.11	176-210	64	4
H	DR pcp-deficit	SPRD male rat	α5IA-II	30	i.p.	30 min	Tween 5 %	18.10	222-261	10	4

Reference nr.	Habi. to lab.	Lab	Nr. pr. cage i	Date for expe	Groups
A1	04.09	BS.18	4	05.09-14.09	veh, veh+e.shock, 0.4+e.shock, 1.33+e.shock, 4+e.shock
A2	10.08	BS.18	2	14.08-23.08	veh+e.shock, veh, 1, 1+e.shock, 3, 3+e.shock, 10, 10+e.shock
A3	06.10	BS.18	4	10.10-19.10	veh, veh+e.shock, 0.3+e.shock, 1+e.shock, 3+e.shock
B1	10.11	BS.18	4	13.11-16.11	veh, veh+e.shock, 0.4+e.shock, 1.33+e.shock, 4+e.shock
B2	20.10	BS.18	4	24.10-27.10	veh+e.shock, veh, 1+e.shock, 3+e.shock, 10+e.shock
B3	03.11	BS.18	4	07.11-10.11	veh, veh+e.shock, 0.3+e.shock, 1+e.shock, 3+e.shock
C	24.11	BS.18	4	28.11-01.12	veh(nTFC), veh+e.shock(nTFC), veh+e.shock(TFC), 1+e.shock(TFC), 3+e.shock(TFC)
D1	31.08	BS.18	8	01.09	veh, 5, 10, 20
D2	14.09	BS.18	4	15.09	veh, 5, 10, 20
E1	15.09	BS.18	4	18.09-21.09	veh, 1.25, 2.5, 5
E2	26.09	BS.18	4	27.09-28.09	veh, 1.25, 2.5, 5
E3	28.09	BS.18	4	28.09-29.09	veh, 1.25, 2.5, 5
F1	31.10	BS.18	4	01.11	veh, 0.4, 1.33, 4, veh+pcp, 0.4+pcp, 1.33+pcp, 4+pcp
F2	04.10	BS.18	4	05.10-06.10	veh, 1, 3, 10, veh+pcp, 1+pcp, 3+pcp, 10+pcp
F3	27.10	BS.18	4	30.10	veh, 0.3, 1, 3, veh+pcp, 0.3+pcp, 1+pcp, 3+pcp
G	23.11	BS.21/18	4	24.11	veh, 0.3, 1, 3, veh+amp, 0.3+amp, 1+amp, 3+amp
H	26.10	BS.11	2	27.10	veh, veh+pcp, 30, 30+pcp

Appendix 3.1.

Appendix 3.2.

Fear conditioning, alprazolam (A1)

2006-08-28 40 SPDR rats reach NeuroSearch and get stabled in the animal stable, five rats per cage

2006-09-04 The rats are placed in laboratory BS.18
The rats are rearranged in laboratory BS.18 and placed four rats per cage

2006-09-05 The animals are handled in one minute and weighted and marked
The Startle boxes are calibrated to 150, ”Soerens test, 110dB”

2006-09-06 Habituation of the animals, executed as described in the experimental instruction (Line)

2006-09-07 The solutions are made. Alprazolam 0.4, 1.33, 4, i.p - 2 ml/kg in 5% cremophor

The conditioning experiment is executed with 30 minutes pretreatment time
The cages are changed

2006-09-08 The test experiment is executed

2006-09-14 The re-test experiment is executed, a repetition of the test experiment
The animals are destroyed by CO_2

Observations:
The rats which where injected with alprazolam became drowsy and lay prostrate. The rats which were injected with 4 mg/kg were completely flaccid and had their hind legs stretched behind and lay prostrate. Their hind part of the body did not move at all

Appendix 3.3.

Fear conditioning, α5IA-II (A2)

2006-08-09	64 SPDR rats reach NeuroSearch and get stabled in the animal stable, five rats per cage
2006-08-10	The rats are rearranged in the animal stable and placed two rats per cage
2006-08-11	The rats are placed in laboratory BS.18
2006-08-14	The animals are handled in one minute and weighted and marked. Following they are placed in clean cages The Startle boxes are calibrated to 150, ”Soerens test, 110dB”
2006-08-15	Habituation of the animals, executed as described in the experimental instruction
2006-08-16	The solutions are made. α5IA-II 1, 3, 10 mg/kg, i.p - 2 ml/kg in Tween 5% The conditioning experiment are executed with 30 minutes pretreatment time
2006-08-17	The test experiment is executed. The cages are changed
2006-08-23	The re-test experiment is executed, a repetition of the test experiment The animals are destroyed by CO_2

Appendix 3.4.

Fear conditioning, NS.A (A3)

2006-10-04	40 SPDR rats reach NeuroSearch and get stabled in the animal stable, four rats per cage
2006-10-06	The rats are placed in laboratory BS.18, four per cage
2006-10-10	The Startle boxes are calibrated to 150, ”Soerens test, 110dB” The animals are handled in one minute and weighted and marked. Following they are placed in clean cages
2006-10-11	Habituation of the animals, executes as described in the experimental instruction (Merete)
2006-10-12	The solutions are made. NS.A 0.3, 1, 3, i.p - 2 ml/kg in tween 5% The conditioning experiment is executed with 30 minutes pretreatment time
2006-10-13	The test experiment is executed (Line) The cages are changed
2006-10-19	The re-test experiment is executed (Merete), a repetition of the test experiment The animals are destroyed by CO_2

Observations:
Conditioning day: The rats at dose b and c were very drowsy and easy to place in the startle cages. There was not notable more urine and feces in the feces trays of the rats which received electric shock compared to the rats that did not received electric shock (In experiments with other drugs it has been observed that the electric shock causes elevated urination and amount of feces).

Test day: After the conditioning experiment the rats 33-36 by mistake were placed in the cage that had housed the rats 37-40, whereas the rats 37-40 had been placed in the cage that has housed the rats 33-36. These eight rats had consequently stayed over night in non-familiar cages before the test experiment

Appendix 3.5.

Extended-tone fear conditioning, alprazolam (B1)

2006-11-08 40 SPDR rats reach NeuroSearch and get stabled in the animal stable, four rats per cage

2006-11-10 The rats are placed in laboratory BS.18

2006-11-13 The animals are handled in one minute and weighted and marked (Merete)
The Startle boxes are calibrated to 150, ”Soerens test, 110dB”

2006-11-14 Habituation of the animals, executed as described in the experimental instruction (Line)

2006-11-15 The solutions are made. Alprazolam 0.4, 1.33, 4 mg/kg, i.p - 2 ml/kg in Cremophor 5%

The conditioning experiment is executed with 30 minutes pretreatment time

2006-11-16 The test experiment is executed with the use of the tone extended program” FC test 2006-10 Tone ext twice” (Line)
The animals are destroyed by CO_2

Observations:
Conditioning day: The rats which where injected with alprazolam became very drowsy and their hind part of the body looked paralyzed, this was as well observed for the rats administered the lowest dose of alprazolam (0.4 mg/kg). The rats lay prostrate, had their hind legs stretched behind, rolling over and laid on the side with their hind part of the body dragged

Appendix 3.6.

Extended-tone fear conditioning, α5IA-II (B2)

2006-10-18 40 SPDR rats reach NeuroSearch and get stabled in the animal stable, four rats per cage

2006-10-20 The rats are placed in laboratory BS.18

2006-10-24 The animals are handled in one minute and weighted and marked (Line)
The Startle boxes are calibrated to 150, ”Soerens test, 110dB”

2006-10-25 Habituation of the animals, executes as described in the experimental instruction (Merete)

2006-10-26 The solutions are made. α5IA-II 1, 3, 10 mg/kg, i.p - 2 ml/kg i Tween 5%

The conditioning experiment is executes with 30 minutes pretreatment time

2006-10-27 The test experiment is executed with the use of the tone-extended program” FC test 2006-10 Tone ext twice”
The animals are destroyed by CO_2

Observations:
Conditioning day: All the doses made the rats drowsy, they screw up their eyes and lay prostrate

Appendix 3.7.

Extended-tone fear conditioning, NS.A (B3)

2006-11-01 40 SPDR rats reach NeuroSearch and get stabled in the animal stable, four rats per cage

2006-11-03 The rats are placed in laboratory BS.18

2006-11-07 The animals are handled in one minute and weighted and marked (Line)
The Startle boxes are calibrated to 150, ”Soerens test, 110dB”

2006-11-08 Habituation of the animals, executed as described in the experimental instruction (Merete)

2006-11-09 The solutions are made. NS.A 0.3, 1, 3 mg/kg, i.p - 2 ml/kg in Tween 5%

The conditioning experiment is executes with 30 minutes pretreatment time

2006-11-10 The test experiment is executed with the use of the tone-extended program” FC test 2006-10 Tone ext twice”
The animals are destroyed by CO_2

Observations:
Conditioning day: No diarrhoea and nearly no urine and feces in the feces trays. The rats were fearless; some of them jump from their open cages on the table and down to the floor. Sometimes the rats froze in certain positions

Appendix 3.8.

Trace Fear Conditioning (C)

2006-11-22 64 SPDR rats reach NeuroSearch and get stabled in the animal stable, four rats per cage

2006-11-24 The rats are placed in laboratory BS.18

2006-11-28 The animals are handled in one minute and weighted and marked
The Startle boxes are calibrated to 150, ”Soerens test, 110dB” (Merete)

2006-11-29 Habituation of the animals, executes as described in the experimental instruction. Use the program “FC Habituation 45 dB MKH” (Line)

2006-11-30 The solutions are made. α5IA-II 1 and 3 mg/kg, i.p - 2 ml/kg in Tween 5%

The conditioning experiment is executes with 30 minutes pretreatment time

Use the programs “TFC tone shock no trace KBT” and ”TFC tone shock 60 sec trace KBT”

2006-12-01 The test experiment is executed with the use of the program ”TFC LIS MKH”
The animals are destroyed by CO_2

Appendix 3.9.

Pre-pulse inhibition, dose-response, methylphenidate, mouse (D1)

2006-08-31 32 NMRI female mice are placed in laboratory BS.23, eight per cage

2006-09-01 The methylphenidate-HCl solutions are made. 5, 10, 20 mg/kg, s.c. in scruff of the neck - 10 ml/kg in NaCl 0,9%

The Startle boxes are calibrated to 150, ”Soerens test, 110dB”
The mice are placed in laboratory BS.18 and the pre-pulse experiment is executed

The animals are destroyed by CO_2

Observations:
The mice which were injected with the highest doses (10 and 20 mg/kg) were tremendously more active and run around in their cages. Some of the mice at the highest dose (20 mg/kg) displayed in addition stereotype behavior: exaggerated licking of their nose and they caught hold of the startle cages with their teeths.

Jesper injected the mice which were administered methylphenidate and Merete and Line injected the vehicle mice.

Appendix 3.10.

Pre-pulse inhibition, dose-response, methylphenidate, rat (D2)

2006-09-14 24 SPRD rats are placed in laboratory BS.18, four per cage

2006-09-15 The methylphenidate-HCl solutions are made. according to the dilution calculations made in the excel sheet ”Fortyndingsskema 1509” 5, 10, 20 mg/kg, s.c. in the scruff of the neck - 1 ml/kg i NaCl 0,9%

The Startle boxes are calibrated to 150, ”Soerens test, 110dB”
The pre-pulse inhibition experiment is executed

The animals are destroyed by CO_2

Observations:
The rats which were injected with the highest dose (20mg/kg): stereotype sniffing while they were standing on the hind legs. The rats that were injected with 10 and 20 mg/kg were all hyperactive and difficult to place into the startlecages

Appendix 3.11.

Pre-pulse inhibition, dose-response, 72 h (E1)

2006-09-13	32 SPDR rats reach NeuroSearch and get stabled in the animal stable, five rats per cage
2006-09-15	The rats are placed in laboratory BS.18 The rats are rearranged in laboratory BS.18 and placed four rats per cage
2006-09-18	The solutions are made. 1.25, 2.5, 5 mg/kg s.c in the scruff of the neck - 1 ml/kg in 0,9% NaCl The animals are weighted and marked The animals were injected chronologically from number 1 to 32 in the period between 10 and 11 a.m.
2006-09-19	The Startle boxes are calibrated to 150, ”Soerens test, 110dB”
2006-09-21	The pre-pulse inhibition experiment is executed. NaCl 0.9 % is administered 10 minutes prior to the pre-pulse inhibition experiment. Immediately after the pre-pulse inhibition experiment the rats´ brains are removed and placed on ice by Helle Hvorup Knudsen

Appendix 3.12.

Pre-pulse inhibition, dose-response, 24 h (E2)

2006-09-20 32 SPDR rats reach NeuroSearch and get stabled in the animal stable, five rats per cage

2006-09-26 The rats are placed in laboratory BS.18
The rats are rearranged in laboratory BS.18 and placed four rats per cage

2006-09-27 The solutions are made. 1.25, 2.5, 5 mg/kg, s.c in the scruff of the neck - 1 ml/kg in 0.9% NaCl
The animals are weighted and marked
The animals were injected chronologically from number 1 to 32 in the period between 10:30 and 11:30 a.m.
The Startle boxes are calibrated to 150, ”Soerens test, 110dB”

2006-09-28 The pre-pulse inhibition experiment is executed. NaCl 0.9 % are administered 10 minutes prior to the pre-pulse inhibition experiment.

Immediately after the pre-pulse inhibition experiment the rats´ brains are removed and placed on ice by Rigmor Jensen

Observations:
2006-09-27: The rats which were injected with the highest dose displayed stereotype behaviour and moved the front part of their bodies and their heads from side to side

Appendix 3.13.

Pre-pulse inhibition, dose-response, 10 min (E3)

2006-09-20 32 SPDR rats reach NeuroSearch and get stabled in the animal stable, five rats per cage

2006-09-28 The rats are placed in laboratory BS.18
The rats are rearranged in laboratory BS.18 and placed four rats per cage

The animals are weighted and marked

2006-09-29 The solutions made the 27th of September were used.
The pre-pulse inhibition experiment is executed. The rats are injected according to the dose group they belong to and 10 minutes prior to the pre-pulse inhibition experiment

Immediately after the pre-pulse inhibition experiment the rats´ brains are removed and placed on ice by Britta Karlsson

Observations:
2006-09-28: Rat number seven were very small (200 g), dehydrated, stressed (having blood around its eyes) and it has a bristly fur

Appendix 3.14.

Pre-pulse inhibition, PCP deficit, alprazolam (F1)

2006-10-25 64 SPDR rats reach NeuroSearch and get stabled in the animal stable, four rats per cage

2006-10-31 The rats are placed in laboratory BS.18 (Four of the cages are placed outside the Scantainer without filter paper)

2006-11-01 The Startle boxes are calibrated to 150, ”Soerens test, 110dB”
The animals are weighted and marked
The solutions are made. PCP 2.5 mg/kg, s.c in the scruff of the neck - 1 ml/kg in 0.9% NaCl and alprazolam 0.4, 1.33, 4 mg/kg, i.p – 2 ml/kg in 5% Cremophor
The pre-pulse inhibition experiment is executed. The rats are injected according to the dose group they belong to. Alprazolam were injected 30 minutes prior to the experiment and PCP 10 minutes prior to the experiment

The animals are destroyed by CO_2

Observations:
The PCP-rats displayed stereotyped behaviour. They turned around them selves and moved the front part of their body from side to side. The rats injected with alprazolam became very drowsy. This was especially noticeable for the rats injected with the highest dose. They nearly did not move and the hole of their body was very flaccid. Likewise the PCP+alprazolam rats were stereotype and drowsy. Alprazolam neutralised not the PCP provoked stereotypic behaviour, but the stereotype behaviour of the rats became slower and drowsier – they rolled their body around. In addition the hairs on their throat were missing and there were hair in the feces trays after the experiment.

Appendix 3.15.

Pre-pulse inhibition, PCP deficit, α5IA-II (F2)

2006-09-27 64 SPDR rats reach NeuroSearch and get stabled in the animal stable, five rats per cage

2006-10-03 The Startle boxes are calibrated to 150, ”Soerens test, 110dB”

2006-10-04 The rats are rearranged in the animal stable and placed four rats per cage
The rats are placed in laboratory BS.18 (Four of the cages are placed outside the Scantainer without filter paper)

2006-10-05 The animals are weighted and marked
The solutions are made. PCP 2.5 mg/kg, s.c i nakken - 1 ml/kg in 0.9% NaCl and α5IA-II1, 3, 10 mg/kg, i.p – 2 ml/kg in 5% tween
The pre-pulse inhibition experiment is executed. The rats are injected according to the dose group they belong to. α5IA-II were injected 30 minutes prior to the experiment and PCP 10 minutes prior to the experiment

POWER CUT: The rats numbered 29 to 44 were exclude from the experiment

The rats 1-28 are destroyed by CO_2

2006-10-06 The pre-pulse inhibition experiment was continued with the rats 45 to 64. The remainder α5IA-II and PCP made the day before were used. The solutions had been wrapped in tinfoil and kept in the refrigerator over night
The rats 29-64 are destroyed by CO_2

Observations:
The PCP-rats displayed stereotype behaviour. They turned around them selves and moved the front part of their bodies from side to side

Appendix 3.16.

Pre-pulse inhibition, PCP deficit, NS.A (F3)

2006-10-18 14 SPDR rats reach NeuroSearch and get stabled in the animal stable, four rats per cage (two rats in the last cage)

2006-10-25 50 SPDR rats reach NeuroSearch and get stabled in the animal stable, five rats per cage (two rats in the last cage)

2006-10-27 All of the rats are placed in laboratory BS.18 (Four of the cages are placed outside the Scantainer without filter paper)

2006-10-30 The Startle boxes are calibrated to 150, ”Soerens test, 110dB”
The animals are weighted and marked
The solutions are made. PCP 2.5 mg/kg, s.c in nakken - 1 ml/kg in 0.9% NaCl and NS.A 0.3, 1, 3 mg/kg, i.p – 2 ml/kg in 5% tween
The pre-pulse inhibition experiment is executed. The rats are injected according to the dose group they belong. NS.A were injected 30 minutes prior to the experiment and PCP 10 minutes prior to the experiment

The animals are destroyed by CO_2

Observations:
The PCP-rats displayed stereotype behaviour. They turned around them selves and moved the front part of their bodies from side to side

Appendix 3.17.

Pre-pulse inhibition, amphetamine deficit, NS.A (G)

2006-11-22 64 SPDR rats reach NeuroSearch and get stabled in the animal stable, four rats per cage

2006-11-23 The rats are placed in laboratory BS.21 (Four of the cages are placed outside the Scantainer without filter paper)

2006-11-24 The rats are moved to laboratory BS.18 (Four of the cages are placed outside the Scantainer without filter paper)
The Startle boxes are calibrated to 150, ”Soerens test, 110dB”
The animals are weighted and marked
The solutions are made. Amphetamine 4 mg/kg, s.c i nakken - 1 ml/kg in 0.9% NaC and NS.A 0.3, 1, 3 mg/kg, i.p – 2 ml/kg in 5% tween
The pre-pulse inhibition experiment is executed. The rats are injected according to the dose group they belong to. Amphetamine and NS.A were injected 30 minutes prior to the experiment. The rats were injected s.c and then turned around and injected i.p. and then placed back in their cage

The animals are destroyed by CO_2

Observations:
Amphetamine and NS.A: Slow movements, elevated feces and urine in the feces trays after the experiment, bristly fur, laid prostrate and on their side, had their forepaws placed under their chests, very vigorous, ran around, vigorous sniffing, stereotyped sniffing on their hind legs and overturned down from their standing position on the hind legs and landed on the side, slow movements (maybe there was a tendency that the rats administered the highest dose of NS.A were having the slowest movement).
Taken together it seemed like the rats either were drowsy and laid down or they were very energic and jumped around when they were frightened (maybe NS.A neutralize the energic behaviour induced by amphetamine and causes the more slow movements)

Amphetamine alone: bristly fur, nervous jumps, ran around with their legs stretched and their body raised from the ground, no urine or feces in the feces trays after the experiment, stiffening around the cage, energic, a lot of the time they stand on their hind legs

NS.A alone: elevated drinking (three rats tried to drink from one water tap at the same time), elevated feces and urine in the feces trays after the experiment

In general: The rats jumped very much, jumped around when they where handled before and after the pre-pulse inhibition experiment

Appendix 3.18.

Dose-response, PCP deficit, α5IA-II (H)

2006-10-26 10 SPDR rats are placed in laboratory BS.11, two per cages (The cages are not placed in a Scantainer, but placed on a table with filter paper)

2006-10-27 The animals are weighted and marked

The solutions are made. PCP 2.5 mg/kg, s.c in the scruff of the neck - 1 ml/kg in 0.9% NaCl and α5IA-II 10 and 30 mg/kg, i.p – 2 ml/kg in 5% tween

α5IA-II was administered 30 minutes prior to the observation start and PCP 10 minutes prior to the start of the observations.
The animals were observed by Line Sørensen, Karin Sandager Nielsen and Naheed Mirza

The animals are destroyed by CO_2.

Groups
1: Vehicle (ip) + PCP
2: Vehicle (ip) + PCP
3: α5IA-II (30 mg/kg) + PCP
4: α5IA-II (30 mg/kg) + PCP
5: α5IA-II (30 mg/kg) + Vehicle (sc)
6: α5IA-II (30 mg/kg) + Vehicle (sc)
7: α5IA-II (30 mg/kg) + PCP
8: α5IA-II (30 mg/kg) + Vehicle (sc)
9: Vehicle (ip) + PCP
10: Vehicle (ip) + Vehicle (sc)

Observations:
1: Stereotype behaviour; rolling around, turns on to the side of the body, move the head from side to side
2: Normal behaviour (PCP was administered wrongly)
3: Did not move, drowsy, lie drowsy and on the side of the body, had the eyes closed (PCP was administered wrongly)
4: Stereotype: walks around in a circle, rolling the front part of the body from side to side, open eyes
5: Did not move, lay prostrate, half closed eyes
6: Half closed eyes
7: Stereotype: rolling, turns around it selves, walks around in a circle, open eyes
8: Lay totally still on the side of the body, nearly closed eyes
9: Stereotype behaviour, walk around it selves
10: Normal behaviour

Conclusion:
α5IA-II do not neutralize the PCP-behaviour at all

Appendix 3.19.

Fear conditioning, alprazolam (A1)

Date/init:	20060905-0914/MKH LIS	Drug:	alprazolam in 5% cremophor
Strain:	Male SPRD	Doses:	a=0,4, b=1,33, c=4 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Habituation	Conditioning	Testing
1	veh	0,50	1	249			
2	veh + e.sti	0,51	2	253			
3	a + e.sti	0,49	3	243			
4	b + e.sti	0,49	4	247			
5	c + e.sti	0,53	5	265			
6	veh	0,47	6	237			
7	veh + e.sti	0,51	7	254			
8	a + e.sti	0,52	8	260			
9	b + e.sti	0,50	1	250			
10	c + e.sti	0,48	2	241			
11	veh	0,49	3	244			
12	veh + e.sti	0,51	4	255			
13	a + e.sti	0,46	5	230			
14	b + e.sti	0,53	6	266			
15	c + e.sti	0,51	7	253		Very influenced, lying on the stomach,hind legs stretched behind	
16	veh	0,51	8	256			
17	veh + e.sti	0,52	1	258			
18	a + e.sti	0,52	2	260			
19	b + e.sti	0,49	3	246			
20	c + e.sti	0,50	4	248			
21	veh	0,52	5	258			
22	veh + e.sti	0,48	6	241			
23	a + e.sti	0,48	7	242			
24	b + e.sti	0,48	8	239			
25	c + e.sti	0,50	1	249			
26	veh	0,49	2	243			
27	veh + e.sti	0,49	3	247			
28	a + e.sti	0,50	4	250			
29	b + e.sti	0,50	5	252			
30	c + e.sti	0,51	6	254			
31	veh	0,49	7	244			
32	veh + e.sti	0,50	8	250			
33	a + e.sti	0,52	1	258			
34	b + e.sti	0,51	2	254			
35	c + e.sti	0,50	3	251			
36	veh	0,48	4	240			
37	veh + e.sti	0,47	5	236			
38	a + e.sti	0,49	6	245			
39	b + e.sti	0,49	7	247			
40	c + e.sti	0,50	8	252			

Appendix 3.20.

Fear conditioning, α5IA-II (A2)

Date/init:	20060814-0823/MKH LIS	Drug:	alpha5IA-II in tween 5 %
Strain:	Male SPRD	Doses:	1, 3, 10 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Habituation	Conditioning	Testing
1	veh + e.sti	0,48	1	240			
2	veh	0,48	2	241			
3	1+ e.sti	0,47	3	235			
4	1	0,47	4	234			
5	3 + e.sti	0,53	5	263			
6	3	0,47	6	236			
7	10+ e.sti	0,52	7	262			
8	10	0,45	8	226			
9	10	0,48	1	240			
10	veh + e.sti	0,48	2	240			
11	veh	0,48	3	240			
12	1+ e.sti	0,47	4	236			
13	1	0,47	5	234			
14	3 + e.sti	0,50	6	250			
15	3	0,51	7	256			
16	10+ e.sti	0,47	8	237			
17	10+ e.sti	0,48	1	240			
18	10	0,51	2	257			
19	veh + e.sti	0,50	3	251			
20	veh	0,49	4	246			
21	1+ e.sti	0,49	5	246			
22	1	0,50	6	250			
23	3 + e.sti	0,49	7	243			
24	3	0,48	8	240			
25	3	0,48	1	239			
26	10+ e.sti	0,49	2	243			
27	10	0,52	3	259			
28	veh + e.sti	0,48	4	238			
29	veh	0,48	5	241			
30	1+ e.sti	0,47	6	237			
31	1	0,48	7	242			
32	3 + e.sti	0,51	8	256			
33	3 + e.sti	0,45	1	227			
34	3	0,49	2	243			
35	10+ e.sti	0,48	3	242			
36	10	0,48	4	242			
37	veh + e.sti	0,48	5	238			
38	veh	0,46	6	231			
39	1+ e.sti	0,51	7	256			
40	1	0,51	8	254			

To be continued

Appendix 3.20.

x 41	1	0,50	1	248			
x 42	3 + e.sti	0,50	2	249			
x 43	3	0,50	3	249			
x 44	10+ e.sti	0,47	4	237			
x 45	10	0,46	5	230			
x 46	veh + e.sti	0,48	6	242			
x 47	veh	0,48	7	241			
x 48	1+ e.sti	0,48	8	240			
x 49	1+ e.sti	0,49	1	246			
x 50	1	0,50	2	252			
x 51	3 + e.sti	0,50	3	251			
x 52	3	0,47	4	234			
x 53	10+ e.sti	0,50	5	249			
x 54	10	0,50	6	248			
x 55	veh + e.sti	0,50	7	250			
x 56	veh	0,46	8	232			
x 57	veh	0,48	1	240			
x 58	1+ e.sti	0,46	2	232			
x 59	1	0,50	3	249			
x 60	3 + e.sti	0,50	4	250			
x 61	3	0,51	5	253			
x 62	10+ e.sti	0,50	6	250			
x 63	10	0,48	7	240			
x 64	veh + e.sti	0,48	8	240			

Appendix 3.21.

Fear conditioning, NS.A (A3)

Date/init:	20061010-1019/LIS MKH	Drug:	NS.A in tween 5%
Strain:	Male SPRD	Doses:	a=0,3, b=1, c=3 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Habituation	Conditioning	Testing
1	veh	0,48	1	238			
2	veh + e.sti	0,48	2	240			
3	a + e.sti	0,46	3	231			
4	b + e.sti	0,48	4	238			
5	c + e.sti	0,48	5	238			
6	veh	0,45	6	227			
7	veh + e.sti	0,47	7	235			
8	a + e.sti	0,48	8	240			
9	b + e.sti	0,51	1	255			
10	c + e.sti	0,47	2	233			
11	veh	0,48	3	240			
12	veh + e.sti	0,47	4	235			
13	a + e.sti	0,46	5	228			
14	b + e.sti	0,48	6	240			
15	c + e.sti	0,47	7	233			
16	veh	0,48	8	241			
17	veh + e.sti	0,47	1	237			
18	a + e.sti	0,50	2	252			
19	b + e.sti	0,49	3	245			
20	c + e.sti	0,47	4	235			
21	veh	0,47	5	237			
22	veh + e.sti	0,47	6	233			
23	a + e.sti	0,45	7	227			
24	b + e.sti	0,46	8	232		No data registered	
25	c + e.sti	0,49	1	243			
26	veh	0,47	2	237			
27	veh + e.sti	0,49	3	245			
28	a + e.sti	0,48	4	240			
29	b + e.sti	0,46	5	230			
30	c + e.sti	0,48	6	242			
31	veh	0,48	7	242			
32	veh + e.sti	0,46	8	232			
33	a + e.sti	0,50	1	248			
34	b + e.sti	0,48	2	242			
35	c + e.sti	0,49	3	246			
36	veh	0,50	4	250			
37	veh + e.sti	0,46	5	228			
38	a + e.sti	0,49	6	243			
39	b + e.sti	0,49	7	244			
40	c + e.sti	0,48	8	240			

Appendix 3.22.

Extended-tone fear conditioning, alprazolam (B1)

Date/init:	20061113-16/MKH LIS	Drug:	alprazolam in 5% cremophor
Strain:	Male SPRD	Doses:	a=0,4, b=1,33, c=4 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Habituation	Conditioning	Testing
1	veh	0,50	1	248			
2	veh + e.sti	0,49	2	243			
3	a + e.sti	0,51	3	254			
4	b + e.sti	0,48	4	240			
5	c + e.sti	0,50	5	248			
6	veh	0,50	6	250			
7	veh + e.sti	0,51	7	253			
8	a + e.sti	0,48	8	238			
9	b + e.sti	0,51	1	254			
10	c + e.sti	0,51	2	253			
11	veh	0,49	3	243			
12	veh + e.sti	0,45	4	227			
13	a + e.sti	0,45	5	225			
14	b + e.sti	0,44	6	220			
15	c + e.sti	0,46	7	228			
16	veh	0,47	8	235			
17	veh + e.sti	0,49	1	244			
18	a + e.sti	0,47	2	235			
19	b + e.sti	0,51	3	255			
20	c + e.sti	0,47	4	233			
21	veh	0,46	5	229			
22	veh + e.sti	0,50	6	251			
23	a + e.sti	0,50	7	248			
24	b + e.sti	0,47	8	233			
25	c + e.sti	0,49	1	243			
26	veh	0,47	2	234			
27	veh + e.sti	0,46	3	230			
28	a + e.sti	0,48	4	240			
29	b + e.sti	0,49	5	244			
30	c + e.sti	0,49	6	244			
31	veh	0,49	7	244			
32	veh + e.sti	0,48	8	239			
33	a + e.sti	0,49	1	246			
34	b + e.sti	0,51	2	255			
35	c + e.sti	0,51	3	256			
36	veh	0,50	4	248			
37	veh + e.sti	0,50	5	250			
38	a + e.sti	0,49	6	245			
39	b + e.sti	0,48	7	240			
40	c + e.sti	0,50	8	250			

Appendix 3.23.

Extended-tone fear conditioning, α5IA-II (B2)

Date/init:	20061024-1027/LIS MKH	Drug:	alpha5IA-II in 5% tween
Strain:	Male SPRD	Doses:	a=1, b=3, c=10 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Habituation	Conditioning	Testing
1	veh	0,42	1	212			
2	veh + e.sti	0,48	2	241			
3	a + e.sti	0,41	3	206			
4	b + e.sti	0,47	4	235			
5	c + e.sti	0,47	5	235			
6	veh	0,45	6	225			
7	veh + e.sti	0,45	7	227			
8	a + e.sti	0,46	8	232			
9	b + e.sti	0,47	1	236			
10	c + e.sti	0,48	2	239			
11	veh	0,40	3	202			
12	veh + e.sti	0,50	4	250			
13	a + e.sti	0,44	5	220			
14	b + e.sti	0,46	6	232			
15	c + e.sti	0,48	7	238			
16	veh	0,45	8	224			
17	veh + e.sti	0,44	1	221			
18	a + e.sti	0,47	2	236			
19	b + e.sti	0,45	3	227			
20	c + e.sti	0,48	4	240			
21	veh	0,50	5	248			
22	veh + e.sti	0,42	6	210			
23	a + e.sti	0,43	7	216			
24	b + e.sti	0,44	8	218			
25	c + e.sti	0,43	1	217			
26	veh	0,45	2	223			
27	veh + e.sti	0,44	3	221			
28	a + e.sti	0,46	4	228			
29	b + e.sti	0,46	5	229			
30	c + e.sti	0,48	6	238			
31	veh	0,43	7	214			
32	veh + e.sti	0,42	8	210			
33	a + e.sti	0,46	1	232			
34	b + e.sti	0,46	2	228			
35	c + e.sti	0,43	3	215			
36	veh	0,41	4	207			
37	veh + e.sti	0,44	5	220			
38	a + e.sti	0,44	6	221			
39	b + e.sti	0,44	7	220			
40	c + e.sti	0,44	8	220			

Appendix 3.24.

Extended-tone fear conditioning, NS.A (B3)

Date/init:	20061107-1110/LIS MKH	Drug:	NS.A in 5% tween
Strain:	Male SPRD	Doses:	a=0.3, b=1, c=3 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Habituation	Conditioning	Testing
1	veh	0,49	1	245			
2	veh + e.sti	0,48	2	240			
3	a + e.sti	0,48	3	241			
4	b + e.sti	0,50	4	250			
5	c + e.sti	0,51	5	253			
6	veh	0,49	6	244			
7	veh + e.sti	0,50	7	248			
8	a + e.sti	0,50	8	251			
9	b + e.sti	0,49	1	244			
10	c + e.sti	0,50	2	248			
11	veh	0,48	3	241			
12	veh + e.sti	0,48	4	240			
13	a + e.sti	0,49	5	244			
14	b + e.sti	0,47	6	235			
15	c + e.sti	0,49	7	245			
16	veh	0,48	8	240			
17	veh + e.sti	0,49	1	245			
18	a + e.sti	0,48	2	238			
19	b + e.sti	0,47	3	235			
20	c + e.sti	0,49	4	247			
21	veh	0,51	5	253			
22	veh + e.sti	0,48	6	240			
23	a + e.sti	0,49	7	247			
24	b + e.sti	0,48	8	242			
25	c + e.sti	0,47	1	237			
26	veh	0,46	2	232			
27	veh + e.sti	0,48	3	240			
28	a + e.sti	0,48	4	242			
29	b + e.sti	0,50	5	252			
30	c + e.sti	0,49	6	247			
31	veh	0,48	7	239			
32	veh + e.sti	0,50	8	248			
33	a + e.sti	0,48	1	240		Box opened by mistake to time 5 min 25 s	
34	b + e.sti	0,48	2	240			
35	c + e.sti	0,49	3	246			
36	veh	0,49	4	244			
37	veh + e.sti	0,47	5	236			
38	a + e.sti	0,48	6	240			
39	b + e.sti	0,47	7	235			
40	c + e.sti	0,50	8	248			

Appendix 3.25.

Trace Fear Conditioning (C)

Date/init:	20061128-01/LIS MKH	Drug:	alpha5IA-II in 5% tween
Strain:	Male SPRD	Doses:	a=1, b=3

Animal no.	Drug	ml	Startle box	Weight	Habituation	Conditioning	Testing
1	veh+e.shock(TFC)	0,44	1	222			
2	a+e.shock(TFC)	0,43	2	213			
3	b+e.shock(TFC)	0,48	3	240			
4	veh+e.shock(TFC)	0,45	4	223			
5	veh(nTFC)	0,44	5	222			
6	veh+e.shock(nTFC)	0,45	6	223			
7	veh(nTFC)	0,46	7	232			
8	veh+e.shock(nTFC)	0,47	8	233			
9	veh+e.shock(nTFC)	0,45	1	227		Traceprogram (TFC)	
10	veh(nTFC)	0,45	2	224		Traceprogram (TFC)	
11	veh+e.shock(nTFC)	0,47	3	234		Traceprogram (TFC)	
12	veh(nTFC)	0,47	4	233		Traceprogram (TFC)	
13	a+e.shock(TFC)	0,44	5	220			
14	b+e.shock(TFC)	0,46	6	232			
15	veh+e.shock(TFC)	0,47	7	236			
16	a+e.shock(TFC)	0,45	8	225			
17	b+e.shock(TFC)	0,48	1	238			
18	veh+e.shock(TFC)	0,46	2	230			
19	a+e.shock(TFC)	0,45	3	225			
20	b+e.shock(TFC)	0,43	4	216			
21	veh+e.shock(TFC)	0,47	5	233			
22	a+e.shock(TFC)	0,49	6	243			
23	b+e.shock(TFC)	0,50	7	248			
24	veh+e.shock(TFC)	0,46	8	229			
25	veh(nTFC)	0,45	1	225			
26	veh+e.shock(nTFC)	0,45	2	225			
27	veh(nTFC)	0,44	3	219			
28	veh+e.shock(nTFC)	0,46	4	228			
29	b+e.shock(TFC)	0,45	5	225			
30	veh+e.shock(TFC)	0,49	6	243			
31	a+e.shock(TFC)	0,46	7	230			
32	b+e.shock(TFC)	0,47	8	236			
33	a+e.shock(TFC)	0,46	1	230			
34	b+e.shock(TFC)	0,47	2	233			
35	veh+e.shock(TFC)	0,46	3	232			
36	a+e.shock(TFC)	0,46	4	228			
37	veh+e.shock(nTFC)	0,46	5	232			
38	veh(nTFC)	0,45	6	227			
39	veh+e.shock(nTFC)	0,42	7	210			
40	veh(nTFC)	0,48	8	241			

Appendix 3.26.

Pre-pulse inhibition, amphetamine deficit, NS.A (G)

Date/init:	20061124/MKH LIS	Drug:	NS.A in tween 5%
Strain:	Male SPRD	Doses:	a=0.3, b=1, c=3 mg/kg

Animal no	Drug	ml NS.A	Methampf	ml amp	Weight	Inj NS.A 30m	Inj Amph 30 m	PPI
1	veh ip	0,40	veh sc	0,20	200			
2	a ip	0,37	veh sc	0,19	186			
3	b ip	0,40	veh sc	0,20	200			
4	c ip	0,39	veh sc	0,19	194			
5	veh ip	0,38	amp sc	0,19	190			
6	a ip	0,39	amp sc	0,20	196			
7	b ip	0,40	amp sc	0,20	200			
8	c ip	0,39	amp sc	0,20	195			
9	c ip	0,40	amp sc	0,20	200	The rat escape rat cage during experiment and ran around the startle box		
10	veh ip	0,37	veh sc	0,19	185			
11	a ip	0,39	veh sc	0,19	193			
12	b ip	0,35	veh sc	0,17	173			
13	c ip	0,37	veh sc	0,19	186			
14	veh ip	0,37	amp sc	0,18	183			
15	a ip	0,39	amp sc	0,20	196			
16	b ip	0,37	amp sc	0,18	183			
17	b ip	0,39	amp sc	0,20	195			
18	c ip	0,40	amp sc	0,20	200			
19	veh ip	0,40	veh sc	0,20	200			
20	a ip	0,39	veh sc	0,19	194			
21	b ip	0,38	veh sc	0,19	189			
22	c ip	0,39	veh sc	0,19	193			
23	veh ip	0,35	amp sc	0,18	176			
24	a ip	0,39	amp sc	0,20	197			
25	a ip	0,38	amp sc	0,19	189			
26	b ip	0,37	amp sc	0,18	183			
27	c ip	0,38	amp sc	0,19	188			
28	veh ip	0,38	veh sc	0,19	191			
29	a ip	0,41	veh sc	0,21	207			
30	b ip	0,40	veh sc	0,20	198			
31	c ip	0,38	veh sc	0,19	192			
32	veh ip	0,37	amp sc	0,19	187			
33	veh ip	0,40	amp sc	0,20	202			
34	a ip	0,39	amp sc	0,19	194			
35	b ip	0,39	amp sc	0,19	193			
36	c ip	0,38	amp sc	0,19	191			
37	veh ip	0,39	veh sc	0,20	195			
38	a ip	0,40	veh sc	0,20	199			
39	b ip	0,38	veh sc	0,19	190			
40	c ip	0,39	veh sc	0,20	197			

To be continued

Appendix 3.26.

x 41	c ip	0,39	veh sc	0,20	195			
x 42	veh ip	0,39	amp sc	0,20	197			
x 43	a ip	0,42	amp sc	0,21	208			
x 44	b ip	0,40	amp sc	0,20	200			
x 45	c ip	0,38	amp sc	0,19	191			
x 46	veh ip	0,40	veh sc	0,20	198			
x 47	a ip	0,40	veh sc	0,20	200			
x 48	b ip	0,39	veh sc	0,20	195			
x 49	b ip	0,40	veh sc	0,20	202			
x 50	c ip	0,39	veh sc	0,20	195			
x 51	veh ip	0,41	amp sc	0,21	207			
x 52	a ip	0,40	amp sc	0,20	200			
x 53	b ip	0,40	amp sc	0,20	200			
x 54	c ip	0,39	amp sc	0,20	195			
x 55	veh ip	0,42	veh sc	0,21	210			
x 56	a ip	0,39	veh sc	0,19	194			
x 57	a ip	0,38	veh sc	0,19	191			
x 58	b ip	0,41	veh sc	0,21	205			
x 59	c ip	0,39	veh sc	0,20	197			
x 60	veh ip	0,38	amp sc	0,19	192			
x 61	a ip	0,39	amp sc	0,20	195			
x 62	b ip	0,41	amp sc	0,21	207			
x 63	c ip	0,40	amp sc	0,20	202			
x 64	veh ip	0,42	veh sc	0,21	210			

Appendix 3.27.

Pre-pulse inhibition, dose-response, methylphenidate, rat (D2)

Session: **PPI pp4, 8, 16, 24, 110dB pu** **Strain:** **SPDR** **Dato/init:** **2006-09-15/MKH+LIS**

Stof + veh: **methylphenidate (5-20 mg/kg s.c. i** **Pretreatm:** **-15 min** **Admin.** **s.c.**

Stof + veh: **Pretreatm:** **Admin.** **s.c.**

System Old: normal; ***New: bold italics***

Animal nr	Group	Box nr.	Weight	Comments
1	veh	1	305	
2	5	2	305	
3	10	3	320	
4	20	4	316	
5	***5***	*5*	306	
6	***10***	*6*	323	
7	***20***	*7*	293	Don´t know how much drug it received
8	***veh***	*8*	307	
9	10	1	282	
10	20	2	326	
11	veh	3	310	
12	5	4	310	
13	***20***	*5*	304	
14	***veh***	*6*	303	
15	***5***	*7*	317	
16	***10***	*8*	304	
17	veh	1	285	
18	5	2	283	
19	10	3	308	
20	20	4	292	
21	***5***	*5*	292	
22	***10***	*6*	293	
23	***20***	*7*	310	
24	***veh***	*8*	310	

Appendix 3.28.

Pre-pulse inhibition, dose-response, 72 h (E1)

Date/init:	20060918-0921/MKH LIS	Drug:	PCP in Nacl 0,9%
Strain:	Male SPRD	Doses:	a=1,25, b=2,5, c=5 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Injection 72h	PPI
1	veh	0,23	1	234		
2	a	0,22	2	222		
3	b	0,22	3	224		Box opened to time 10 min
4	c	0,23	4	227		
5	veh	0,25	5	245		
6	a	0,24	6	240		
7	b	0,24	7	235		
8	c	0,23	8	232		
9	c	0,24	1	237		
10	veh	0,23	2	232		
11	a	0,23	3	230		
12	b	0,24	4	237		
13	c	0,23	5	234		
14	veh	0,23	6	229		
15	a	0,22	7	220		
16	b	0,23	8	225	Injected wrongly	
17	b	0,22	1	215		
18	c	0,24	2	238		
19	veh	0,24	3	235		
20	a	0,23	4	228		
21	b	0,23	5	230	Some drops was lost	
22	c	0,24	6	241		
23	veh	0,23	7	230		
24	a	0,24	8	244		
25	a	0,21	1	212		
26	b	0,23	2	229		
27	c	0,23	3	227		
28	veh	0,23	4	229		
29	a	0,24	5	238		
30	b	0,23	6	234		
31	c	0,23	7	230		
32	veh	0,22	8	220		

Appendix 3.29.

Pre-pulse inhibition, dose-response, 24 h (E2)

Date/init:	20060927-0928/MKH LIS	Drug:	PCP in Nacl 0,9%
Strain:	Male SPRD	Doses:	a=1,25, b=2,5, c=5 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Injection 24h	PPI
1	veh	0,25	1	252		
2	a	0,25	2	250		
3	b	0,25	3	253		
4	c	0,25	4	253		
5	veh	0,26	5	255		
6	a	0,26	6	256		
7	b	0,25	7	251		
8	c	0,24	8	240		
9	c	0,25	1	248	Maybe some drops was lost	
10	veh	0,26	2	257		
11	a	0,25	3	251		
12	b	0,23	4	233		
13	c	0,24	5	242		
14	veh	0,24	6	240		
15	a	0,26	7	256		
16	b	0,27	8	265		
17	b	0,25	1	252		
18	c	0,25	2	253		
19	veh	0,24	3	240		
20	a	0,25	4	250		
21	b	0,24	5	243		
22	c	0,24	6	243		
23	veh	0,24	7	240		
24	a	0,25	8	250		
25	a	0,25	1	253		
26	b	0,24	2	242		
27	c	0,25	3	253		
28	veh	0,25	4	252		
29	a	0,25	5	250		
30	b	0,24	6	239		
31	c	0,25	7	253		
32	veh	0,25	8	250		

Appendix 3.30.

Pre-pulse inhibition, dose-response, 10 min (E3)

Date/init:	20060929/MKH LIS	Drug:	PCP in Nacl 0,9%
Strain:	Male SPRD	Doses:	a=1,25, b=2,5, c=5 mg/kg

Animal no.	Drug	ml	Startle box	Weight	Injection 10n	PPI
1	veh	0,25	1	250		
2	a	0,25	2	252		
3	b	0,26	3	255		
4	c	0,25	4	251		
5	veh	0,24	5	239		
6	a	0,25	6	251		
7	b	0,20	7	200	Stressed (blood around the eyes) dehydrated, bristly fur	
8	c	0,25	8	250	Blood drops	
9	c	0,24	1	237		
10	veh	0,24	2	235		
11	a	0,25	3	252		
12	b	0,25	4	251	Drop out	
13	c	0,26	5	255		
14	veh	0,26	6	259		
15	a	0,25	7	249		
16	b	0,25	8	249		
17	b	0,25	1	246		
18	c	0,25	2	246		
19	veh	0,25	3	250		
20	a	0,26	4	261		
21	b	0,24	5	239	Lost drop	
22	c	0,25	6	247		
23	veh	0,25	7	250		
24	a	0,22	8	223		
25	a	0,25	1	251	Lost a lot	
26	b	0,26	2	258		
27	c	0,25	3	251		
28	veh	0,24	4	240		
29	a	0,25	5	246		
30	b	0,25	6	252		
31	c	0,26	7	257		
32	veh	0,26	8	260		

Appendix 3.31.

Pre-pulse inhibition, PCP deficit, alprazolam (F1)

Date/init:	20061101/MKH LIS	Drug:	Alprazolam in 5% cremophor
Strain:	Male SPRD	Doses:	a=0.4, b=1.33, c=4 mg/kg

Animal nc	Drug	ml NS11	PCP	ml PCP	Weight	Inj alprazolar	Inj PCP 10 mir	PPI
1	veh ip	0,45	veh sc	0,22	224			
2	a ip	0,48	veh sc	0,24	242			
3	b ip	0,49	veh sc	0,25	245			
4	c ip	0,46	veh sc	0,23	230			
5	veh ip	0,50	PCP sc	0,25	248			
6	a ip	0,47	PCP sc	0,24	237			
7	b ip	0,50	PCP sc	0,25	251			
8	c ip	0,50	PCP sc	0,25	251			
9	c ip	0,48	PCP sc	0,24	241			
10	veh ip	0,47	veh sc	0,24	235			
11	a ip	0,46	veh sc	0,23	232			
12	b ip	0,51	veh sc	0,26	256			
13	c ip	0,50	veh sc	0,25	248			
14	veh ip	0,49	PCP sc	0,25	246			
15	a ip	0,48	PCP sc	0,24	242			
16	b ip	0,48	PCP sc	0,24	240			
17	b ip	0,48	PCP sc	0,24	238			
18	c ip	0,50	PCP sc	0,25	250			
19	veh ip	0,48	veh sc	0,24	238			
20	a ip	0,47	veh sc	0,24	237			
21	b ip	0,49	veh sc	0,24	243			
22	c ip	0,50	veh sc	0,25	248			
23	veh ip	0,49	PCP sc	0,24	243			
24	a ip	0,50	PCP sc	0,25	252			
25	a ip	0,47	PCP sc	0,24	235			
26	b ip	0,49	PCP sc	0,25	247			
27	c ip	0,48	PCP sc	0,24	240			
28	veh ip	0,48	veh sc	0,24	238			
29	a ip	0,48	veh sc	0,24	242			
30	b ip	0,46	veh sc	0,23	231			
31	c ip	0,47	veh sc	0,23	233			
32	veh ip	0,47	PCP sc	0,24	236			
33	veh ip	0,48	PCP sc	0,24	241			
34	a ip	0,50	PCP sc	0,25	249		Lost drops	
35	b ip	0,52	PCP sc	0,26	259			
36	c ip	0,51	PCP sc	0,25	254			
37	veh ip	0,50	veh sc	0,25	250			
38	a ip	0,46	veh sc	0,23	229			
39	b ip	0,45	veh sc	0,23	227			
40	c ip	0,47	veh sc	0,24	235			

To be continued

Appendix 3.31.

x 41	c ip	0,47	veh sc	0,24	235			
x 42	veh ip	0,51	PCP sc	0,26	257			
x 43	a ip	0,47	PCP sc	0,24	236			
x 44	b ip	0,50	PCP sc	0,25	250			
x 45	c ip	0,48	PCP sc	0,24	240			
x 46	veh ip	0,49	veh sc	0,24	244			
x 47	a ip	0,49	veh sc	0,24	243			
x 48	b ip	0,52	veh sc	0,26	258			
x 49	b ip	0,48	veh sc	0,24	239			
x 50	c ip	0,49	veh sc	0,25	245			
x 51	veh ip	0,49	PCP sc	0,25	245			
x 52	a ip	0,48	PCP sc	0,24	238			
x 53	b ip	0,49	PCP sc	0,25	246			
x 54	c ip	0,46	PCP sc	0,23	228			
x 55	veh ip	0,49	veh sc	0,24	243			
x 56	a ip	0,50	veh sc	0,25	252			
x 57	a ip	0,49	veh sc	0,24	243			
x 58	b ip	0,49	veh sc	0,24	243			
x 59	c ip	0,48	veh sc	0,24	239			
x 60	veh ip	0,48	PCP sc	0,24	242			
x 61	a ip	0,47	PCP sc	0,23	233			
x 62	b ip	0,49	PCP sc	0,24	243			
x 63	c ip	0,51	PCP sc	0,25	253			
x 64	veh ip	0,47	veh sc	0,24	237			

Appendix 3.32.

Pre-pulse inhibition, PCP deficit, α5IA-II (F2)

Date/init:	20061005/MKH LIS	Drug:	alpha5IS-II in tween 5%
Strain:	Male SPRD	Doses:	a=1, b=3, c=10 mg/kg

Animal no	Drug	ml NS10	PCP	ml PCP	Weight	Inj NS10002 3	Inj PCP 10 min	PPI
1	veh ip	0,54	veh sc	0,27	271			
2	1 ip	0,56	veh sc	0,28	280			
3	3 ip	0,54	veh sc	0,27	270			
4	10 ip	0,50	veh sc	0,25	252			
5	veh ip	0,55	PCP sc	0,27	273			
6	1 ip	0,55	PCP sc	0,27	273			
7	3 ip	0,50	PCP sc	0,25	251			
8	10 ip	0,49	PCP sc	0,25	246			
9	10 ip	0,56	PCP sc	0,28	278			
10	veh ip	0,57	veh sc	0,29	286			
11	1 ip	0,53	veh sc	0,27	266			
12	3 ip	0,50	veh sc	0,25	250			
13	10 ip	0,53	veh sc	0,26	264			
14	veh ip	0,52	PCP sc	0,26	260			
15	1 ip	0,53	PCP sc	0,26	264		Lost drops	
16	3 ip	0,52	PCP sc	0,26	260			
17	3 ip	0,51	PCP sc	0,26	255			
18	10 ip	0,51	PCP sc	0,26	255			
19	veh ip	0,55	veh sc	0,28	275			
20	1 ip	0,50	veh sc	0,25	250			
21	3 ip	0,53	veh sc	0,27	267			
22	10 ip	0,50	veh sc	0,25	250			
23	veh ip	0,48	PCP sc	0,24	242			
24	1 ip	0,51	PCP sc	0,25	254			
25	1 ip	0,53	PCP sc	0,27	267			
26	3 ip	0,52	PCP sc	0,26	260			
27	10 ip	0,54	PCP sc	0,27	270			
28	veh ip	0,54	veh sc	0,27	272			
29	1 ip	0,57	veh sc	0,29	285	Power cut		Was in box
30	3 ip	0,53	veh sc	0,26	263	Power cut		Was in box
31	10 ip	0,57	veh sc	0,28	284	Power cut		Was in box
32	veh ip	0,54	PCP sc	0,27	272	Power cut		Was in box
33	veh ip	0,52	PCP sc	0,26	260	Power cut		Was in box
34	1 ip	0,53	PCP sc	0,27	266	Power cut		Was in box
35	3 ip	0,56	PCP sc	0,28	280	Power cut		Was in box
36	10 ip	0,54	PCP sc	0,27	270	Power cut		Was in box
37	veh ip	0,52	veh sc	0,26	262	Power cut		Injected
38	1 ip	0,51	veh sc	0,26	255	Power cut		Injected
39	3 ip	0,54	veh sc	0,27	268	Power cut		Injected
40	10 ip	0,53	veh sc	0,27	265	Power cut		Injected

To be continued

Appendix 3.32.

x 41	10 ip	0,56	veh sc	0,28	280	Power cut	Injected
x 42	veh ip	0,54	PCP sc	0,27	268	Power cut	Injected
x 43	1 ip	0,55	PCP sc	0,27	274	Power cut	Injected
x 44	3 ip	0,51	PCP sc	0,26	255	Power cut	Injected

Experiment was discontinued – the remaining of the experiment was executed the following day the 6th of October

x 45	10 ip	0,57	PCP sc	0,29	285		
x 46	veh ip	0,53	veh sc	0,27	266		
x 47	1 ip	0,55	veh sc	0,28	276		
x 48	3 ip	0,55	veh sc	0,28	276		
x 49	3 ip	0,56	veh sc	0,28	282		
x 50	10 ip	0,55	veh sc	0,27	274		
x 51	veh ip	0,54	PCP sc	0,27	270	Something lost	
x 52	1 ip	0,54	PCP sc	0,27	272	Something lost	
x 53	3 ip	0,53	PCP sc	0,26	263		
x 54	10 ip	0,56	PCP sc	0,28	278		
x 55	veh ip	0,56	veh sc	0,28	282		
x 56	1 ip	0,54	veh sc	0,27	272		
x 57	1 ip	0,56	veh sc	0,28	280		
x 58	3 ip	0,52	veh sc	0,26	262		
x 59	10 ip	0,54	veh sc	0,27	270		
x 60	veh ip	0,55	PCP sc	0,28	275		
x 61	1 ip	0,54	PCP sc	0,27	270		
x 62	3 ip	0,53	PCP sc	0,27	265	Something lost	
x 63	10 ip	0,53	PCP sc	0,27	266		
x 64	veh ip	0,53	veh sc	0,26	263		

Appendix 3.33.

Pre-pulse inhibition, PCP deficit, NS.A (F3)

Date/init:	20061030/MKH LIS	Drug:	NS.A in tween 5%
Strain:	Male SPRD	Doses:	a=0.3, b=1, c=3 mg/kg

Animal nc	Drug	ml NS11	PCP	ml PCF	Weight	Inj NS.A 30m	Inj PCP 10 mir	PPI
1	veh ip	0,46	veh sc	0,23	230			
2	a ip	0,48	veh sc	0,24	238		A drops (small) lost	
3	b ip	0,47	veh sc	0,24	236			
4	c ip	0,45	veh sc	0,23	227			
5	veh ip	0,44	PCP sc	0,22	218			
6	a ip	0,46	PCP sc	0,23	228			
7	b ip	0,45	PCP sc	0,23	226			
8	c ip	0,46	PCP sc	0,23	230		Lost a big drop	
9	c ip	0,46	PCP sc	0,23	232			
10	veh ip	0,45	veh sc	0,22	223			
11	a ip	0,45	veh sc	0,22	224			
12	b ip	0,46	veh sc	0,23	228			
13	c ip	0,44	veh sc	0,22	222			
14	veh ip	0,44	PCP sc	0,22	222			
15	a ip	0,54	PCP sc	0,27	271			
16	b ip	0,52	PCP sc	0,26	262			
17	b ip	0,55	PCP sc	0,28	276			
18	c ip	0,53	PCP sc	0,27	266			
19	veh ip	0,53	veh sc	0,26	264			
20	a ip	0,54	veh sc	0,27	270			
21	b ip	0,55	veh sc	0,28	275			
22	c ip	0,54	veh sc	0,27	270			
23	veh ip	0,52	PCP sc	0,26	259			
24	a ip	0,51	PCP sc	0,25	253			
25	a ip	0,43	PCP sc	0,22	216			
26	b ip	0,44	PCP sc	0,22	220		Lost most of it	
27	c ip	0,47	PCP sc	0,23	233			
28	veh ip	0,45	veh sc	0,22	223			
29	a ip	0,44	veh sc	0,22	220			
30	b ip	0,42	veh sc	0,21	212			
31	c ip	0,47	veh sc	0,24	237	Blood in the syringe, injected in the other side		
32	veh ip	0,46	PCP sc	0,23	228		Lost drop	
33	veh ip	0,51	PCP sc	0,26	256			
34	a ip	0,52	PCP sc	0,26	260		Jump out the startle cage during the test	
35	b ip	0,56	PCP sc	0,28	280			
36	c ip	0,55	PCP sc	0,28	275			
37	veh ip	0,46	veh sc	0,23	230			
38	a ip	0,47	veh sc	0,23	234			
39	b ip	0,44	veh sc	0,22	220			
40	c ip	0,46	veh sc	0,23	230			

To be continued

Appendix 3.33.

x 41	c ip	0,44	veh sc	0,22	222			
x 42	veh ip	0,48	PCP sc	0,24	242			
x 43	a ip	0,45	PCP sc	0,23	227			
x 44	b ip	0,47	PCP sc	0,24	235			
x 45	c ip	0,45	PCP sc	0,22	224			
x 46	veh ip	0,45	veh sc	0,23	226			
x 47	a ip	0,44	veh sc	0,22	222			
x 48	b ip	0,48	veh sc	0,24	241			
x 49	b ip	0,45	veh sc	0,22	224			
x 50	c ip	0,46	veh sc	0,23	230			
x 51	veh ip	0,45	PCP sc	0,22	223		Lost drop	
x 52	a ip	0,43	PCP sc	0,21	214			
x 53	b ip	0,49	PCP sc	0,24	243			
x 54	c ip	0,47	PCP sc	0,24	236			
x 55	veh ip	0,45	veh sc	0,23	226			
x 56	a ip	0,45	veh sc	0,23	225			
x 57	a ip	0,46	veh sc	0,23	232			
x 58	b ip	0,48	veh sc	0,24	240			
x 59	c ip	0,46	veh sc	0,23	231			
x 60	veh ip	0,46	PCP sc	0,23	229			
x 61	a ip	0,43	PCP sc	0,21	213			
x 62	b ip	0,46	PCP sc	0,23	228			
x 63	c ip	0,47	PCP sc	0,23	234			
x 64	veh ip	0,47	veh sc	0,24	235			

Dose-response, PCP deficit, α5IA-II (H)

Date/init:	20061027/LIS	Drug:	alpha5IA-II in tween 5%
Strain:	Male SPRD	Doses:	10 mg/ kg og 30 mg/kg

Animal nc	Drug	ml NS10	PCP	ml PCP	Weight	Inj alpha5IA-	Inj PCP 10 mir	Observationer
1	veh ip	0,49	PCP sc	0,24	243			
2	veh ip	0,48	PCP sc	0,24	241		Wrongly injected	
3	30 ip	0,47	PCP sc	0,23	233		Wrongly injected	
4	30 ip	0,46	PCP sc	0,23	232			
5	30 ip	0,52	veh sc	0,26	261			
6	30 ip	0,44	veh sc	0,22	222			
7	30 ip	0,48	PCP sc	0,24	240			
8	30 ip	0,46	veh sc	0,23	231			
9	veh ip	0,50	PCP sc	0,25	251			
10	veh ip	0,48	veh sc	0,24	240			

Appendix 3.35.

Fear Conditioning in Rats		
NEUROSEARCH Pharmacological Department		NS-Code: **1.1. M650.XXX**
Author: **KBT** Date: 30.maj 2006	Accepted by: Date:	Dyreforsøgstilladelse: 18_15 2000/561-306

FORMÅL: At evaluere et teststofs anxiolytiske, anxiogene eller kognitionsfremmende effekt.

2. TEORI:

Testens princip er at dyrene trænes (konditioneres) til at være bange for en tone som i sig selv ikke er angstfremkaldende.

I konditionerings sessionen bliver dyrene præsenteret for en tone (10sec) i det sidste sekund af tonen får dyrene et elektrisk stød via et gitter i gulvet (0.6mA). 24 timer senere bliver dyrene igen præsenteret for tonen (uden stød).

Når dyret hører tonen bliver det bange, dette kommer til udtryk ved at dyret "freezer" dvs. der ikke er anden bevægelsen end at dyret trækker vejret. Det er denne nedsatte aktivitet som bliver registreret. Gennem hele forsøget bliver dyrene placeret på en transducer (vægt), transduceren måler dyrets bevægelse. Et teststof kan således øge eller reducere dyrets bevægelse.

Test stoffer kan blive administreret inden konditionering eller inden test. Kognitionsfremmende stoffer (eg. Nikotin) administreres inden konditionering. De har ingen rigtig effekt på konditioneringen men 24 timer senere under testen vil dyrene bevæge sig mindre, dette tolkes til at dyrene enten har været mere opmærksomme under konditioneringen eller at de husker konditioneringen bedre.
Anxiolytiske eller anxiogene stoffer vil som oftest blive administreret inden testen. Et anxiolytisk stof vil øge aktiviteten hvorimod et anxiogent stof vil nedsætte aktiviteten.

Appendix 3.35.

FORSØGETS UDFØRELSE:

Dyr:
SPRD han rotter (200-250g)
Rotterne huses i små kasser med to i hver.
Når dyrene er bestilt fra dyrestalden til BS.18, er det kun den der skal udføre forsøget der har kontakt med dyrene. Det er derfor en god ide at skifte dyrene når der håndteres den første dag. Herved risikerer man heller ikke at dyrene skal skiftes under forsøget. (hvilket kan føre til at forsøget ikke fungere).

Udstyr:
TSE startle bokse (BS.18)

Inden forsøgets start

a. opret en folder under 'J:\In vivo Pharmacology\Models in Pharmacology\Anxiety\Fear conditioning in rats\Results' svarende til det forsøg der skal køres.
b. Lav en forsøgsprotokol. Skabelonen ligger i mappen worksheets, der er angivet hvilken dosis de enkelte dyr skal have, anvendes denne rækkefølge bliver databehandlingen lige til at gå til.
c. Lav en informationsseddel til scantaineren. Skabelonen findes under: J:\Andet\Dyreforsøg\Information

Brug af TSE startle bokse (BS.18)

1. Transducerne **skal** håndteres **forsigtigt**! De kan **IKKE** tåle hårde slag!
2. Rotte transducerene monteres i startle boksene. Der står et nummer på hver af transducerne (1 til 8) svarende til startle boksene (boks nr. 1 er boks nr 1 i det øverste system, boks nr. 8 er nr 4 i det nederste system). Det er vigtigt at transducerne bliver monteret inden systemet starter op.

Computeren

3. Tænd for control boksene (knappen sidder bag på boksen).
4. Tænd for computerne
   - Bruger: startle
   - Password: startle1
5. Der er kun en skærm til de to computere, skærmen kan bruges til begge computere. Der står en 'Transfer boks' oven på computerne. Der er et lille grønt lys på 'transfer boksen', der viser hvilket system du arbejder på. Der er to måder at skifte mellem de to systemer du kan trykke på knappen under lyset eller du kan trykke to gange på Ctrl så skifter du automatisk. Du kan skifte mellem de to systemer på et hvilket som helst tidspunkt, det er lige meget om du kører forsøg, det påvirker ikke systemerne.

Inden du starter skal der oprettes foldere hvor rådata skal gemmes. Der skal oprettes to foldere en til calibrerings data og en til data fra hele dit forsøg.

6. Åben 'Shortcut to rawdata'
7. Vælg 'calibration' → vælg 'rat'
8. Opret en ny folder, navngives med datoen (YYYYMMDD). → luk folderen ned igen.
9. Åben 'Shortcut to rawdata' igen
10. Vælg 'Rat FC'
11. Opret en ny folder, der navngives med de dage du kører forsøget over (YYYYMMDD-DD)

Opstart af programmet

12. Åben 'Startle response'
    - Name: System
    - Password: System
    - Tryk OK

System indstillinger og test. Dette foretages inden hvert forsøg (habituation, conditionering eller test)

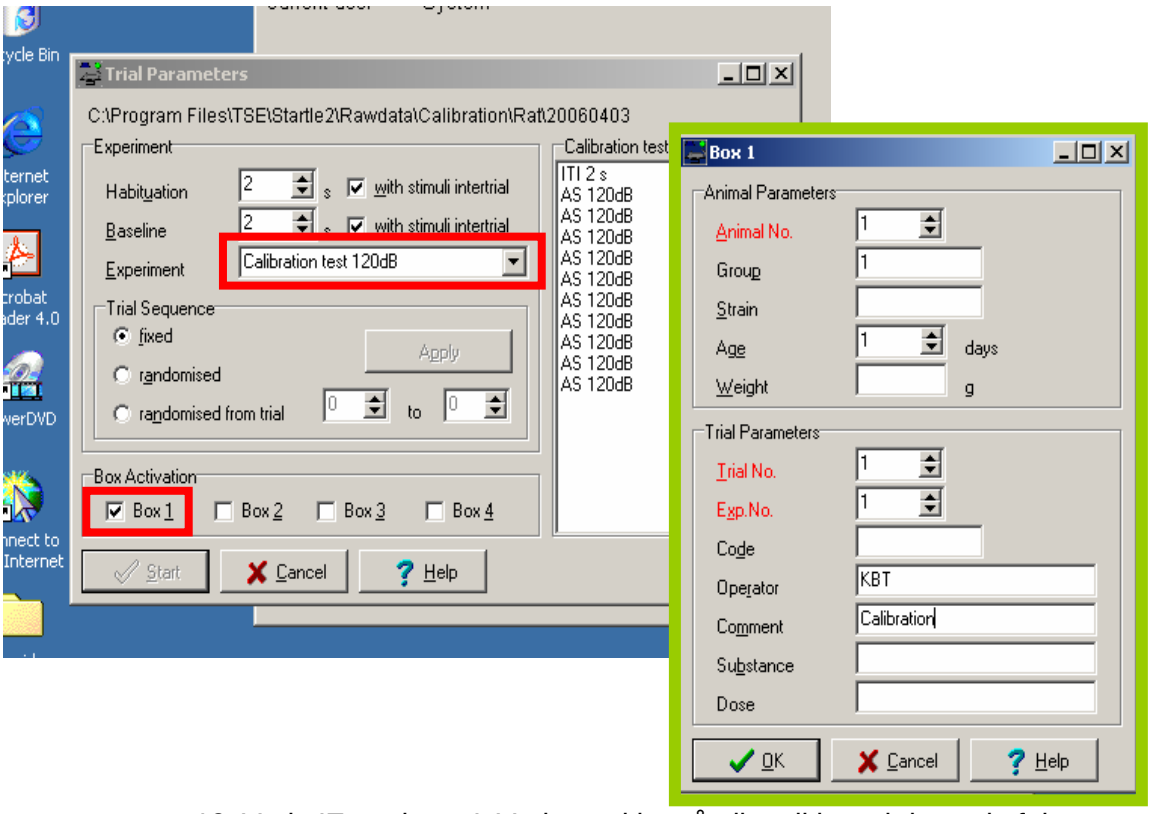
13. Tryk på 'Parameters' → vælg 'Program parameters'
    - 'Sampling interval' indstilles til 2ms til kalibrering af transducerne og 4ms når de øvrige forsøg køres. Det er **MEGET VIGTIGT** at ændre 'samping interval til 4ms under test. Ellers er det ikke muligt at foretage data analyserne. Når der ændres på 'Sampling interval' skal programmet genstarte.
    - 'Amplification Sensor': 500
    - Tryk OK
14. Tryk på 'System Test'
    - Der popper et nyt vindue op med en fejlmeddelelse hvis du ikke har tændt controlboksen.
    - Tjek at der er lyd → tryk på Sound og Noise og du vil kunne hører lyde fra startle boksene
    - Tryk på E-stim du vil kunne hører en klikkende lyd fra kontrol boksen.
    - Forneden ser man 'Time Control'. Der må ikke være mere en 5% forskel på de to tal, det første er det man har indstillet 'Samplinger Interval' til tidligere (pkt.13).
      2ms intervallet må da være 1.9ms – 2.1ms
      4ms intervallet må da være 3.8ms – 4.2ms
    - Tryk 'Close'

Appendix 3.35.

Kalibrering af transducerne.

Tranducerne kalibreres før et forsøg startes op, dvs. på dag 1.

15. Den kunstige rotte placeres på transducer nr. 1, ledningen fra "rotten" skal pege mod døren i startle boksen.
16. Åben 'Startle Response'. Vælg 'Archive' → vælg 'Data Directory'
17. Find stien til de fil du oprettede tidligere (pkt. 8). I denne fil vil de data du kører blive gemt. → tryk 'OK'
18. Vælg 'Experiment' → Tryk 'Experiment'. Følgende skærmbillede åbner op:



19. Vælg 'Experiment'. Ved at trykke på pilen til høre i det røde felt kan man vælge programmet.
20. 'Box Activation'. Klik på 'Box 1'. Et nyt vindue popper op. (den grønne boks).
    - Animal no. Indstilles svarende til nummeret på boksen (1-8)
    - Trial no. Indstilles svarende til den omgang man kører, der køres min. 3 runder med hver boks. Første runde er bare for at se hvordan boksen er indstillet nu. Anden runde indstilles systemet (mere om det nedenfor). Tredje runde er igen for at se om der er indstillet korrekt.
21. Tryk OK → Tryk Start.
22. Når første runde er slut tjekkes resultaterne.
23. Åben Analysis → Vælg Data Selection
24. Der er to felter for oven er de forsøg der er kørt, i 'Selected Data

Records' kan man se de forsøg der kan vises/eksporteres. Dobbelklik på den nederste fil i det øverste felt, denne vil nu blive kopieret ned til 'Selected Data Records'. → Tryk 'OK'

25. Åben Analysis → vælg 'Results Table'
26. Nedenfor vises den tabel der så vises.

TSE Startle Response
NeuroSearch S/A, Ballerup, DK (2004-2133)

Analysis (Mean)
19.04.2006

Animal No.	1
Trial No.	2
Exp. No.	1
Date	04/19/2006
Start Time	10:02:16 AM
ADC-Interval	2 ms
Trigger	43.5 g (Trigger from Baseline Phase * 1.0/ min. 0.0 g)
max. Delay	20 ms

Trial AS 120dB	Reaction ms	Duration ms	Maximum g	Maximum ms
1	14	24	153.3	18
2	14	40	152.3	18
3	14	40	160.6	18
4	12	50	166.5	18
5	14	40	157.2	18
6	12	42	157.7	18
7	14	40	157.7	18
8	12	50	163.1	18
9	12	50	169.9	18
10	12	50	[illegible]	18
Mean	13.0	42.6	161.2	18.0

27. I det røde felt ses gennemsnittet af de ti målinger der er i et forsøg. Denne værdi skal helst være omkring 150 (±2). Dette gentages en runde mere hvor der indstilles på potentimeteret på kontrol boksen. Der er et potentimeter til hver startle boks, potentimeteret er en lille skrue i hullet på fronten af kontrol boksen. Sæt skruetrækkeren forsigtigt ind i hullet, ved at dreje til højre eller venstre kan man indstille responset. Drejes med uret sænkes outputtet, drejes mod uret øges outputtet. På grafen skal outputtet være omkring 300 for at mean værdien bliver ca. 150.
28. Kalibrerings proceduren gentages indtil alle otte bokse er kalibreret. Og klar til forsøg.

Du skulle nu være klar til at starte selve forsøget op.

Appendix 3.35.

Procedure:
Forsøget tager fire dage, det skal være den samme person der kører hele forsøget. Kalibreringen skal være foretaget inden du skal til at køre selve forsøget, dvs. sætte rotter i startle boksene.

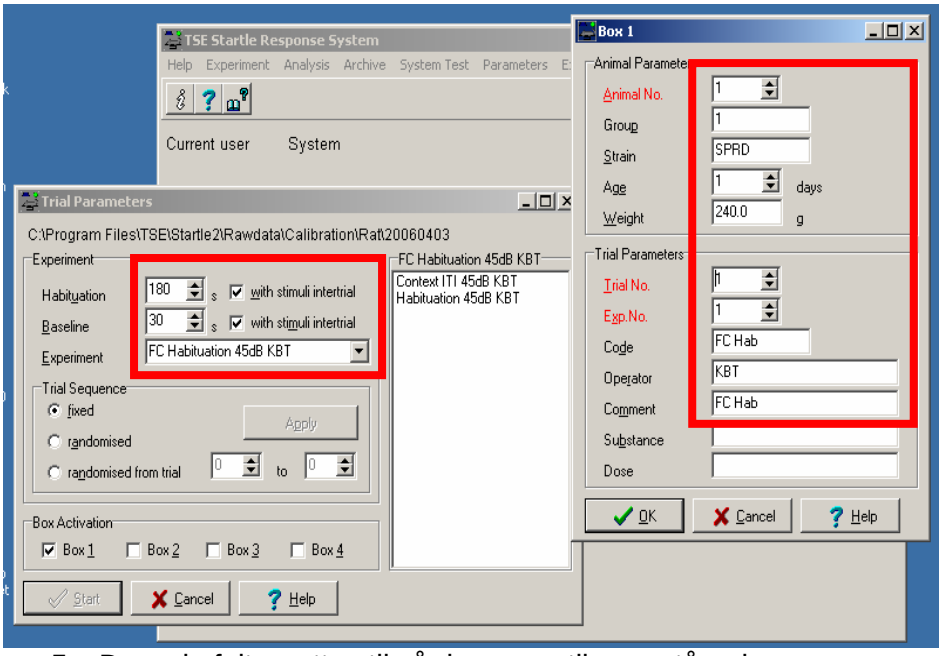
Dag 1: Håndtering (ca. 1 time)
Dyrene håndteres (ca. 1 min pr dyr), dyrene skal vendes til at blive håndteret af den der udfører forsøget. Dyrene mærkes og afvejes, vægten noteres.

På hver af de følgende dage gennemgåes to procedurer inden du er klar til at køre forsøg.

I. Archieve indstilles så det passer til forsøget. Vælg 'Archive' → vælg 'Data Directory' → tilpas stien så data fra alle tre dage kommer I den same sti.
II. System indstillinger **(4ms)** og test (pkt. 13-14 ovenfor)

Dag 2: Habituering (ca. 1½ time)

1. Tænd for computerne og kontrol boksene.
2. Åben 'Startle response'
   a. Name: System
   b. Password: System
   c. Tryk OK
3. Tryk på 'Experiment' → Vælg 'Experiment'
4. Skærmbilledet indstilles som vist nedenfor.

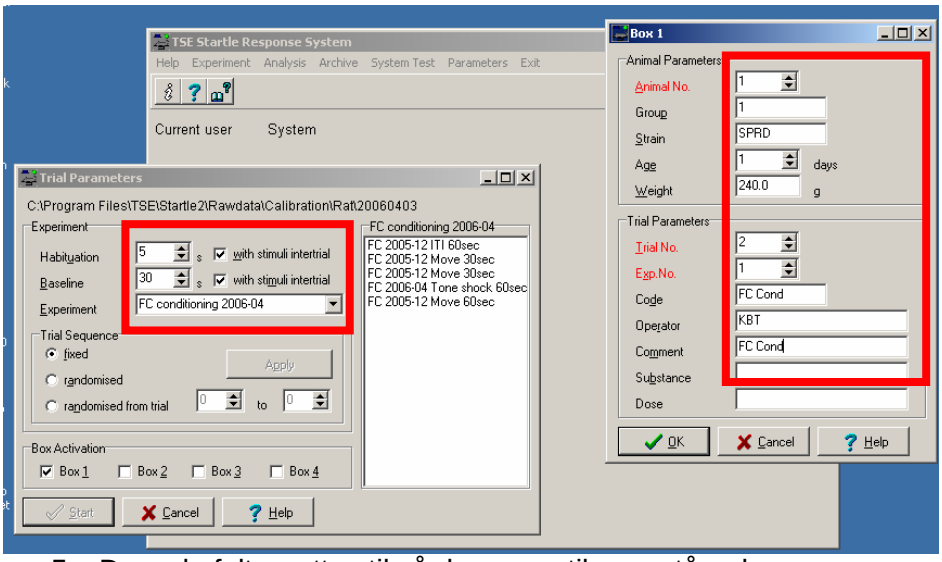


5. De røde felter rettes til så de svarer til ovenstående.
6. Alle fire bokse aktiveres (der skal være en tick ud for hver boks).
7. En rotte placeres i hver af startle kasserne og ind i startle kammeret. Når alle rotter til et system (4stk) er klar trykkes på 'Start' og systemet starter.
8. Når eksperimentet er slut kommer et nyt vindue frem. 'The experiment is finished' → Tryk 'OK'
9. Gentag proceduren indtil alle rotter har været I startle kammeret.

Dag 3: Conditionering (ca. 2 timer, afhænger af

forbehandlingstiden)

1. Tænd for computerne og kontrol boksene.
2. Åben 'Startle response'
    a. Name: System
    b. Password: System
    c. Tryk OK
3. Tryk på 'Experiment' → Vælg 'Experiment'
4. Skærmbilledet indstilles som vist nedenfor.

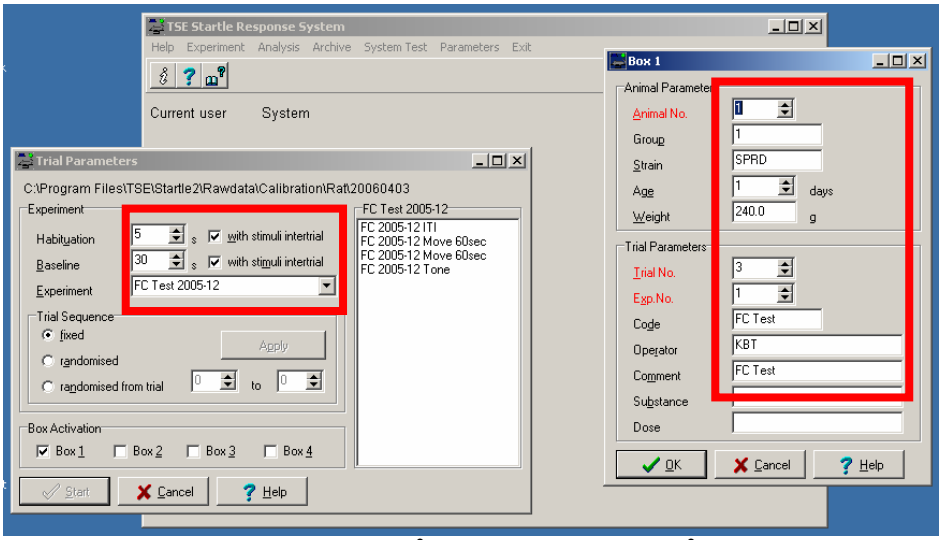


5. De røde felter rettes til så de svarer til ovenstående.
6. Alle fire bokse aktiveres (der skal være en tick ud for hver boks).
7. Undersøges effekten af et stof. Kan stoffet blive administreret inden conditioneringen, således at tiden nul er når der trykkes på start.
8. En rotte placeres i hver af startle kasserne og ind i startle kammeret. Der er et stik i startle kassen, dette leder strømmen så forsøgsdyret får et stød under konditioneringen. Er stikket ikke sat ind får dyret ikke stød.
9. Når alle rotter til et system (4stk) er klar trykkes på 'Start' og systemet starter.
10. Når eksperimentet er slut kommer et nyt vindue frem. 'The experiment is finished' → Tryk 'OK'
11. Gentag proceduren indtil alle forsøgsdyrene er blevet konditioneret.

Appendix 3.35.

Dag 4: Test (ca. 2 timer, afhænger af forbehandlingstiden)
Rotten sættes ned i startle kassen og ind i startle boksen. Når fire rotter er sat ind i et system (nyt foroven og gammel for neden). Gå over til computeren.

1. Tænd for computerne og kontrol boksene.
2. Åben 'Startle response'
    d. Name: System
    e. Password: System
    f. Tryk OK
3. Tryk på 'Experiment' → Vælg 'Experiment'
4. Skærmbilledet indstilles som vist nedenfor.



5. De røde felter rettes til så de svarer til ovenstående.
6. Alle fire bokse aktiveres (der skal være en tick ud for hver boks).
7. Undersøges effekten af et stof. Kan stoffet blive administreret inden testen, således at tiden nul er når der trykkes på start.
8. En rotte placeres i hver af startle kasserne og ind i startle kammeret.
9. Når alle rotter til et system (4stk) er klar trykkes på 'Start' og systemet starter.
10. Når eksperimentet er slut kommer et nyt vindue frem. 'The experiment is finished' → Tryk 'OK'
11. Gentag proceduren indtil alle forsøgsdyrene er blevet testet.

Appendix 3.35.

Når alle forsøg er afsluttet kan **data eksporteres.**

1. Vælg 'Analysis' → vælg 'Data Selection'
2. Vælg det dyr der skal eksporteres (dobbelt klik så det kommer ned i 'Selected Data Records'. Der kan kun eksporteres et dyr af gangen.
3. Tryk 'OK'
4. Vælg 'Analysis' → tryk 'Export Analog Data' → vælg stien til den mappe du oprettede i starten af forsøget gem rådata filerne under \Rawdata\From TSE computer. Eksporteres både konditionerings og test data skal disse deles i to foldere. Navnet på den enkelte fil skal svare til dyrets nummer. → Tryk 'Save'
5. Når alle dyr fra begge systemer er eksporteret enkeltvis, skal Baseline værdierne eksporteres.
6. Vælg 'Analysis' → vælg 'Data Selection' → Tryk 'ALL' (knap nederst). Alle data filerne vil nu transporteres ned i 'Selected Data Records' → Tryk 'OK'
7. Vælg 'Analysis' → tryk 'Export Tabel' → gem tabellen sammen med rådata husk at der er to tabeller en fra hvert system.

Programmet lukkes ned.
HUSK at slukke for kontrol boksene, da de ikke kan tåle at stå tændt natten over. Har de stået tændt skal de først køle ned inden man kan køre forsøg – dette kan godt tage en halv dag.

Parametre:

Appendix 3.35.

DATA-BEHANDLING:

Databehandlingen tager en hel dag. Der er følgende trin i databehandlignen:

A. Eksporter data fra TSE computeren i BS.18 (procedurer beskrevet ovenfor).
B. Sæt data ind i template I (baseline)
C. Importer data i template I og før det frem til template III
D. Sæt data ind i Template IV
E. Kør statestik på data
F. Lav grafer af data
G. Lav powerpoint siderne af data
H. Ret protokollen til med statestik og vægt.

Baseline værdierne skal beregnes. Under punkt 2 kan det være at tabellen ser forkert ud data kan være trukket sammen i en kolonne elelr er spredt over flere kolonner. Er dette tilfældet skal du ændre din computers regional settings til German. (Tryk på Start, vælg 'control panel', vælg ikonet 'Regional and Language Options'. Under reginal options kan man vælge 'German (germany)', Tryk 'Apply', tryk OK.)

1. Åben folderen Templates (J:\In vivo Pharmacology\Models in Pharmacology\Anxiety\Fear conditioning in rats).

2. Åben 'Template IV (data for graphs)' og gem filen i rawdata folderen. Filen navngives: YYYY-MM-DD-DD-compound-batch-rFC-route pretreatment-initals. Åben sheetet 'means' I kolonne A er der bogstaverne A-E – disse refererer til de fem stofgrupper, der har været anvendt I forsøget. I kolonne B kan du således indtaste hvilken gruppe der passer til de enkelte bogstaver.
3. Lad templaten være åben du skal bruge den om lidt.

4. Åben 'Template I (baseline)'
5. Åben tabellerne fra det system I og system II, der ligger under rawdata. I tabellen fra system II markeres fra A4 og resten af filen, kopier den markerede del af filen. Dette pastes ind i filen fra system II. → Marker hele datasættet som nu står i den nye fil. → kopier.
6. I Template I pastes det markerede datasæt ind i sheetet der hedder : 'Tabel paste'.
7. Templaten sætter nu data op på en lidt anden måde således
8. Åben sheetet der hedder 'Til template IV' og kopier data fra den blå kasse. → gå til 'Template IV' og paste data ind i den blå boks i 'Means'-sheetet.
9. Tryk GEM. Template I gemmes under rawdata folderen. Den gemmes bare til alt databehandling er afsluttet.

Databehandlingen kan tage ret lang tid, det mest tidskrævende er at importere data ind i template II (import data). Dette kræver en del tålmodighed, men det sparer dig for en masse tid. Det er Frosty (FLO), der har lavet den første template, er der problemer kan han være behjælpelig med at modificere templaten.

Når en datafil eksporteres fra TSE systemet er den meget stor, der er

optaget data hvert 4ms hvilket betyder at der er ca. 60.000 linier i en excel fil – hvilket alt for stort (et excel ark har 65.000 linier). Det Frostys template gør er, helt automatisk at beregne et gennemsnit for hvert sekund. Hvilket gør det mere håndterbart for den videre dataanalyse.

10. Åben template II (import data). Et nyt vindue popper op hvor du skal trykke på knappen, der hedder 'Enable macros' (vælger du en af de andre fungere templaten ikke). Template I kan bruges til både konditionerings og test data.
11. Gem templaten i den folder der blev oprettet i begyndelsen af forsøget under rawdata. Templaten navngives: YYYY-MM-DD_compound-batch-rFC -route-pretreatment-inital s.
12. I kolonne A er der en knap/ikon, hvor der står Import data.
13. Klik på knappen. → Et nyt vindue åbner nu op.
14. Find stien hvor du gemte rådatafilerne fra TSE-computeren. → Vælg fil nr. 1. → tryk OK.
15. Når programmet er klar igen vil du igen kunne klikke på Import data. Alle datafilerne importeres det er meget vigtigt at de står i nummerorden ellers vil du ikke kunne bruge den efterfølgende templates.
16. Der kan være at programmet lukker ned og spørger om der skal sendes en fejlreport tryk på "Don't send", og VENT. Det kan godt tage 15 min men computeren genskaber templaten. Vælger du at lukke ned, er der stor risiko for at der ikke er gemt noget og så kan du bare starte forfra.
17. Når alle filerne er importeret tjekkes det om alle filerne nu også er der og står i den rigtige rækkefølge.

Databehandling af konditionerings data

18. Åben 'Template III - COND (means in groups)'
19. Gå tilbage til template II og kopier hele data sættet fra kolonne B og resten af datasættet.
20. Paste datasættet ind i sheetet der hedder Rawdata i template III.
21. Marker hele datasættet fra sheetet og kopier.
22. Hele datasættet kopieres nu ind i Template IV (som allerede er åben!) i det sheet der hedder 'COND data'.

Databehandling af test data

23. Åben 'Template III - TEST (means in groups)'
24. Gå tilbage til template II og kopier hele data sættet fra kolonne B og resten af datasættet.
25. Paste datasættet ind i sheetet der hedder Rawdata i template III.
26. Marker hele datasættet fra sheetet og kopier.
27. Hele datasættet kopieres nu ind i Template IV (som allerede er åben!) i det sheet der hedder 'TEST data'.
28. Både for konditionerings og test data kan du tjekke at de rette dyr er blevet overført til den rette gruppe. Har du kørt forsøget i den rækkefølge der er angivet ovenfor skal følgende dyr være i hver gruppe

    A: 1,6,11,16,21,26,31,36
    B: 2,7,12,17,22,27,32,37
    C: 3,8,13,18,23,28,33,38
    D: 4,9,14,19,24,29,34,39

Appendix 3.35.

E: 5,10,15,20,25,30,35,40

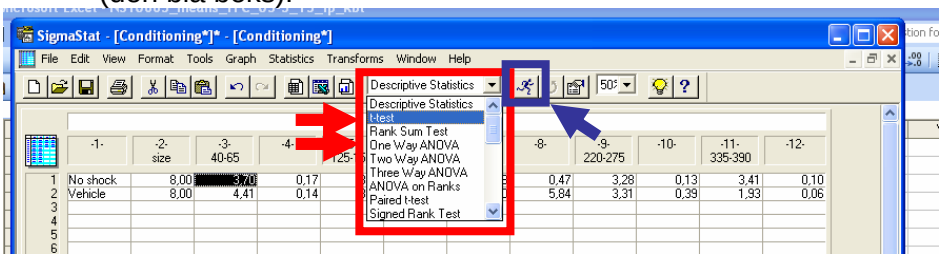
Du er nu klar til at køre statestik!

Statestik

1. Åben 'Templates' → vælg 'Statistics'

Analyse af konditioneringsdata

2. Åben 'conditioning'. → Gem templaten i den folder du startede forsøget med at oprette i den folder der hedder 'statistics'. Navngives efter følgende regel: compound-batch-route-pretreatment-date-initals.
3. Templaten er delt i to dele, kolonne 1-14 skal anvendes til at analysere om et shock har haft en effekt på forsøgsdyrene, dvs. om de har lært testen. Kolonne 16-29 anvendes til at se om der er en stofeffekt.
4. Gå til 'Template IV' → klik på sheetet 'means'. → marker cellen J14-U14 og J15-U15 → kopier området.
5. Åben 'conditioning' templaten igen og sæt cursoren i den kolonne, der hedder 3 (40-65) i den første række. → paste.
6. Gå nu tilbage til 'Template IV' → klik på sheetet 'means'. → marker cellen J15-U15 og J18-U18 → kopier området.
7. Åben 'conditioning' templaten igen og sæt cursoren i den kolonne, der hedder 18 (40-65) i den første række. → paste.
8. I kolonnerne der hedder size indtastes det antal dyr der er anvendt i de enkelte grupper.
9. Der skal nu køres en lang række analyser. Det er en god ide at printe protokollen så man slipper for at skulle klikke frem og tilbage når man skal skrive værdier ind.
10. I den røde boks neden for kan du se de to test der skal anvendes. I første omgang skal der køres t-test på første del af data sættet. Tryk på den lille pil til højre for Descriptive Statistics, herved bliver rullegardinet synligt (det i den røde boks). → klik på t-test → klik herefter på den løbende mand (den blå boks).

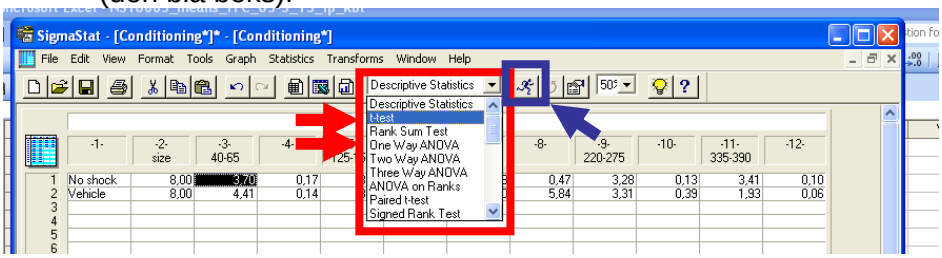


11. Et nyt vindue popper op. Under data format vælg: 'Mean, size, standard error'. → Tryk 'Next'
12. Klik på kolonnen med mean (ex. 40-65mean) → klik på kolonnen size → klik på kolonnen med sem. → klik finish
13. Et nyt vindue popper op. Hvor man kan se resultatet af analysen. Noter om der er signifikans i protokollen (røde tal). Er der ikke signifikans skriv 'n.s.' (=not significant).
14. Herefter testes om der er effekt af stof. Vælg nu One Way ANOVA → klik herefter på den løbende mand (den blå boks).

15. Et nyt vindue popper op. Under data format vælg: 'Mean, size, standard error'. → Tryk 'Next'
16. Klik på kolonnen med mean (ex. 40-65) → klik på kolonnen size → klik på kolonnen med sem. → klik finish
17. Et nyt vindue popper op. Enten hvor man kan se resultatet af analysen. Eller hvor man får mulighed for at køre en post hoc test. Vælg Dunnetts → Sammenligningen skal være overfor vehikel gruppen (Row 1) → Tryk 'Finish'. Et nyt vindue popper op med resultatet af analysen. Noter om der er signifikans i protokollen (røde tal). Er der ikke signifikans skriv 'n.s.' (=not significant).

Analyse af test data

18. Åben 'test'. → Gem templaten i den folder du startede forsøget med at oprette i den folder der hedder 'statistics'. Navngives efter følgende regel: compound-batch-route-pretreatment-date-initals.
19. Templaten er delt i to dele, kolonne 1-14 skal anvendes til at analysere om et shock har haft en effekt på forsøgsdyrene, dvs. om de har lært testen. Kolonne 16-29 anvendes til at se om der er en stofeffekt.
20. Gå til 'Template IV' → klik på sheetet 'means'. → marker cellen J4-U4 og J5-U5 → kopier området.
21. Åben 'conditioning' templaten igen og sæt cursoren i den kolonne, der hedder 3 (C1) i den første række. → paste.
22. Gå nu tilbage til 'Template IV' → klik på sheetet 'means'. → marker cellen J5-U5 og J8-U8 → kopier området.
23. Åben 'conditioning' templaten igen og sæt cursoren i den kolonne, der hedder 18 (C1) i den første række. → paste.
24. I kolonnerne der hedder size indtastes det antal dyr der er anvendt i de enkelte grupper.
25. Der skal nu køres en lang række analyser. Det er en god ide at printe protokollen så man slipper for at skulle klikke frem og tilbage når man skal skrive værdier ind.
26. I den røde boks neden for kan du se de to test der skal anvendes. I første omgang skal der køres t-test på første del af data sættet. Tryk på den lille pil til højre for Descriptive Statistics, herved bliver rullegardinet synligt (det i den røde boks). → klik på t-test → klik herefter på den løbende mand (den blå boks).



27. Et nyt vindue popper op. Under data format vælg: 'Mean, size, standard error'. → Tryk 'Next'
28. Klik på kolonnen med mean (ex. C1) → klik på kolonnen size → klik på kolonnen med sem. → klik finish
29. Et nyt vindue popper op. Hvor man kan se resultatet af

analysen. Noter om der er signifikans i protokollen (røde tal). Er der ikke signifikans skriv 'n.s.' (=not significant).

30. Herefter testes om der er effekt af stof. Vælg nu One Way ANOVA → klik herefter på den løbende mand (den blå boks).
31. Et nyt vindue popper op. Under data format vælg: 'Mean, size, standard error'. → Tryk 'Next'
32. Klik på kolonnen med mean (ex. C1) → klik på kolonnen size → klik på kolonnen med sem. → klik finish
33. Et nyt vindue popper op. Enten hvor man kan se resultatet af analysen. Eller hvor man får mulighed for at køre en post hoc test. Vælg Dunnetts → Sammenligningen skal være overfor vehikel gruppen (Row 1) → Tryk 'Finish'. Et nyt vindue popper op med resultatet af analysen. Noter om der er signifikans i protokollen (røde tal). Er der ikke signifikans skriv 'n.s.' (=not significant).

Analyse af Baselineværdierne.

34. Først sammenlignes vehikel dyrene, dvs de dyr der har fået stød mod de dyr der ikke har fået stød. Benyt t-test.
35. Dernæst analyseres stofgrupperne mod vehiklen. Der anvendes en ONE-WAY ANOVA. Se ovenfor hvordan Sigma stat anvendes.

Så skal der laves grafer. Der er to typer af grafer der er 'timelines' og 'means'.

MEAN GRAF

36. Åben folderen 'Templates' → Kopier folderen 'Graphs' → Åben den folder der blev dannet i starten af forsøget → paste folderen 'Graphs' ind i folderen.
37. Den første graf er af gennemsnit (means) denne skal bruges til at sætte ind i powerpoint siden, den giver et hurtigt overblik over hvordan forsøget er gået.
38. Åben 'Graphs' → Åben 'Means' → åben 'mean graphs for powerpoint'. → Gem grafen. Navn gives som følger: Compound-batch-route-pretreatment-date-initals. Der skal gemmes to gange, den ene graf er til konditionerings data den anden er til test data. Beskrivelsen for de to grafer er ens. Det er derfor meget vigtigt at få skrevet på grafen om det er konditionering eller test!
39. Åben 'Template IV' → åben sheetet 'means' → kopier data fra 'Baselines' → paste dem ind under baselines i graf data sheetet.
40. Gå tilbage til 'Template IV' og 'means' sheetet. → kopier data fra C1-C2-tone-C3-C4-context → paste data i de respektive kolonner i graf-sheetet.
41. Klik på graf-siden → tilpas tidsintervaller under grafen.
    - C1: 40-70 (konditionering) og 40-100 (test)
    - C2: 125-155 (konditionering) og 105-165 (test)
    - Tone: 210-220 (konditionering) og 170-180 (test)
    - C3: 220-285 (konditionering) og 180-230 (test)
    - C4: 335-395 (konditionering) og230-280 (test)
    - Context: Det er gennemsnittet af C1-C2-C3-C4 det vil

sige hele perioden hvor der ikke er tone.

42. Sæt stjerner på graferne svarende til den statistiske analyse.
43. Marker alt og kopier graferne over på powerpoint siden (der ligger i den folder der er blevet oprettet til forsøget).

TIMELINE GRAF

Timeline graferne giver et mere detaljeret billede af forsøget. Der er to sæt grafer, det første sæt er kontrol-forsøget, hvor vehikel gruppen der ikke har fået stød bliver sammenlignet med vehikel dyrene der har fået stød. Den anden gruppe af grafer er sammenligningen af vehikler med stofgrupperne.

Konditioneringsdata no shock vs shock

44. Gå til 'Template IV' → tryk på sheetet 'COND' → kopier C5-D5 til C42-D42. → tilbage til grafen.
45. Åben 'Conditioning data' →sæt cursoren i kolonne 2 række 1 (under no shock) → tryk paste. Gå tilbage til template IV og kopier det tilsvarende datasæt fra vehikel og paste det ind i graf templaten.
46. Gå tilbage til 'Template IV' → åben 'means' → kopier J14-J15 til U14-U15. → gå tilbage til graf-templaten og sæt cursoren i kolonne 7 række 1 → tryk paste
47. Åben grafsiden og tilpas teksten og noter den statistiske analyse på grafen.

Test-data no shock vs shock

48. Åben 'Graphs' → Åben 'Timelines' → åben 'no shock vs shock-test. → åben baseline data sheetet.
49. Åben 'Template IV' → åben 'means' sheetet → kopier C4-C5 til H4-H5.
50. Gå tilbage til grafens data sheet. → sæt cursoren i kolonne tre i række et og paste. → gå til graf siden tilpas teksten og noter den statistiske analyse på grafen.
51. Gå til 'Template IV' → tryk på sheetet 'TEST' → kopier C5-D5 til C50-D50. → tilbage til grafen.
52. Åben 'Test data' →sæt cursoren i kolonne 2 række 1 (under no shock) → tryk paste. Gå tilbage til template IV og kopier det tilsvarende datasæt fra vehikel og paste det ind i graf templaten.
53. Gå tilbage til 'Template IV' → åben 'means' → kopier J4-J5 til U4-U5. → gå tilbage til graf-templaten og sæt cursoren i kolonne 7 række 1 → tryk paste
54. Åben grafsiden og tilpas teksten og noter den statistiske analyse på grafen.

CONDITIONING-graf

55. Åben 'Graphs' → Åben 'Timelines' → åben 'CONDITIONING-compound-batch-route-pretreatment-date-initials' → åben baseline data sheetet.
56. Åben 'Template IV' → åben 'means' sheetet → kopier C5-C8 til H5-H8.
57. Gå tilbage til grafens data sheet. → sæt cursoren i kolonne tre i række et og paste. → gå til graf siden tilpas teksten og noter den statistiske analyse på grafen.

58. Gå til 'Template IV' → tryk på sheetet 'COND' → kopier G5-H5 til G42-H42. → tilbage til grafen.
59. Åben 'Data 0.3mg/kg) →sæt cursoren i kolonne 2 række 1 (under vehicle) → tryk paste. Gå tilbage til template IV og kopier det tilsvarende datasæt fra 0.3mg/kg og paste det ind i graf templaten.
60. Gå tilbage til 'Template IV' → åben 'means' → kopier J15-J16 til U15-U16. → gå tilbage til graf-templaten og sæt cursoren i kolonne 7 række 1 → tryk paste
61. Åben grafsiden og tilpas teksten og noter den statistiske analyse på grafen.
62. Pkt. 58-61 gentages for de næste to doser af stofgruppen.

TEST-graf

63. Åben 'Graphs' → Åben 'Timelines' → åben 'Test-compound-batch-route-pretreatment-date-initials' → åben baseline data sheetet.
64. Åben 'Template IV' → åben 'means' sheetet → kopier C5-C8 til H5-H8.
65. Gå tilbage til grafens data sheet. → sæt cursoren i kolonne tre i række et og paste. → gå til graf siden tilpas teksten og noter den statistiske analyse på grafen.
66. Gå til 'Template IV' → tryk på sheetet 'TEST' → kopier G5-H5 til G50-H50. → tilbage til grafen.
67. Åben 'Data 0.3mg/kg) →sæt cursoren i kolonne 2 række 1 (under vehicle) → tryk paste. Gå tilbage til template IV og kopier det tilsvarende datasæt fra 0.3mg/kg og paste det ind i graf templaten.
68. Gå tilbage til 'Template IV' → åben 'means' → kopier J5-J6 til U5-U6. → gå tilbage til graf-templaten og sæt cursoren i kolonne 7 række 1 → tryk paste
69. Åben grafsiden og tilpas teksten og noter den statistiske analyse på grafen.
70. Pkt. 66-70 gentages for de næste to doser af stofgruppen.

Der skal nu printes.
Følgende skal printes og sættes i 'Rat Fear Conditioning' mappen:

- Protokol (med statestik) og forsøgssedlen der er brugt i laboratoriet med vægt. (vægt beregnes og skrives på protokollen)
- Powerpoint side for konditionering, powerpoint side for test.
- No shock vs shock (alle grafer)
- Vehikel vs. Stofgrupper (alle grafer)

REFERENCES.:

Appendix 3.36.

Prepulse Inhibition (PPI) in Mice or Rats		
NEUROSEARCH Pharmacological Department		NS-Code: **2.1. ????.XXX**
Author: Date:	Accepted by: Date:	Dyreforsøgstilladelse: 18_15 2000/561-306

FORMÅL
TEORI

Appendix 3.36.

FORSØGETS UDFØRELSE

Inden forsøgets start

1. Der laves en forsøgsprotokol ud fra skabelonen ’PPI forsøgsprotokol’, der findes under ’J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Work sheets’.

2. Der laves en informationsseddel til scantaineren. Skabelonen hedder ’informations seddel dyr 2005-06-01’ og kan findes under ’J:\Andet\Dyreforsøg\Information’ (evt. udfylde det meste og lægge under ’J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Work sheets’)

Indstilling af udstyr

3. Der er forskellige transducere til mus og rotter, og det tjekkes om de korrekte transducere sidder i boksene. Hvis de skal skiftes hives ledningen ud foran, transducerne udskiftes, og ledningen sættes i igen. Knappen foran på de grå bokse skal indstilles til den type dyr forsøget køres med.

4. Tænd computerne og log på begge systemer med user name: **startle** og password: **startle1**.
   Switch knap: For at spare plads er der installeret en swich-box og kun en skærm. Switch boksen har to knapper, knap hører til cpu 1 (det nye system) og knap to hører til cpu 2 (det gamle system). Ved hver af knapperne er der to små lys, de orange er altid tændt og det grønne referer til hvilket system man arbejder på. Man skifter mellem systemerne ved at trykke to gange på crtl-knappen. Der kan frit skiftes mellem de to systemer, også mens et forsøg kører.

5. Tænd bagpå de grå bokse (interface), der står mellem computeren og startle boksene

6. Der oprettes tre foldere til forsøgsresultaterne.
   a. Opret en folder under ‘J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Results’ under den type stof, der testes i forsøget. Dette gøres ved at kopiere den eksisterende mappe ’Stoftype XXXX’ og omdøbe den nye mappe til navnet på det stof, der testes. I denne mappe findes to biblioteker ’Raw data’ og ’Statistics’, og resultaterne fra forsøget lægges under ’Raw data’ når de eksporteres.
   b. Tryk på ’Desktop’ → ’Shortcut to Rawdata’ → ’Calibration’ → ’Mouse/Rat (i system 2: Mouse transducers/Rat transducer)’. Der oprettes en ny folder, som navngives med dags dato (YYYYMMDD), hvor kalibreringsdata gemmes.
   c. Tryk på ’Desktop’ → ’Shortcut to Rawdata’ → ‘PPI2006’ → ‘Mouse/Rat’. Der oprettes en folder, som navngives dags dato (YYYYMMDD). I denne folder gemmes al rådata inden det eksporteres til den mappe, der blev oprettet under pkt. 6.a.

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Opstart af programmet

7. Start startle programmet for hvert system på desktoppen. Log ind i programmet med user name: **System** og password: **System**.

8. Check under Program Parameters: Amplification sensor= 500 (rotter). Sampling interval skal være 2 ms. Hvis der ændres på 'Samling interval' skal programmet genstartes

9. Tryk på 'System Test'. Hvis boksene ikke er tændt kommer der en fejlmeddelelse på skærmen. Når der er trykkes på 'Sound' og 'Noise', skal der høres lyde fra startle boksene.

Kalibrering af transducerne

10. Transducerne skal kalibreres hver gang et nyt forsøg køres, og transducerne kalibreres én ad gangen. Den kunstige rotte placeres på transduceren i box nr. 1 ca. på midten og ledningen placeres, så den ikke hiver i 'rotten'.

11. Vælg 'Archive' og 'Data Directory'. Vælg den folder, der blev oprettet under pkt. 6.b. Resultaterne fra kalibreringen bliver gemt i denne folder.

12. Vælg 'Experiment' og 'Experiment'. Både 'Habituation' og 'Baseline' skal været indstillet til 2 sekunder. Under 'Experiment' vælges for system 1: 'Soren-test, 110dB' og for system 2: 'Soren-test'

13. Under 'Box Activation' trykkes der på hver af boxene, og et nyt vindue kommer nu frem. Under 'Animal No.' skrives nummeret på boksen, og under 'Trial No.' Skrives nummeret på den omgang der køres for hver boks. Første runde indstilles der ikke på systemet. Tryk 'OK' og 'Start'.

14. Efter første runde tjekkes resultaterne ved at vælge 'Analysis' og 'Data Selection'. Der kommer nu et vindue op med to felter. I det øverste felt ses alle forsøg, der er gemt i det pågældende bibliotek. Først trykkes der på 'None' så alle data fra den nederste liste fjernes, og derefter dobbeltklikkes på det forsøg, der ønskes vist. Tryk 'OK'.

15. Vælg 'Analysis' og 'Results Table', hvorefter et nyt vindue kommer frem.

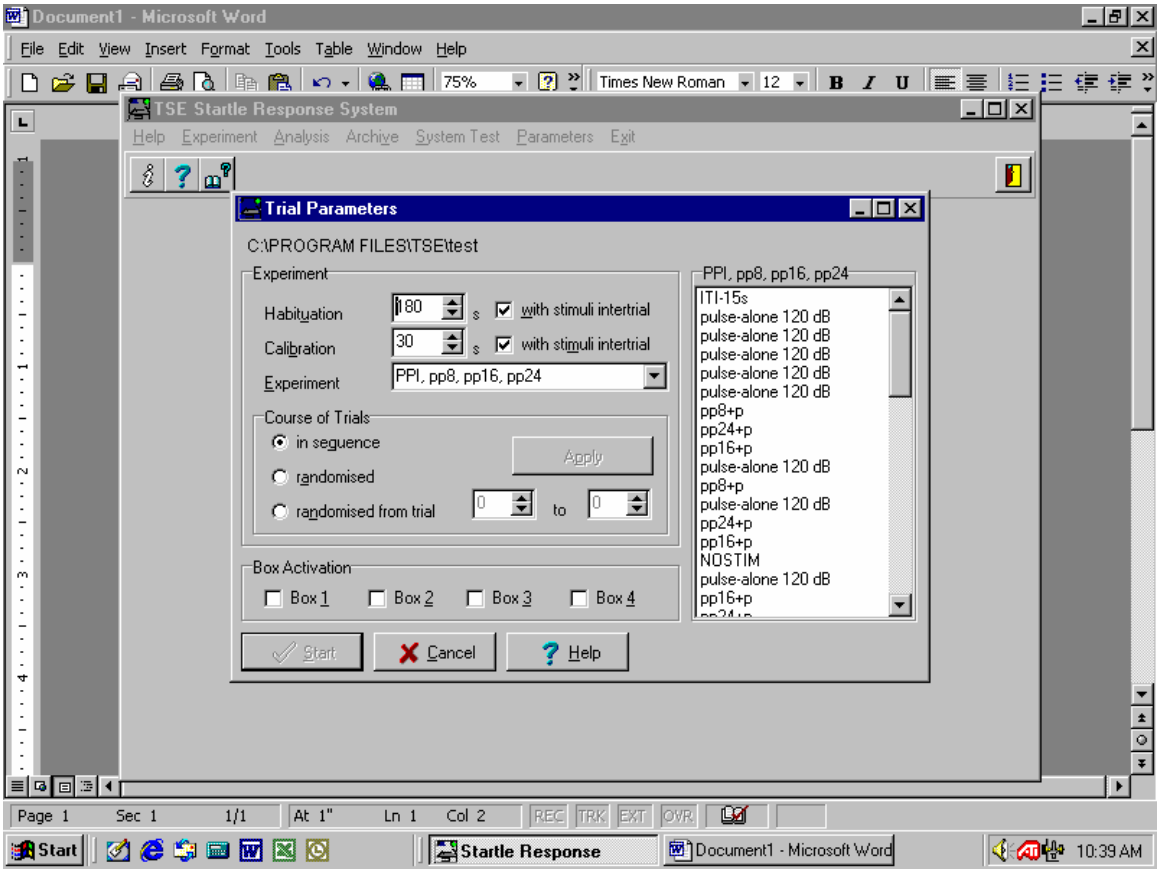
16. I nederste linje ses gennemsnittet af de ti målinger, der er foretaget i forsøget, og den skal ligge omkring 150 (± 2). Hvis værdien ligger udenfor dette område, indstilles der på potentimeteret på den grå boks vha. skruetrækkeren, der ligger i øverste skuffe. Skruetrækkeren sættes forsigtigt ind i hullet og ved at dreje med uret sænkes responset og omvendt. På grafen skal outputtet være omkring 300, for at gennemsnitsværdien bliver ca. 150. Herefter køres en ny runde, og dette gentages indtil gennemsnitsværdien ligger inden for intervallet. Til sidst køres en runde, hvor der ikke indstilles på systemet, mens forsøget kører.

17. Kalibreringsproceduren gentages indtil alle otte bokse er kalibreret.

Udførelse af forsøget

18. Vælg 'Archive' og 'Data Directory'. Vælg den folder, der blev oprettet under pkt. 6.c. Resultaterne fra forsøget bliver nu gemt i denne folder.

19. Tryk på 'Eksperiment' → 'Experiment' og vælg for system 1: 'PPI,pp4, 8, 16, 24, 110dB pulse, all+5db' og for system II: 'PPI, pp4, 8, 16, 24, 110dB pulse'. Habituation og calibration sættes til hhv. 180 og 30 sek. (default indstilling). 'Trial Sequence' skal være sat til 'fixed'. Boksene vælges én ad gangen, og ved hver box skrives 'Animal nr.' og under 'Comment' skrives hvilken dosis det pågældende dyr har fået.



20. Dyr 1-4 og 5-8 doseres med veh, dose 1, dose 2, dose 3.

21. Efter forbehandlingstiden, kommes dyrene i burene - pas på ikke at klemme haler og fødder! Burene sættes på de små aluminiumsbakker og sættes ind i startle boksene. Dyr 1-4 sættes ind i system 1 og dyr 5-8 sættes ind i system 2.

22. Luk lågerne og klik på start. Programmet varer ca. 21½ min, og der er 82 trials i alt.

23. Dyr 9-12 og 13-16 doseres med dose 1, dose 2, dose 3 og veh. For hver runde forskubbes doserne på denne måde, så forsøget bliver balanceret.

24. Mellem hver runde renses bure og aluminiumsbakker med en våd serviet og der tørres efter med en tør serviet.

Overførsel af rådata fra computeren til J-drevet

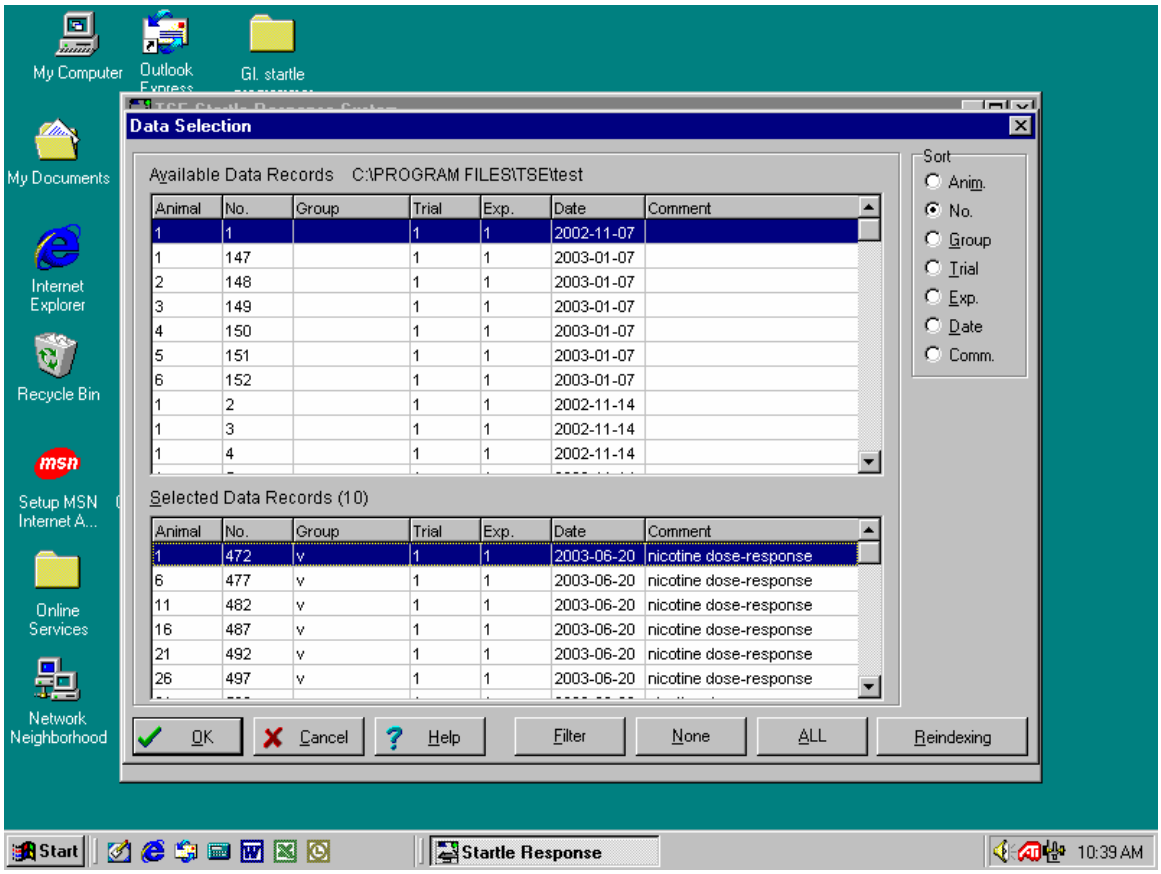
25. Vælg Analysis → Analysis Parameters. På højre side af dette billede er der en lang række parametre. Følgende punkter skal være markerede:
    a. Animal Number
    b. Trial Number
    c. Experiment Number
    d. Date
    e. Start Time

    De øvrige skal ikke markeres da de ellers ikke vil passe til templaten.
    Tryk OK

26. Vælg i menuen: Analysis → Data Selection. I vinduet ses der nu 2 lister. Den øverste indeholder alle data, der er lavet med programmet i den pågældende mappe. Vha. f.eks. Animal No. og dosen kan man lokalisere de data, man gerne vil eksportere. Vha. Filter kan man sortere data efter f.eks. dato. På den nederste liste vises de data, der er valgt fra den øverste liste.

27. Tryk først på knappen 'None', så alle data fra den nederste liste i vinduet fjernes. Herefter finder man de første dyr man vil eksportere (fx vehicle gruppen) fra den øverste liste og ligger dem ned i den nederste liste ved at dobbeltklikke på dem én efter én (hvis man kommer til at overføre et forkert dyr, kan det sættes tilbage på den øverste liste igen ved at dobbeltklikke på det).

Appendix 3.36.



28. Når de første dyr er valgt, klikkes OK og i menuen vælges Analysis → Export analogue data. Filnavnet opgives som 'dosis' for eksempel: 'veh' og desuden tilføjes 'system 1' eller 'system 2' alt efter hvilket system man arbejder på. Gem filen i den folder, der blev oprettet under pkt. 6.a: 'J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Results\Stoftype\Stofnavn\Raw data'

29. Pkt. 25 og 26 gentages for de næste stofgrupper indtil al data er overført til J-drevet.

Databehandling

30. Det eksporterede data kan nu behandles fra alle computere, der har adgang til J-drevet. For hver stofgruppe åbnes de to filer fra system 1 og 2, der lægges sammen til én. I filen fra system 2 kopieres fra celle A4 og resten af datasættet, og det indsættes under datasættet i filen fra system 1. For at datasættet passer til templaten slettes kolonne C 'Trial No.'. Det sammenlagte datasæt gemmes nu under navnet 'dosis'. Hvis tallene ikke er inddelt i kolonner, kan man gøre følgende: Markér kolonne J og vælg i menuen: Data → Text to columns. Her vælges Delimited → Tab slås fra, og space og semicolon slås til → Under Advanced… vælges Punktum som decimal separator, og Space (dvs. det blanke felt) vælges som Thousands separator. Nu skulle tallene gerne være delt ind i kolonner, og tallene i kolonne J skal stå i højre side af cellen (det betyder, at

Excel genkender dem som tal og ikke som tekst). Dette kan også gøres ved at gå ind i computerens Settings → Control Panel → Regional options → Numbers, og sætte Decimal symbol til punktum, og List separator til komma (husk at trykke Apply).

31. Åbn filen med det sammenlagte datasæt for vehicle gruppen. Kopier hele arket ved at trykke Ctrl+A → copy.

32. Åbn den relevante rådata-template, som ligger under 'J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Templates'. Tryk 'Enable Macros' og Indsæt det kopierede ark i celle A1 i det første sheet ('Rådata 8 dyr').

33. Gå til det sidste sheet i filen ('Beregning til graf'). Skriv den rigtige dosis i celle A1.

34. Kopier kolonne A-G. Åbn Graf-templaten, som ligger under 'J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Templates'. Svar 'No' til at update links.

35. De kopierede kolonner indsættes i A1 ved at højreklikke og derefter trykke 'Paste Special' → 'Paste value'.

36. Ved behandling af data fra næste dosisgruppe gentages punkt 31-34. De kopierede kolonner indsættes nu under næste dose (i Graf-templatens celle T1), og dette gentages for alle grupper.

37. Filen med den færdige graf gemmes under 'J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Results\Stoftype\Stofnavn'. Evt. outliers (ud fra kriterier i kolonne O-R med rød skrift) fjernes (både kolonne I-L og kolonne O-R) – denne graf gemmes under samme bibliotek.

38. Fra sheet "Til SigmaPlot" kopieres tallene (start celle A4) over i Sigma Plot-templaten '…', der ligger under 'J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Templates'. Filen gemmes under 'J:\In vivo Pharmacology\Models in Pharmacology\Schizophrenia\Prepulse Inhibition in mice (or rats)\Results\Stoftype\Stofnavn'

Appendix 3.37.

Programs used in traditional fear conditioning

FC Habituation

Context ITI 45dB KBT
- After 0ms for 50ms, Noise, 45dB
- 50ms to 50ms, Intertrial

Habituation 45 dB
- After 0ms for 390000ms, Noise, 45dB
- After 0ms for 100ms, Acquisition

FC Conditioning 2006-04

FC2005-12 ITI 60 sec
- After 0ms for 60000ms, Noise, 45dB
- 60000ms to 60000ms, Intertrial

FC2005-12 Move 30 sec
- After 0ms for 30000ms, Noise, 45dB
- After 0ms for 30000ms, Acquisition

FC2005-12 Move 30 sec
- After 0ms for 30000ms, Noise, 45dB
- After 0ms for 30000ms, Acquisition

FC2006-04 Tone Shock 60 sec
- After 0ms for 10000ms, Sound, 5000 Hz, 80dB
- After 0ms for 70000ms, Acquisition
- After 9000ms for 10000ms, E-stim, 0.6mA
- After 10000ms for 60000ms, Noise, 45dB

FC2005-12 Move 60 sec
- After 0ms for 60000ms, Noise, 45dB
- After 0ms for 60000ms, Acquisition

FC Test 2005-12

FC2005-12 ITI
- After 0ms for 10000ms, Noise, 45dB
- 10000ms to 10000ms, Intertrial

FC2005-12 Move 60 sec
- After 0ms for 60000ms, Noise, 45dB
- After 0ms for 60000ms, Acquisition

FC2005-12 Move 60 sec
- After 0ms for 60000ms, Noise, 45dB
- After 0ms for 60000ms, Acquisition

FC2005-12 Tone
- After 0ms for 10000ms, Sound, 5000Hz, 80dB
- After 0ms for 11000ms, Acquisition
- After 10000ms for 100000ms, Noise, 45dB

Appendix 3.38.

Program used on test day in extended-tone fear conditioning

FC Test 2006-10 Tone-ext twice

- FC 2005-12 ITI
  - After 0ms for 10000ms, Noise, 45dB
  - 10000ms to 10000ms, Intertrial
- FC 2006-10 Move 40sec
  - After 0ms for 40000ms, Noise, 45dB
  - After 0ms for 40000ms, Acquisition
- FC 2006-10 Tone-ext 90sec
  - After 0ms for 90000ms, Sound, 5000Hz, 80dB
  - After 0ms for 90000ms, Acquisition
- FC 2006-10 Move 40sec
  - After 0ms for 40000ms, Noise, 45dB
  - After 0ms for 40000ms, Acquisition
- FC 2006-10 Tone-ext 90sec
  - After 0ms for 90000ms, Sound, 5000Hz, 80dB
  - After 0ms for 90000ms, Acquisition

Appendix 3.39.

Programs used in trace fear conditioning

FC Habituation 45dB MKH

- Context ITI 45dB KBT
  - After 0ms for 50ms, Noise, 45dB
  - 50ms to 50ms, Intertrial
- Habituation 45dB MKH
  - After 0ms for 390000ms, Noise, 45dB
  - After 0ms for 270000ms, Acquisition

TFC tone shock no trace KBT

- TFC ITI 50ms KBT
  - After 0ms for 50ms, Noise, 45dB
  - 50ms to 50ms, Intertrial
- TFC tone shock no trace KBT
  - After 0ms for 10000ms, Noise, 45dB
  - After 0ms for 30000ms, Acquisition
  - After 10000ms for 10000ms, Sound, 5000Hz, 80dB
  - After 19000ms for 1000ms, E-stim, 0.6mA
  - After 20000ms for 30000ms, Noise, 45dB
  - After 20000ms for 10000ms, Noise, 45dB

TFC tone shock 60sec trace KBT

- TFC ITI 50ms KBT
  - After 0ms for 50ms, Noise, 45dB
  - 50ms to 50ms, Intertrial
- TFC tone shock 60sec trace KBT
  - After 0ms for 10000ms, Noise, 45dB
  - After 0ms for 90000ms, Acquisition
  - After 10000ms for 10000ms, Sound, 5000Hz, 80dB
  - After 20000ms for 70000ms, Noise, 45dB
  - After 79000ms for 1000ms, E-stim, 0.6mA

TFC LIS MKH

- TFC ITI 50ms KBT
  - After 0ms for 50ms, Noise, 45dB
  - 50ms to 50ms, Intertrial
- TFC 140sec acquisition
  - After 0ms for 140000ms, Acquisition
  - After 0ms for 140000ms, Noise, 45dB
  - After 15000ms for 10000ms, Sound, 5000Hz, 80dB
  - After 25000ms for 75000ms, Noise, 45dB

Appendix 3.40.

Program used in pre-pulse inhibition

PPI,pp4, 8, 16, 24, 110dB pulse, all+5dB

ITI-15s with 70dB background
- After 0ms for 20000ms, Noise, 70dB
- 10000ms to 20000ms, Intertrial

Start pulse alone 110 dB, all+5dB
- After 0ms for 40ms, Noise, 115dB
- After 0ms for 64ms, Acquisition
- After 40ms for 24ms, Noise, 70dB

Start pulse alone 110 dB, all+5dB
Start pulse alone 110 dB, all+5dB
Start pulse alone 110 dB, all+5dB
Start pulse alone 110 dB, all+5dB
Pp8+pp110, all+5dB
- After 0ms for 20ms, Noise, 78dB
- After 20ms for 80ms, Noise, 70dB
- After 100ms for 40ms, Noise, 115dB
- After 100ms for 65ms, Acquisition
- After 140ms for 24ms, Noise, 70dB

Pp4+pp110, all+5dB
- After 0ms for 20ms, Noise, 74dB
- After 20ms for 80ms, Noise, 70dB
- After 100ms for 40ms, Noise, 115dB
- After 100ms for 65ms, Acquisition
- After 140ms for 24ms, Noise, 70dB

Pp16+p110, all+5dB
- After 0ms for 20ms, Noise, 86dB
- After 20ms for 80ms, Noise, 70dB
- After 100ms for 40ms, Noise, 115dB
- After 100ms for 65ms, Acquisition
- After 140ms for 24ms, Noise, 70dB

Pulse-alone 110dB, all…
- After 0ms for 40ms, Noise, 115dB
- After 0ms for 64ms, Acquisition
- After 40ms for 24ms, Noise, 70dB

Pp24+p110
- After 0ms for 20ms, Noise, 94dB
- After 20ms for 80ms, Noise, 70dB
- After 100ms for 40ms, Noise, 115dB
- After 100ms for 65ms, Acquisition
- After 140ms for 24 ms, Noise, 70dB

Pp8+p110
Pulse-alone 110dB
Pp4+p110
Pp16+p110
NOSTIM with 70dB background

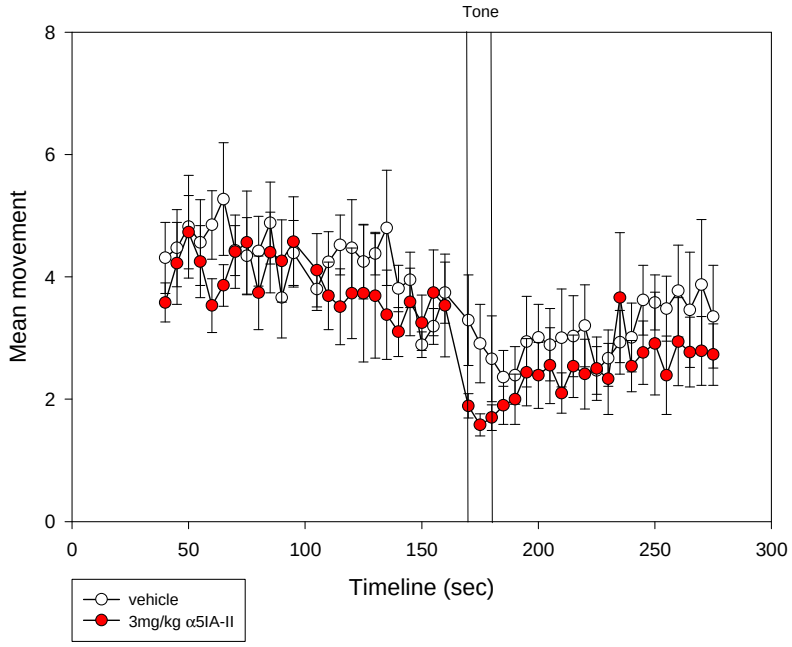
After 0ms for 164ms, Noise, 70dB
After 100ms for 65ms, Acquisition
NOSTIM with 70dB background
Pp24+p110
Pp24+p110
Pulse-alone 110dB
Pp16+p110
Pp4+p110
Pp4+p110
NOSTIM with 70dB background
Pp8+p100
Pp24+p110
NOSTIM with 70dB background
Pp8+p110
Pp16+p110
Pulse-alone 110dB
Pp24+p110
NOSTIM with 70dB background
Pp4+p110
Pp8+p110
Pp4+p110
Pp16+p110
NOSTIM with 70dB background
Pulse-alone 110dB
Pp24+p110
Pp16+p110
Pp8+p110
Pulse-alone 110dB
NOSTIM with 70dB background
Pulse-alone 110dB
Pp8+p110
Pulse-alone 110dB
Pp4+p110
Pp24+p110
Pp16+p110
Pp8+p110
NOSTIM with 70dB background
Pp4+p110
Pp16+p110
Pp240p110
Pp8+p110
Pp4+p110
Pp24+p110
Pp16+p110
Pp4+p110
Pulse-alone 110dB
Pp8+p110
NOSTIM with 70dB background

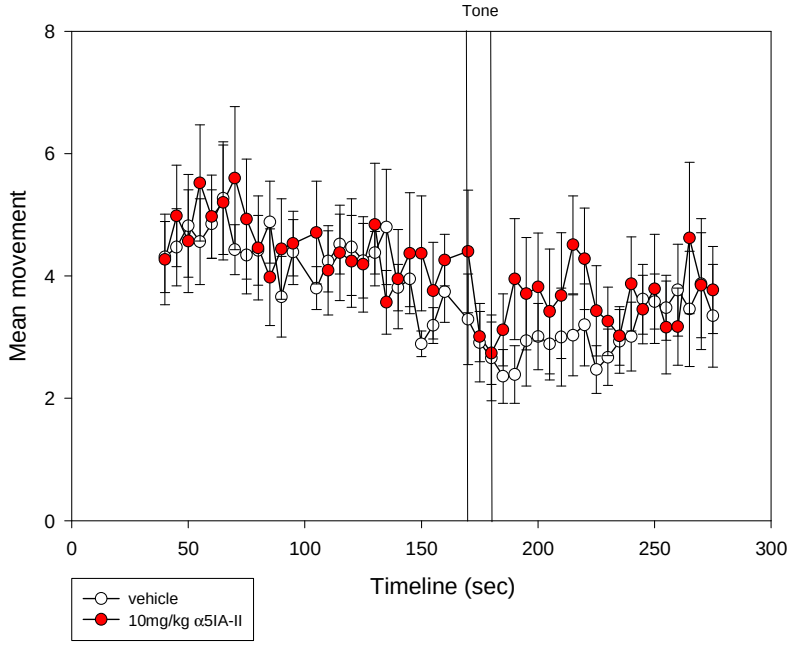
Appendix 3.40.

Pp24+p110
Pulse-alone 110dB
NOSTIM with 70dB background
Pp16+p110
Pp16+p110
Pp8+p110
Pp16+p110
Pp4+p110
Pulse-alone 110dB
Pp8+p110
NOSTIM with 70dB background
Pp24+p110
Pulse-alone 110dB
Pp4+p110
Pp24+p110
NOSTIM with 70dB background
Slut pulse alone 110dB
Slut pulse alone 110dB
Slut pulse alone 110dB
Slut pulse alone 110dB
Slut pulse alone 110dB

Appendix 5.1.

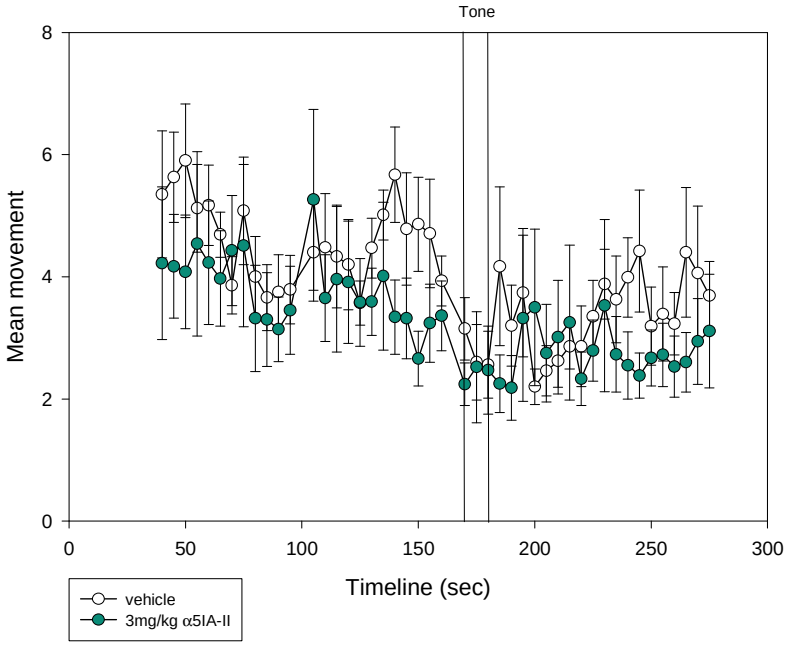
Behaviour of α5IA-II rats on traditional FC test day

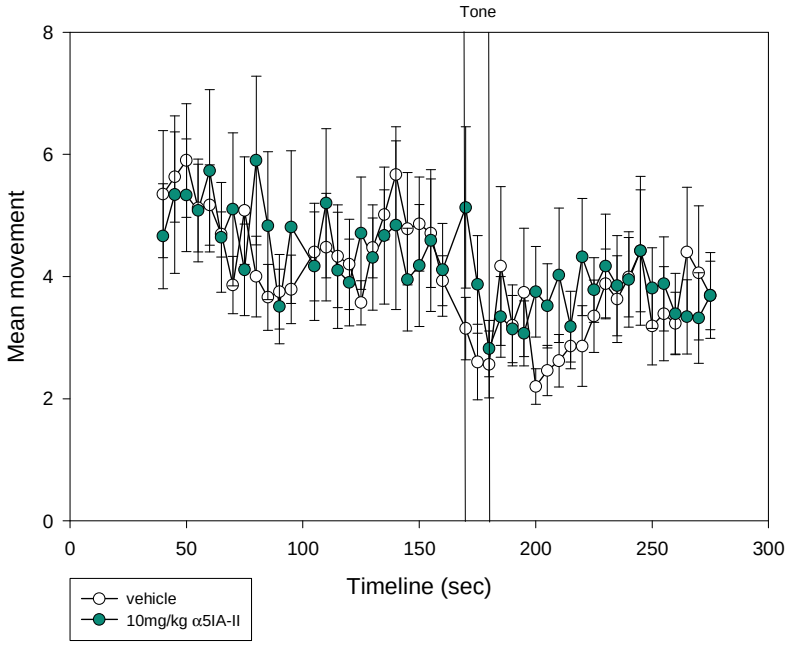




Appendix 5.1.

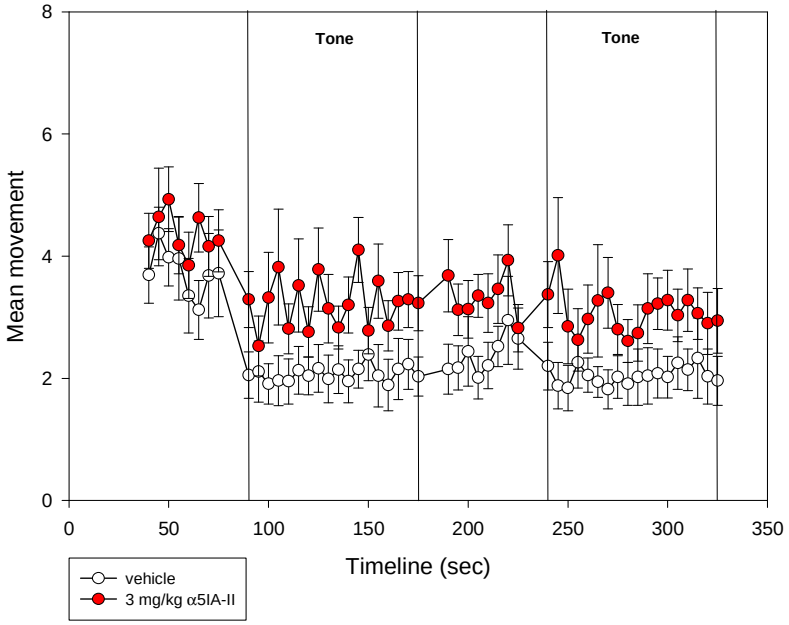
Behaviour of α5IA-II rats on traditional FC re-test day

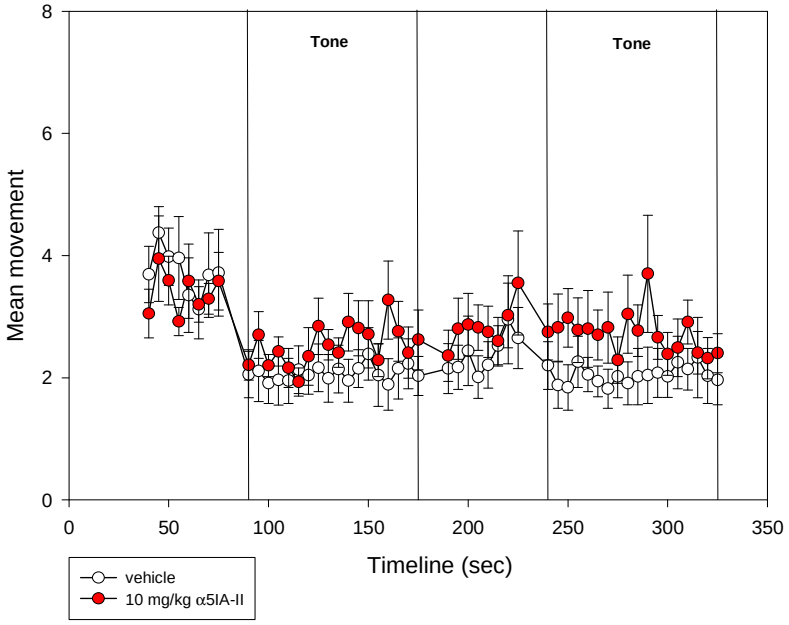




Appendix 5.2.

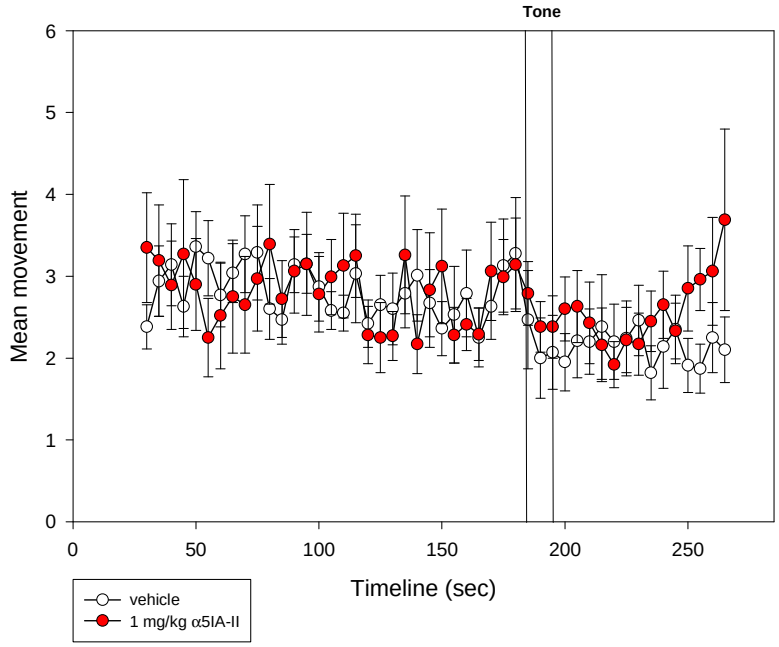
Behaviour of α5IA-II rats on extended-tone FC test day





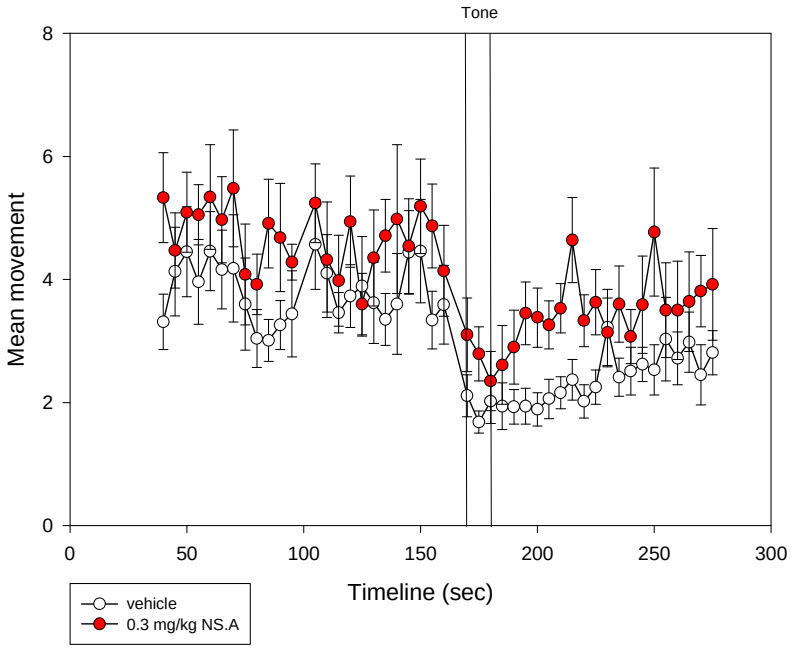
Appendix 5.3.

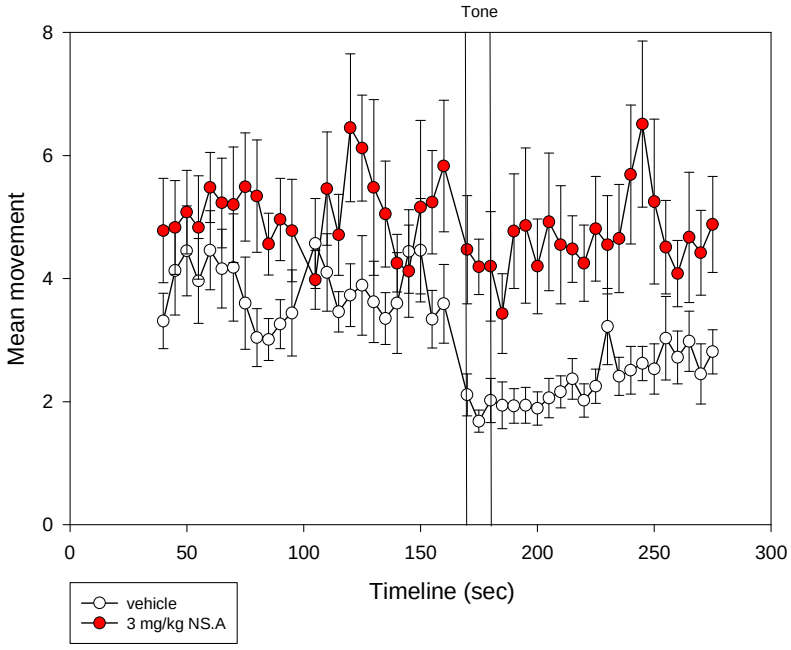
Behaviour of α5IA-II rats on trace FC test day



Appendix 5.4.

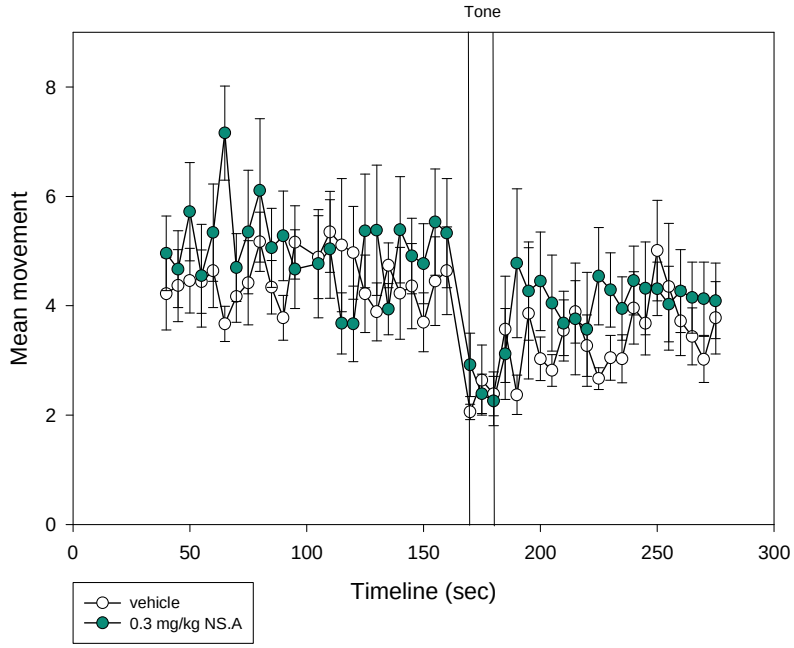
Behaviour of NS.A rats on traditional FC test day

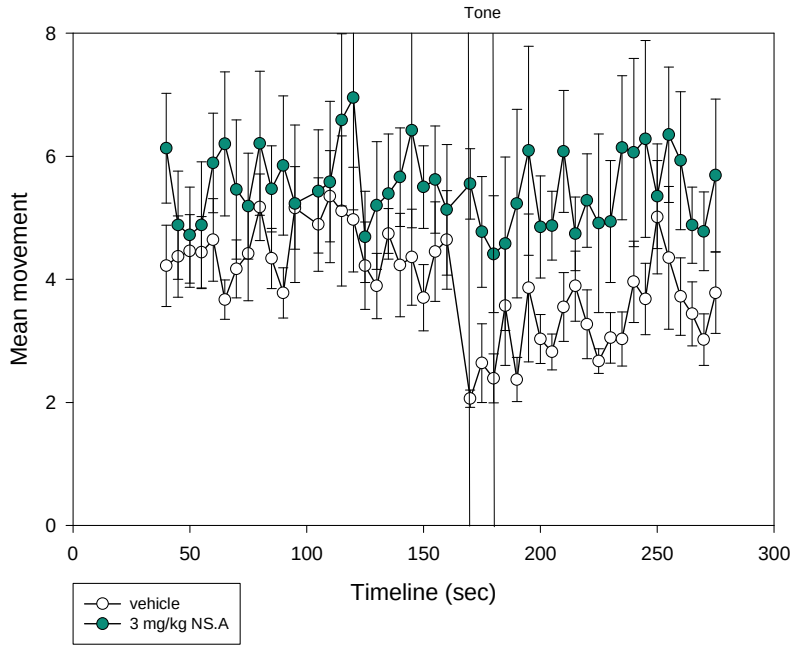




Appendix 5.4.

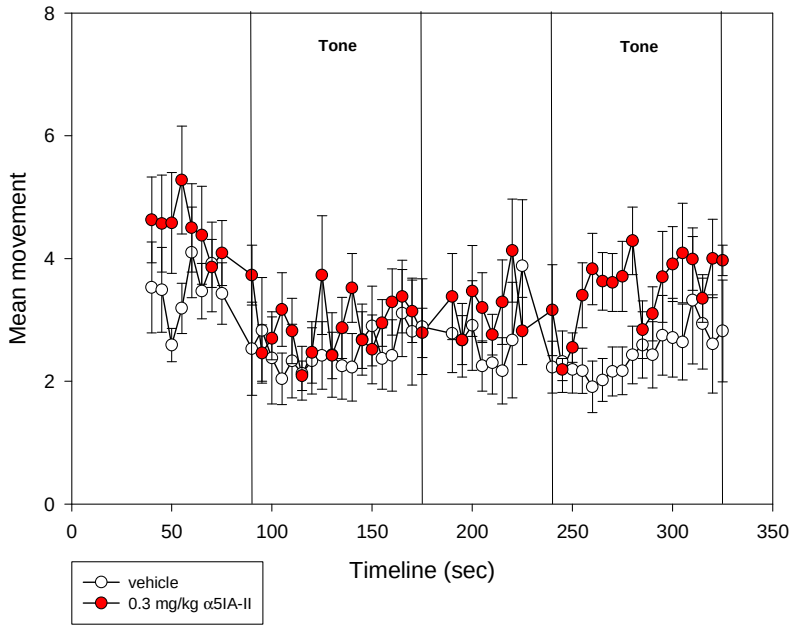
Behaviour of NS.A rats on traditional FC re-test day

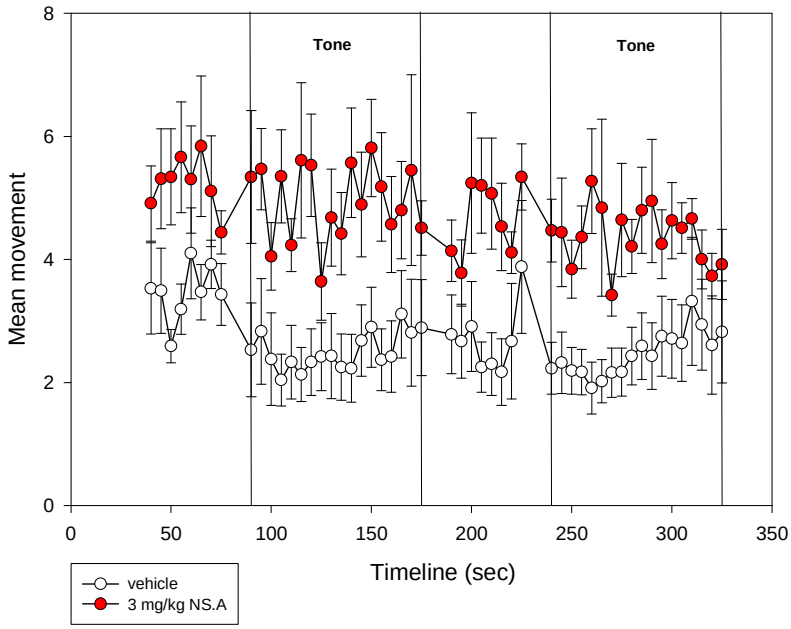




Appendix 5.5.

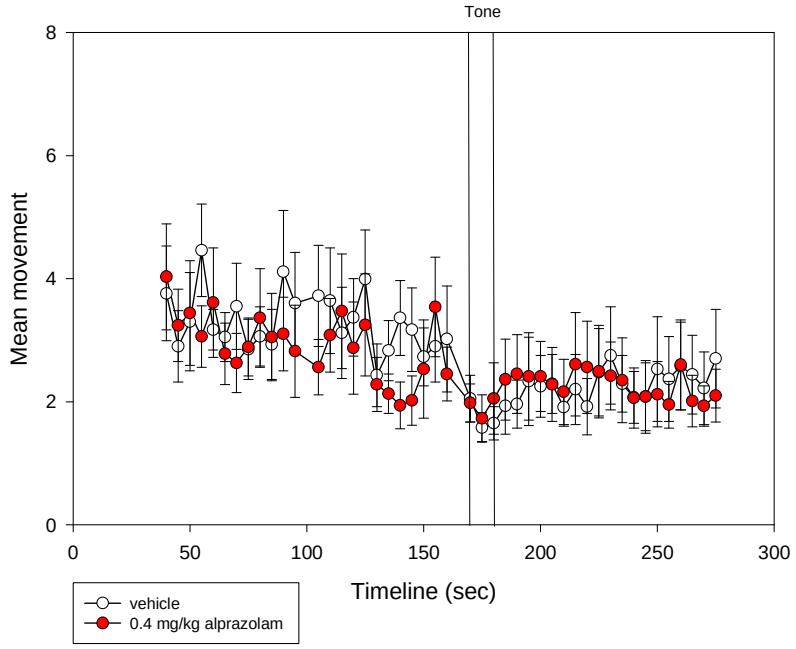
Behaviour of NS.A rats on FC extended-tone test day

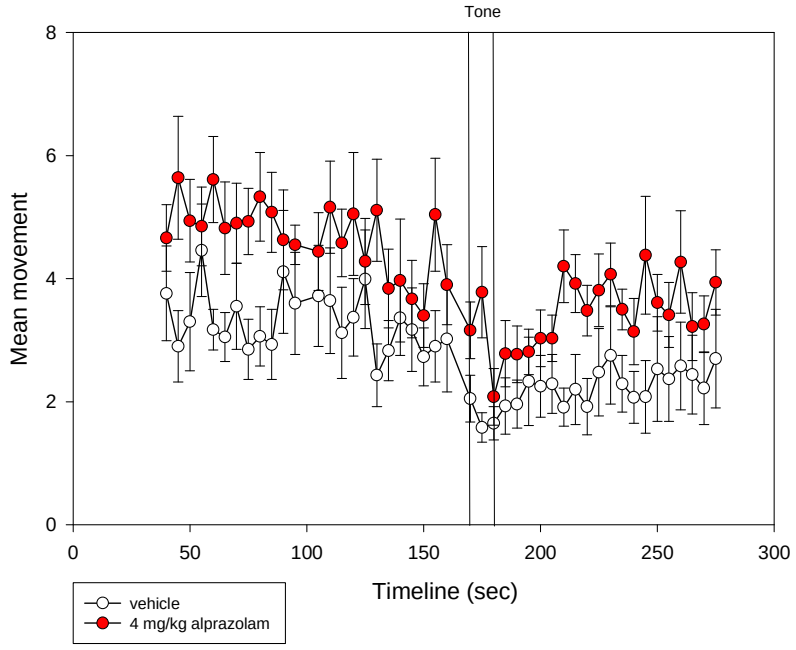




Appendix 5.6.

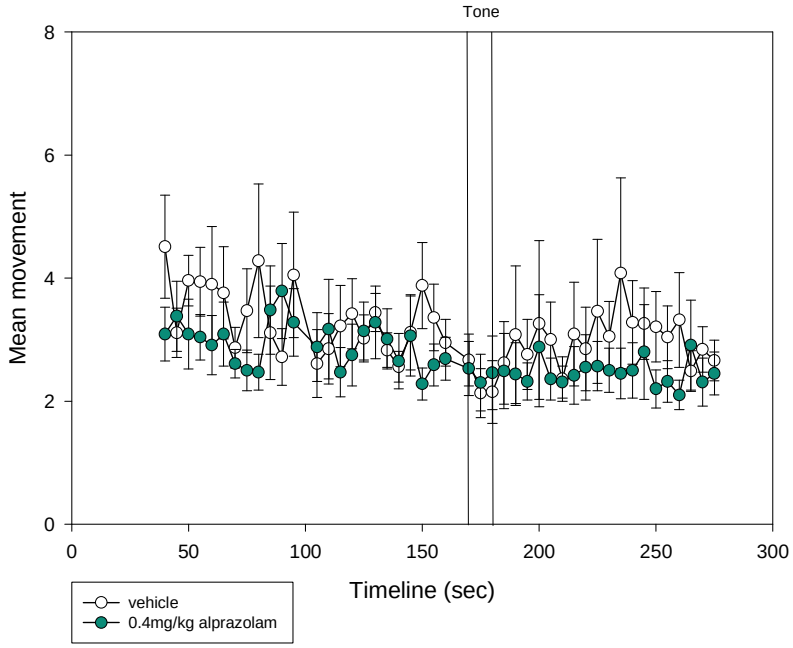
Behaviour of alprazolam rats on traditional FC test day

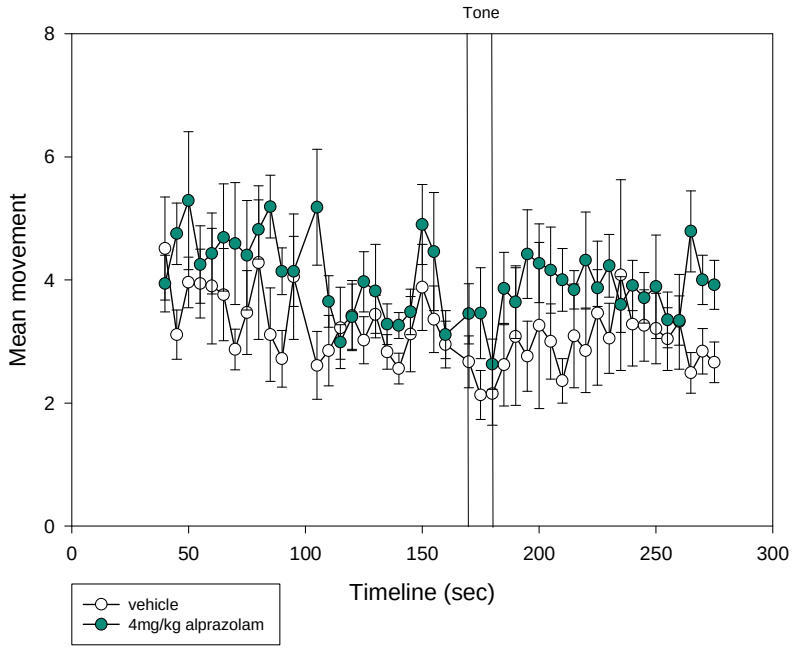




Appendix 5.6.

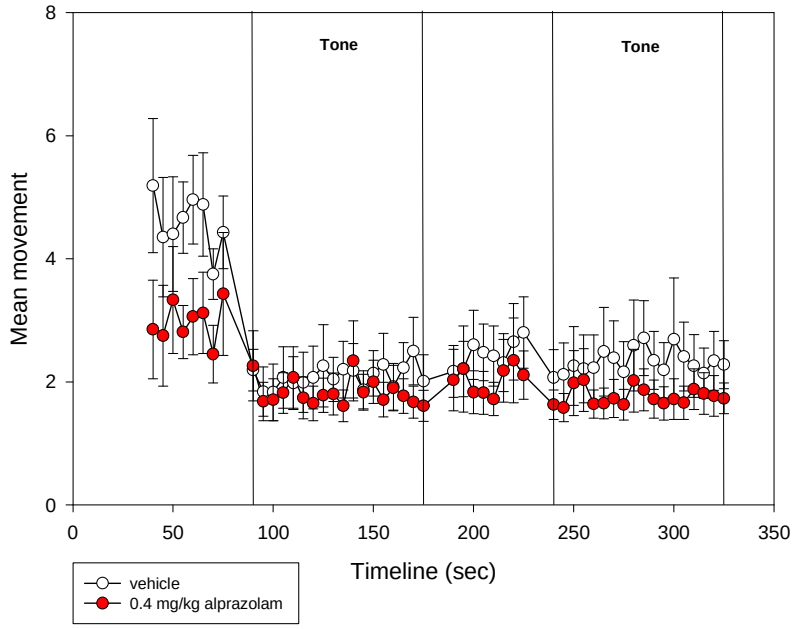
Behaviour of alprazolam rats on traditional FC re-test day

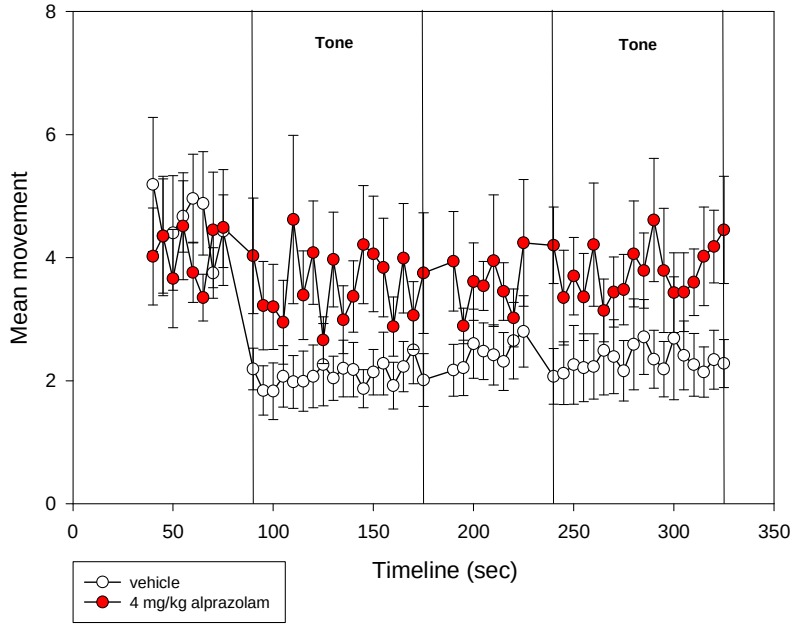




Appendix 5.7.

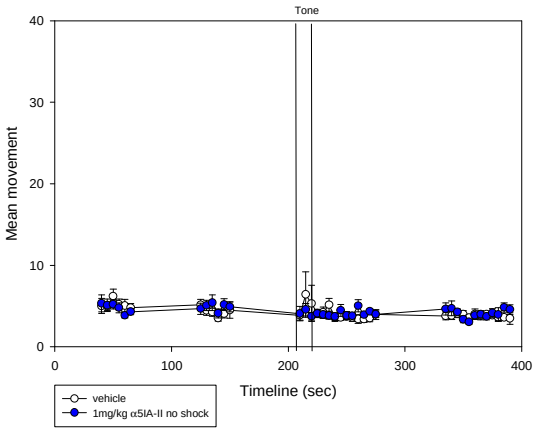
Behaviour of alprazolam rats on FC extended-tone test day

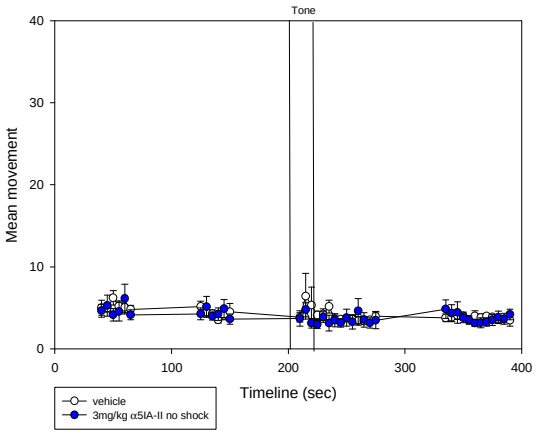


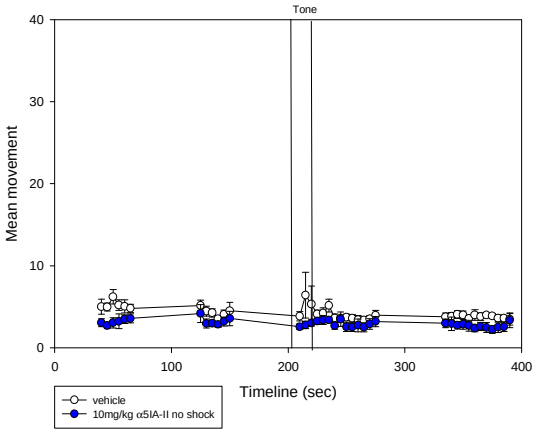


Appendix 5.8.

Behaviour of α5IA-II rats on 'conditioning' day without any unconditioned stimulus

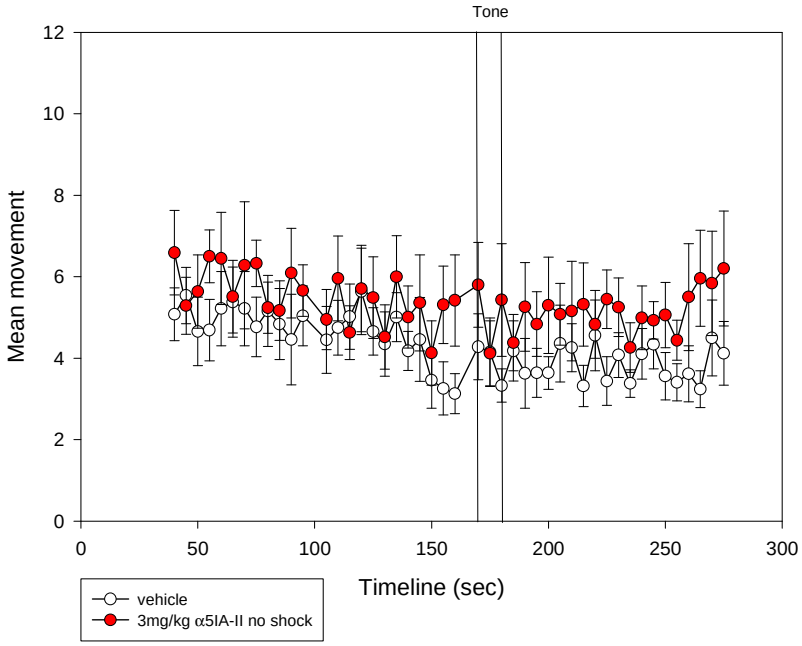


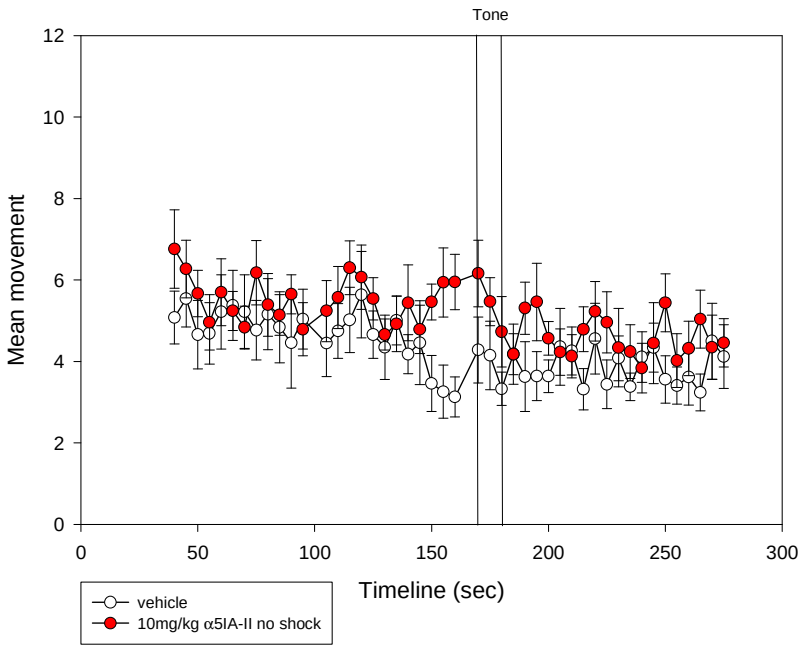




Appendix 5.9.

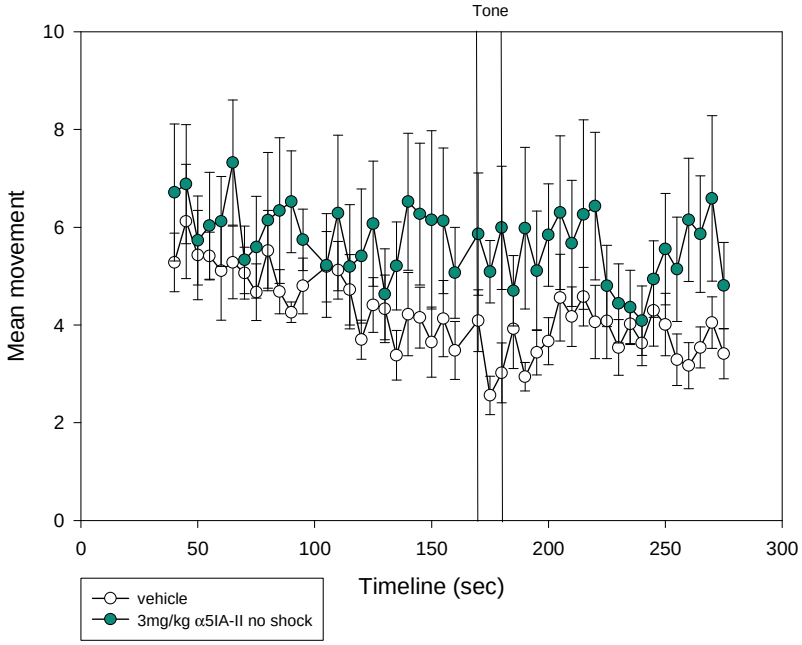
Behaviour of α5IA-II rats on FC test day without previous unconditioned stimulus

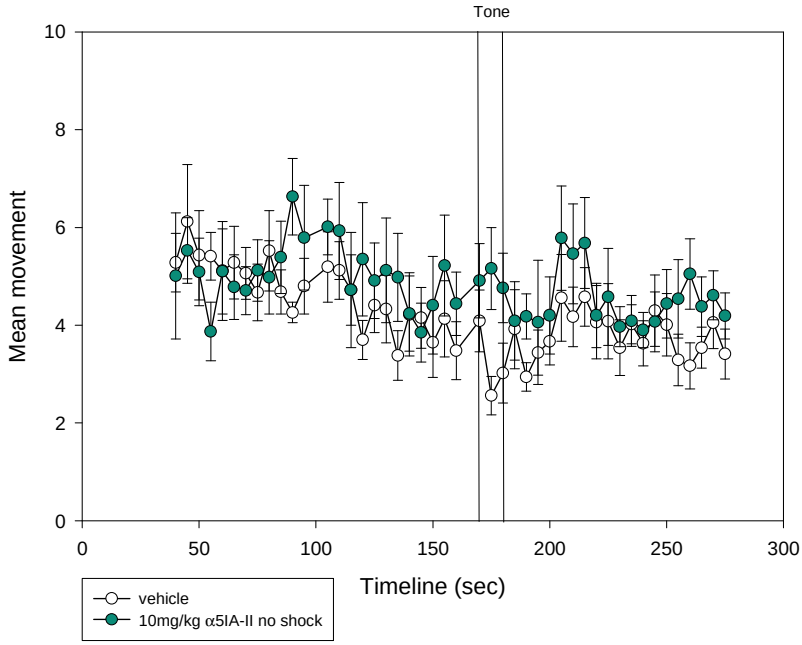




Appendix 5.9.

Behaviour of α5IA-II rats on FC re-test day without previous unconditioned stimulus





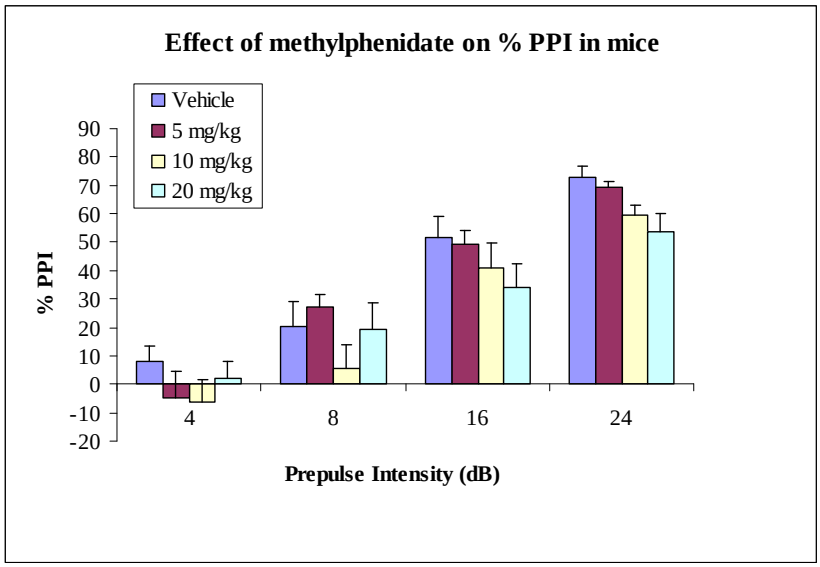
Appendix 5.10.

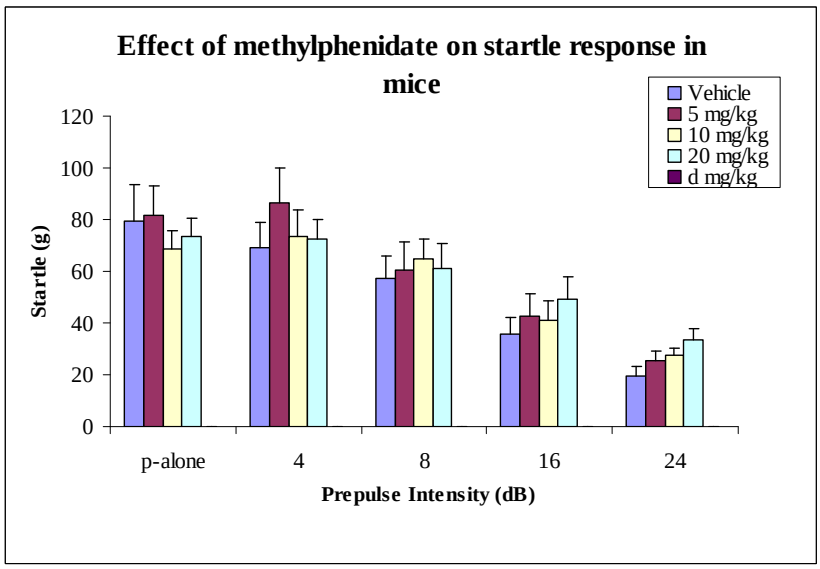
Pre-pulse inhibition experiments with mice versus rats

The results from the experiment investigating the ability of methylphenidate to cause impairment in pre-pulse inhibition are outlined in this section.

Methylphenidate administered to mice

The pre-pulse inhibition and the startle response are seen in the figure below





Appendix 5.10.

In the analysis the *p*-values are

	p-value
Intercept	<0.0001
Treatment	0.7439
Prepulse	<0.0001
System	0.8480
Treatment:Prepulse	<0.0001

The *p*-values of the significant terms from the *t*-test are

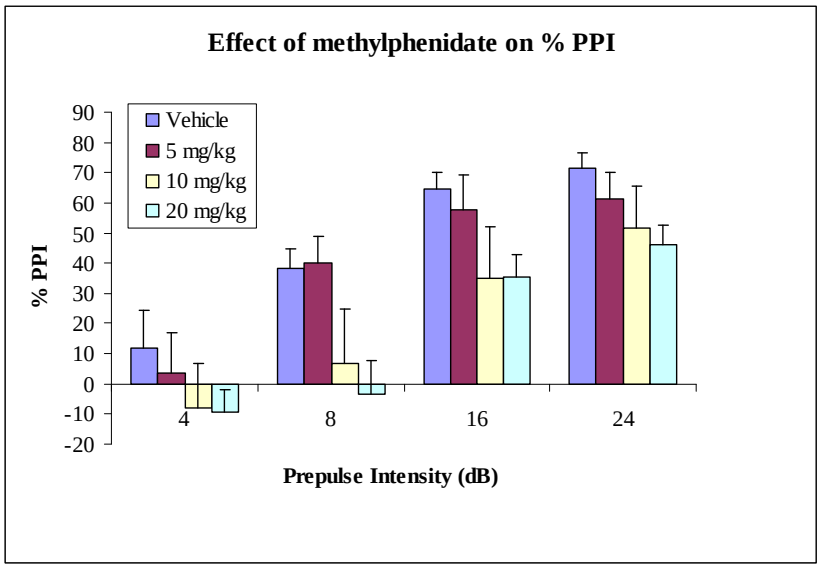
	p-value
Treatment(10mg/kg):Prepulse(pp8)	0.0212
Treatment(10mg/kg):Prepulse(pp16)	0.0047
Treatment(20mg/kg):Prepulse(pp16)	0.0001
Treatment(5mg/kg):Prepulse(pp24)	0.0277
Treatment(10mg/kg):Prepulse(pp24)	<0.0001
Treatment(20mg/kg):Prepulse(pp24)	<0.0001

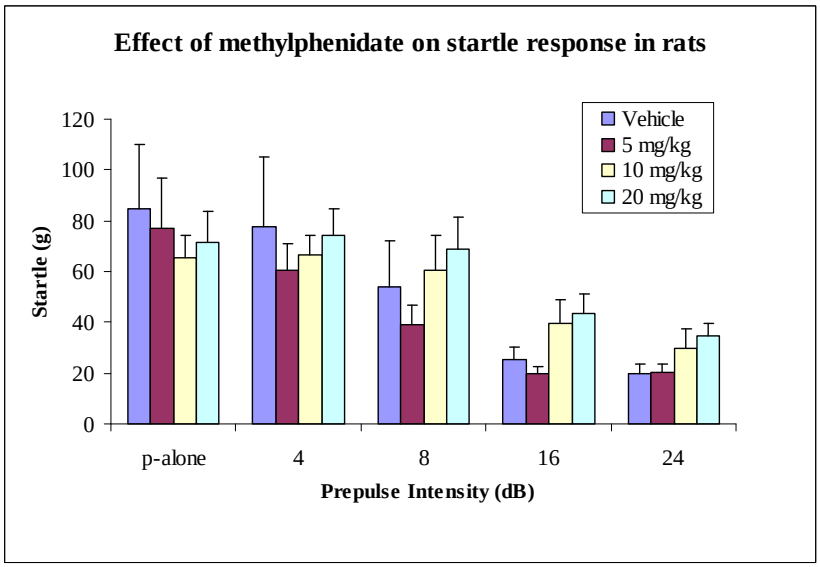
The two highest doses cause impairment in pre-pulse inhibition for pre-pulse 16 and 24. Although this is of statistical significance biologically the impairment caused by the drug is not judged to be sufficient in order to set up a robust model of pre-pulse inhibition impairment.

Appendix 5.10.

Methylphenidate administered to rats

The pre-pulse inhibition and the startle response are seen in the figure below





Appendix 5.10.

In the analysis the *p*-values are

	p-value
Intercept	<0.0001
Treatment	0.4566
Prepulse	<0.0001
System	0.5154
Treatment:Prepulse	<0.0001

The *p*-values of the significant terms in the *t*-test were

	p-value
Treatment(20mg/kg):Prepulse(pp8)	0.0017
Treatment(10mg/kg):Prepulse(pp16)	0.0020
Treatment(20mg/kg):Prepulse(pp16)	<0.0001
Treatment(5mg/kg):Prepulse(pp24)	0.0199
Treatment(10mg/kg):Prepulse(pp24)	0.0033
Treatment(20mg/kg):Prepulse(pp24)	<0.0001

For pre-pulse 24 all doses show significant impairment in pre-pulse inhibition. For pre-pulse 16 the same is shown for the two highest doses as well as the highest dose for pre-pulse 8. But as concluded in the experiment with mice, methylphenidate does not cause sufficient impairment in rats in order set up a robust pre-pulse inhibition impairment model.