Behavioural study of an animal model for AD/HD -the Spontaneously Hypertensive Rat- and control strains

Leander Bindewald
Diploma Thesis

Fakultät für Biologie
Albert-Ludwigs-Universität
Freiburg im Breisgau
March 2007

Supervised by Prof. Klaus Vogt

Research supervised by and done in the laboratory of

Prof. Vivienne Russell
Faculty of Health Sciences
University of Cape Town
South Africa
2006

Zusammenfassung

In der Forschung über die Aufmerksamkeitsdefizit/Hyperaktivitätsstörung (AD/HS) liefern Tiermodelle wertvolle Einsichten über zu Grunde liegenden neurophysiologischen Fehlfunktionen und die Wirkung neuer Medikamente. Die "spontaneously hypertensive" Ratte (SHR) ist bekannt dafür, alle Verhaltensstörungen menschlicher AD/HS Patienten aufzuweisen. Sie ist das meist untersuchte und am besten verstandene Tiermodell für AD/HS. Als Inzuchtstamm hat die SHR eine starke genetische Veranlagung die Symptome von AD/HS zu entwickeln. Da AD/HS jedoch eine Entwicklungsstörung ist, gilt es auch besonders Umwelteinflüsse in der frühen Jungend als auslösende Faktoren zu berücksichtigen. Die vorliegende Studie untersuchte die SHR mit zwei unterschiedlichen Ansätzen: (1) das frühe post-natale Umfeld wurde auf seine Wirkung auf die Ausprägung von AD/HS typischen Verhaltens untersucht. Dazu wurden neugeborene SHR zu Muttertieren der Wistar-Kyoto (WKY) und des Sprague-Dawley (SD) Stämme in Pflege gegeben und im Alter von 30 Tagen (P30) auf ihr Verhalten in "open field" (OF) und "elevated plus maze" (EPM) Apparaten untersucht; (2) in einem parallelen Experiment wurden getestet ob SHR auch die Komorbidität von AD/HS und Suchtkrankheiten aufweist. Dazu wurde die Empfindlichkeit von SHR für die belohnenden Wirkung der Droge Ketamine mit dem "conditioned place preference" (CPP) Paradigma untersucht. Anschließende OF Tests untersuchten die Auswirkungen von Ketamine auf das Bewegungsverhalten von SHR, WKY und SD Ratten. Um die neuronalen Korrelate des Ketamine bedingten Verhaltens zu charakterisieren, wurde versucht die c-fos Expression in Prefrontalem Kortex und Nucleus accumbens von SHR, WKY und SD zu quantifizieren. Diese Studie konnte keine Auswirkungen der unterschiedlichen Pflegemütter auf SHR finden, was die starke genetische Festlegung dieses Tiermodelles unterstreicht. Im Alter P30 wies SHR deutliche Verhaltensunterschiede zu beiden Kontroll-Stämmen auf. Im Alter P60 war das Verhalten der SHR jedoch nicht verschieden von dem des SD Stammes. Diese Befunde untermauern eine kritische Haltung gegenüber SHR als Tiermodell für AD/HS in diesem Alter. Ketamine hatte unterschiedliche Wirkungen auf SHR und WKY Ratten. OF Tests zeigten einen stimulierenden Ketamine-Effekt auf das Bewegungsverhalten nur in SHR. Ketamine-CPP, bisher nicht in Ratten nachgewiesen, wurde in WKY festgestellt, nicht aber in SHR. Die Prävalenz für Suchtstörungen bei AD/HS wurde in SHR nicht nachgewiesen.

Acknowledgements

First of all I wish to express my most cordial gratitude to Prof. Vivienne Russell who generously invited me to her laboratory in Cape Town and supervised this study. I owe the great opportunity to study at the University of Cape Town and a year of invaluable experience primarily to her engagement and courtesy.

My thankfulness equally extents to Prof. Klaus Vogt from my home University of Freiburg for his trust and support across the continents.

Dr. Dirk Lang and Prof. Sue Kidson and Ms. Sharon Werthen for their help to overcome the technical and bureaucratic hurdles at a foreign institution.

Special thanks to Dr. Laurie Kellaway, Prof. Graham Louw, Mrs. Toni Wiggins, Mrs. Liz van de Merve and Ms. Regina Lindau for their most appreciated advice and friendly help with so many technical aspects of my study.

Prof. Willie Daniels from the University of Stellenbosch for making the Ethovision computer setup available for our research.

A special thanks to Ms. Shula Johnson and Mr. Tlhogi Selaledi for breeding and taking such good care of the rats used in this study.

Mr. Charles Harris and Mr. Iekraam Fakier for constructing and maintaining the behavioural apparatuses, they were always at hand for last minute trouble shooting, Mr. Nazeem Damon for keeping the technical equipment in spotless conditions.

Last but not least I would like to thank my fellow students Fleur Howells, Bryony Dobson, Musa Mabandla, Heleen Soeters and Dean Hodgskiss for their support. Shared trouble is half the trouble, shared joy is twice the joy.

Contents

List of Figures

List of Tables

Abbreviations

ABC	Avidin-Biotin-Complex
AD/HD	Attention-deficit/Hyperactivity disorder
ANOVA	Analysis of variance
BSA	Bovine serum albumin
CPP	Conditioned place Preference
DAB	Diaminobenzidine
DAPI	4',6-Diamidino-2-phenylindol
dH_2O	distilled water
EPM	Elevated Plus Maze
i.p.	Intra-peritoneal injection
MP	Methylphenidate
NHS	Normal horse serum
NMDA	*N*-methyl-D-aspartate
OF	Open field
OSA	Oral self administration
P2	Postnatal day 2
PB	Phosphate buffer
PBS	Phosphate buffered saline
SD	Sprague Dawley (rat strain)
SHR	Spontaneoulsy hypertensive rat
SHR12	SHR injected with 12 mg/kg ketamine
SHR20	SHR injected with 20 mg/kg ketamine
SUD	Substance use disorder
UCT	University of Cape Town
WKY	Wistar Kyoto (rat strain)
WKY12	WKY injected with 12 mg/kg ketamine
WKY20	WKY injected with 20 mg/kg ketamine

Abstract

Animal models for attention-deficit/hyperactivity disorder (AD/HD) provide valuable insight into the neurophysiology of the disease and serve to test novel treatments. The spontaneously hypertensive rat (SHR) mimics all behavioural characteristics of AD/HD. It is the most widely used and best understood animal model for AD/HD. As an inbred strain, the SHR has a strong genetic disposition for AD/HD-like behaviour. However, the developmental pathology of AD/HD stresses the importance of environmental influences especially during early ages. This study sought to further characterize the SHR in two different ways: (1) the influence of the early postnatal environment on the expression of AD/HD-like behaviour was tested in an experiment in which SHR pups were cross-fostered onto dams of the Wistar Kyoto (WKY) and Sprague-Dawley (SD) control strains and tested thirty days after birth (P30) for AD/HD-like behaviour in the open field (OF) and elevated plus maze (EPM) apparatus. (2) in parallel experiments SHR were tested to mimic a specific aspect of AD/HD in humans, namely its comorbidity with substance abuse disorder (SUD) in adolescence. The susceptibility of SHR to the rewarding effects of ketamine was tested with the conditioned place preference (CPP) paradigm. OF tests were employed to study the behavioural effects of ketamine on SHR, WKY and SD rats. To investigate neural correlates of ketamine-induced behaviour an attempt was made to quantify c-fos expression in the prefrontal cortex and the nucleus accumbens of SHR and control strains. This study showed no effect of cross-fostering on the behaviour of the SHR, confirming its strong genetic determination. At P30 SHR displayed behaviour that was different from both control strains. However at P60, locomotor activity was not different between SHR and SD rats. These findings challenge the notion that SHR is a good model for AD/HD post-puberty. Ketamine was shown to have a differential effect on SHR and WKY. The OF tests revealed a stimulatory effect of ketamine on locomotor behaviour only in the SHR. Ketamine CPP, not shown in rats before, was found in WKY but not in SHR. The prevalence for SUD in AD/HD was not mimicked by SHR.

Chapter 1

Introduction

1.1 Background of the Study

1.1.1 Attentiondeficit/Hyperactivity Disorder

The high prevalence of attention-deficit/hyperactivity disorder (AD/HD) especially amongst children is a phenomenon recognized by medics and researchers as well as the general public worldwide. The economic and social impact of 3-9% of children and 4% of adults [3] affected, justifies the efforts and funds invested in research of possible causal factors and treatments of the disorder.

As the name suggests AD/HD is a heterogeneous behavioural disorder combining several sets of symptoms, of which attention deficits, hyperactivity and impulsiveness are the most common, often coinciding and easily identified traits.

Being a behavioural condition AD/HD only becomes apparent and a disorder as such, when patients fail to interact with their environment in the societal norms of social integration. Also AD/HD is a developmental disorder affecting mostly children from early years through to adolescence, persisting into adulthood when physiological and behavioural adaptations do not take place. Commonly it first becomes apparent when children stand out against the necessarily highly regulated behavioural requirements of school education. At the extremes of the normal range of demeanour, they are the ones who

fail to achieve learning targets and have poor social interaction with fellow pupils.

Today medication such as methylphenidate (Ritalin), amphetamine and buprion are available and effectively helps AD/HD patients to cope with the demands of their environment. Concern about the wide use of these substances, being psychostimulants, to treat children with AD/HD arises from the documented overlap between AD/HD and substance abuse disorders (SUD) in adolescents (15% - 30%) and adults (35% - 55%) [4]. Especially the administration of Ritalin, the most common medictation, is questioned, despite studies showing no evidence that stimulant treatment increases the risk of later SUD in patients with AD/HD [5]. Effective medication was identified prior to an understanding of the underlying defects of the disorder [6].

Today the psychopathophysiology of AD/HD is still under debate. Various genetic alterations seem to be responsible for the development of AD/HD. Some directly affecting the neurophysiology, some influencing the susceptibility of the young individuals to their environment [7].

Concerted efforts are being made to further investigate candidate genotypes associated with AD/HD [8]. The best studied gene variants associated with AD/HD are those encoding neurotransmitter transporter proteins especially for dopamine and the SNAP-25 protein which co-regulates presynaptic Ca^{2+} responsiveness and glutamate receptor allocation [9, 10]. Well proven environmental factors that increase the risk of developing AD/HD are maternal smoking during pregnancy and low birth-weight/prematurity [7].

Concerning the physiology of AD/HD research on animal models revealed several alterations in the dopaminergic and noradrenergic systems of the brain. Hypofunctional dopamine systems and hyperfunctional norepinephrine pathways involving the frontal cortices seem to have a top down effect on the the modulation of thalamo-striatal circuits responsible for the expression of different behaviours (reviewed in [11] [6] [1]).

Dopamine and norepinephrine were amongst the first candidates for a causal

impairment because methylphinedate targets their transporter proteins, inhibiting the reuptake of extracellular dopamine and thus normalizing intersynaptic dopamine levels [6].

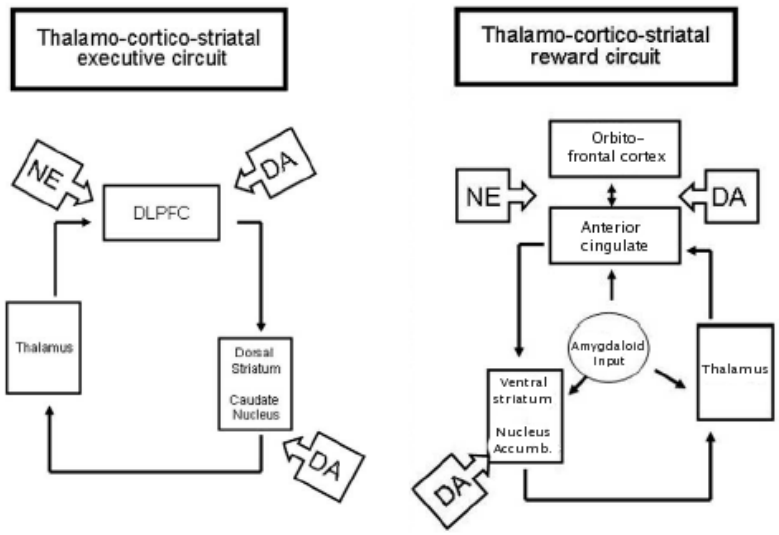


Figure 1.1: Simple model of the two neural circuits possibly involved in AD/HD, DA Dopamine, NE Norepinephrin, DLPFC dorsolateral prefrontal cortex; adapted from Sonuga-Barke '05 [1]

Figure 1.1 depicts a common model of the circuitry effected by dopamine impairments. The effects shown on the right hand side in the thalamo-striatal and limbic structures can influences motor, motivational and reward related behavioural output [12]. The model on the left serves to explain higher order impairments like inattention and learning deficits which could stem from altered modulation of top down circuits including the PFC [13].

Explaining AD/HD as a physiological disorder like this can account for the most behavioural traits of the disorder but not for example for the intra-individual variablility in performance in repetitive tasks [14].

Sonuga-Barke [1] suggests that future modelling of the disease must therefore incorporate and emphasize the developmental aspect of AD/HD opening the aetiology up to environmental influences [1]. Impairments in dopamine and norepinephrin regulation of behavioural circuits can then be challenged as not being the cause but rather adaptations to an underlying deficit yet to be determined [15, 9]. This deficit could again be brought about by environmental or genetic conditions or an interaction between different factors. This view would regard the various genetic variations, for example in transporter protein expression, not necessarily as determinants for the disorder but rather as predisposing factors that limit natural dynamic adaptations to underlying defects and environmental challenges to produce a pathological phenotype.

With respect to such underlying deficiencies, reduced energy supply by glial astrocytes was proposed recently [14] as the causal impairment leading to the development of AD/HD. This hypothesis suggests, that astrocytes fall short in producing enough lactate for the transiently enhanced energy requirements of (a) myelination of axons during development and of (b) rapid firing neurons during sustained local brain activity at any age. The first effect (a) could account for alterations in modulatory systems such as the dopaminergic and the adrenergic as adaptions to signals delivered at lower speed and intensity due to a lack of myelination. Also the neuropathological findings of reduced brain volume in animal models of AD/HD [6] and particularely reduced white matter in humans with AD/HD [14] are well in line with this hypothesis. The rapid depletion of local energy stores (b) could account for impaired attention when sustained focus on one task requires continuous firing of the relevant neural circuits. Impaired signaling of delayed reward, previously suggested to be a fundamental deficit in AD/HD patients [13], could also be explained by this hypothesis since the inability to keep the anticipated value of future rewards present in the association cortex would cause the individual to choose the lesser, but immediate gratification. Physiologically the transient deficiency of energy could translate into fewer action potentials from the same neuron in a certain time, longer refractory times for the re-establishment of membrane potentials and impairement of intra- and inter-

cellular Ca^{2+}signalling [14].

This would further point to the causal impairment of AD/HD to be found in higher order brain structures like the association cortices, with the observed monoamine alterations being secondary compensatory adjustments [12].

1.1.2 SHR and WKY Strains of Rats

Today advanced imaging techniques have opened the door to studying the neurological differences between AD/HD patients and healthy humans. However, research on animal models of AD/HD provides the possibility to study the neurophysiology of behavioural traits, explore new treatments aimed at specific defects and study their immediate and long term effects.

The spontaniously hypertensive rat (SHR) is the best studied and most broadly validated animal model for AD/HD [16]. It has been selectively bread in the 1960s from the Wistar Kyoto (WKY) strain for the display of hypertension during adulthood [17]. Later it was found to display all mayor behavioural characteristics of AD/HD namely hyperactivity, impulsiveness and poor performance in sustained attention tests [16]. These characteristics are already observed in the rats' prepuberty (3 to 4 weeks of age), whereas the SHR's hypertension, which does not model AD/HD behaviour in humans, is only pronounced in adulthood. For the exploration and validation of SHR typical behaviour, rats are often studied in open field (OF) and elevated plus maze (EPM) test and in different operant tasks with varying cue and reinforcement paradigms [16, 18, 19, 20, 21, 22, 23]. The SHR was shown to be hyperactive compared to WKY in the OF [23, 22, 18] especially in a familiar environment [18]. This finding was challenged to be only apparent in young SHR [21]. In the EPM, SHR show less anxious behaviour, entering the open arms more frequently than WKY [22]. In other tasks SHR were found to be more impulsive, defined as the inability to inhibit inapropiate responses ([24] in [9], [22]) and be impaired in learning when reinforcement is delayed [25]. In review [16], SHR is the only animal model that exhibits the major behavioural symptoms of AD/HD. Also, drugs used to treat AD/HD in humans also ameliorate symptoms like hyperactivity in SHR [11].

However, the SHR has been criticized as an animal model for AD/HD because some of its behavioural characteristics are only observed when they are compared to its progenitor WKY strain and not in comperison with other Wistar rats [21] [19]. In addition, WKY rats were recently suggested to be a model of depression [26]. For these reasons it is advisable to compare SHR not only to WKY but also to conduct experiments with a second control strain. In the presented study the outbred strain of Sprague Dawley (SD) rats was used.

1.1.3 Ketamine

The comorbitiy of AD/HD and SUD makes studying the rewarding effects of drugs of abuse in SHRs an interesting topic for research. Sagvolden (1996) suggests that most behavioural aspects of children with AD/HD and of SHR could be due to an impairment in reward circuits lowering the reinforcing porperties of delayed rewards [25, 13]. Individuals with AD/HD might be susceptible to the short term rewards of drugs of abuse because the beneficial long term effects of abstaining from drugs can be seen as a delayed rewards.

A well established way to study drug reward in animals is the conditioned place preference (CPP) paradigm (see 2.1.2 on page 23). In our laboratory, Ms. G. Sadi Lelaka investigated the effect of MP treatment on cocaine challenged WKY and SHR rats with CPP. She found the general rewarding effect of 10 and 20 mg/kg cocaine to be greater in WKY than SHR, but MP treatment lowered it in SHR only (see [27] unpublished).

Other work from our laboratory by Mr. M. Lehohla [15] investigated the role of NMDA receptors in the pre frontal cortex (PFC) of SHR rats and found signs of impaired Ca^{2+}regulation as discussed in 1.1.1 on page 12.

Ketamine ((RS)-2-(2-Chlorphenyl)-2-(methylamino)cyclohexan) as a drug of abuse and a NMDA receptor antagonist was chosen for this study to further investigate the SHR in terms of CPP and OF behaviour and immunocytochemistry.

Ketamine binds to the NMDA channel with high affinity, and prevents it from opening when glutamate binds. Ketamine also binds to other receptors/channels

including DA, serotonin and $Ca^{2+}$with lower affinity (for review see [28]). It is related to dizocilpine (MK-801) and phencyclidin (PCP), which exhibit similar pharmacologies. It is a racemic substance with the (S)-stereoisomer having a far greater affinity for the NMDA receptor then the (R)-ketamine [29]. Ketamine is widely used as a anaesthetic agent in veterinary medicine. In human medicine it is not generally used as an anaesthetic because of its hallucinogenic effects. Since it is not depressing respiration, it is however still applied in emergencies medicine especially when the medical background of the patient is not known [30]. It acts as a dissociative agent, apparently separating body experiences from personal perception. As such it is used as an analgesic in some cases, and recently described as an antidepressant [31] Its hallucinogenic and dissociative properties contributed to ketamine becoming a drug of abuse in the 1960s. It was internationally recognized as such and put on schedule III status in the US in 1999 and class C in the UK in 2006. The effects of ketamine have been proposed to model schizophrenia in human and animal studies [28, 32, 33].

CPP with ketamine is only documented in one study using mice and ketamine dosages from 1-10 mg/kg [34]. However, in that study, as in many others, ketamine is primarily used as a pretreatment to inhibit or suppress morphine or ethanol CPP [34] [35]. Other studies on CPP induced by similar substances like MK-801 and PCP were in review found to give inconsistent results (see review [36]). Reward and addiction can also be studied in self administration experiments. Rats performed more lever presses when this triggered micro injections of the NMDA receptor antagonist PCP into their brains (PFC and Nucleus accumbens)[37]. A technically easier way to achieve self administration of drugs are oral self administration (OSA) protocols in which animals ingest the substances of interest with water, voluntarily and ad libitum (for review see [38]). Ideally the experimental animals have a free choice of drinking the drug solution or plain water. Ketamine was used for OSA experiments but rewarding effects were confounded because the employed protocols used partial food deprivation or addition of glucose to the drug solution to ensure enhanced uptake [39, 40].

Ketamines indirect effects on the nucleus accumbens (NAc), a candidate striatal structure associated with reward pleasure and addiction phenomena (Fig.

1.1) were investigated in several studies. Ketamine enhanced field potentials in the shell region of the NAc evoked by electrical stimulation in the PFC of freely moving rats injected with 25 mg/kg ketamine [41]. The same study showed elevated glutamate release in the NAc by in-vivo microdialysis [41]. Ketamine also induce high-frequency oscillations in the NAc [42]. These findings are discussed with respect to the association of abnormal neuronal processing in the NAc with schizophrenia [42] but they could also help to explain rewarding and addictive effects found with ketamine.

Locomotor stimulation after sub-anaesthetic ketamine administration served as an additional parameter in many studies [42] [41] [43]. Locomotion is commonly measured as the distance travelled within a certain time in OF apparatuses. Rats were found to increase their locomotive activity in the first fifteen minutes after ketamine injections in a dose dependent manner [43, 42, 41]. This increase in locomotion was found for both (S)- and (R)-racemats of ketamine [29]. An increase of locomotion was also found with administration of the NMDA receptor antagonist MK-801 into the NAc of freely moving rats [44].

Additionally to the total distance travelled in the open field Sams-Dodd [45] studied and classified stereotyped behaviour and ataxia in rats after PCP administration, which proved to be applicable also to the effects of ketamine [41]. Stereotyped behaviour according to his study is forward head searching, side to side weaving or turning, rearing with and without falling, jerky side-to-side head movements and various levels of ataxia. Rearing behaviour was looked at in one other study comparing SHR and WKY and was found to be elevated in SHR [46].

1.1.4 Expression of the immediate early gene "c-fos"

The c-fos gene codes for a subunit of the Fos/Jun protein-complex which is a well described transcription factor in cell growth and plasticity [47]. Transcription of these factors follows stimulation after a relatively short time, generally within two hours after onset of stimuli. Quantification of c-Fos expression in immuno-stained brain sections can be used as a measure of local brain activity [43].

Ketamaine injections between 4 and 16 mg/kg strongly stimulated C-fos expression in the PFC and the NAc of rats [43]. The NAc showed elevated stimulation after administration of various drugs of abuse [48, 49]. Supressing the induction of c-fos with antisense nucleotides prevented morphine CPP [48]. A dose-dependent increase in Fos expression two hours after ketamine injection in rat cortex, but not in the hippocampus, was observed even after anaesthetic dosages of 100 mg/kg [50]. Howerver, a study of long term effects of repeated ketamine injections found elevated c-Fos staining in the hippocampus two weeks after the last ketamine administration [33].

One study compared c-fos expression in the NAc of untreated SHR and WKY rats and found it to be lower in SHR [51].

1.2 Objectives of the study

This study aims at further investigating the SHR as a model of AD/HD by testing if comorbid SUD observed in human patients can be modeled by the rats susceptibility to the rewarding effects of a drugs of abuse. SHR is expected to be more susceptible to the rewarding effects of ketamine, the drug used in this study. This will be studied by using the CPP and OSA paradigms.

Besides the anticipated rewarding effects of ketamine, its directly stimulating effects at subanaesthetic doses on SHR, WKY and SD rats are tested with different behavioural parameters in OF experiments. This study aims to investigate if ketamine has a differential effects on the three rat strains. By quantifying the c-Fos immuno-reativity in the PFC and the NAc it is attempted to find neural correlates for strain-specific ketamine effects.

In a parallel study, using the cross-fostering paradigm, rats are tested in the OF and the elevated plus maze (EPM) to investigate environmental factors as opposed to genetic dispositions for the expression of AD/HD-like behaviour. Acknowledging the importance of the dams interaction with rat pups for a developmental disorder, it is hypothesized that the SHR phenotype is the result not only of its genotype but also determined by poor nurturing of the SHR dams.

Chapter 2

Materials and Methods

2.1 Behavioural Experiments

2.1.1 Animals

This study used rats (Rattus Norwegicus) of three different strains: Spontaneously Hypertensive Rats (SHR), Wistar Kyoto (WKY) and Sprague-Dawley (SD). SHRs and WKYs were obtained from the University of Cape Town Animal Unit and housed in the animal room in the basement of the Anatomy Building. SDs were bred in this facility by Mrs. Shula Johnson. All rats were kept under a 12 hour light-dark-cycle (lights on at 6h00 am, lights off at 18h00). Room temperature was controlled at $\pm 21^{\circ}$C. Animals were kept in plastic containers (42 cm x 26 cm x 15 cm) with grid lids giving them access to food pellets and water bottles ad libitum. Not more than five adult Rats were kept in one cage. One week prior to an experiment, rats were reduced to two animals per cage for the conditioned place preference (CPP) experiments (see 2.1.2 on the following page), two to four animals for the open field (OF) experiments (see 2.1.4 on page 27) and one rat per mouse cage (36 cm x 16 cm x 12 cm) for the oral self administration (OSA) experiment (see 2.1.7 on page 30).

Two days prior to and every morning during the CPP experiments, all tested rats where weighed . Rats that were only tested in the OF received the same amount of handling and weight monitoring as the CPP rats during the week before the OF testing. All rats in the OSA experiment were weighed daily for

the entire duration of the procedure. All rats were handled with bare hands, if not stated otherwise.

2.1.2 Conditioned Place Preference

All Behavioural testing was conducted in Room 3.18 of the Anatomy Building, two floors up from the Animal Room. Two rooms adjacent to a central entrance room were available for the experimental apparatus. One of these had an extra labyrinth entrance allowing access without opening the door. This room was used for the CPP experiments.

CPP Boxes consisted of covered polyethylene containers divided into three compartments. A middle compartment of 10 cm x33 cm x 45 cm connected two outer compartments of 24 cm x 33 cm x 45 cm, all separated by trap doors. The left compartment had white walls and a metal mesh floor, the walls of the right compartment were painted black, the floor here was covered with a metal grid. Colour and texture of the floor served as cues for the rats to associate drug effects with one of the compartments. Since rats are nocturnal animals and very sensitive to light, the brightness in the black and white compartment was balanced with adjustable lights in the lid of the boxes. Prior to the experiments of this study, these lights were changed to DC bulbs to prevent the flickering observed in previous setup. Two identical Boxes were available for this study.

Electronic counters connected to light sensors in all three compartments recorded the time that a rat spent in the left and right compartment while exploring the apparatus. A printer was connected to one of the counters to assess the time spent in the compartments on each entry. It was discovered that the counters did not work at the same frequency, they were individually timed with a stop watch to establish the corresponding coefficients to calculate real time readings (Box 1: x 0.969 ; Box 2: x 0.99315). Readings on the time spent in any compartment were multiplied by these coefficients before analysis.

The CPP procedure was a four-day protocol. Rats were subjected to a pre-conditioning session on the first day, followed by two conditioning days and a post-conditioning test on day four.

For the pre-conditioning test, rats were individually put into the middle com-

partment with trap doors open. The lids were closed and the rats left to explore the apparatus undisturbed for 30 minutes. Of the outer compartments in which the rat spent more time became the saline associated compartment for the conditioning sessions, the opposite outer compartment served as the drug-associated compartment. The difference in time(s) spent in the drug minus the saline-associated compartment was calculated from the counter readings. Since the saline-associated compartment was only determined after this pre-conditioning session, this first time difference is always a negative value.

On the two conditioning days, rats were brought to the behaviour testing room at 9h00, injected intra peritoneally (i.p.) with saline (1 ml/kg) and put into the saline-associated compartment for one hour, with the trap door closed. After two hours, during which the rats were returned to the animal room, the same rat was i.p. injected with ketamine (12 or 20 mg/kg) and put into the opposite, hence drug associated, compartment for one hour. This same procedure was repeated on the third day.

On the post-conditioning day, rats were able to once again roam freely between the three compartments and again the time difference between drug-associated compartment and saline-associated compartments was recorded. The preference for the drug-associated compartment was expressed as the difference between the preconditioning value and the post conditioning value. It is hypothesized that a strongly rewarding drug effect would make the rat spend more time in the drug associated compartment during the post-conditioning session, consequently producing a positive post-conditioning value.

Two rats in one cage were brought to the experimental room immediately prior to every trail. The different rat strains and the different dosages were quasi randomly assigned to the two boxes. After every trial the boxes were wiped with a 10% ethanol solution to eliminate animal-to-animal cues.

Before starting the ketamine experiments, 39 naïve SHR and Wistar rats were used to get accustomed to the method and to make sure, that neither of the two outer compartments was on average preferred over the other. Slight increases in the brightness of the light in a compartment was used as a deterrent factor. The light conditions in the compartments were assessed and controlled at the beginning of every testing day, with a Panlux electronic luxmeter, Gossen, Germany

(see Fig.:2.2 on page 26). Luxmeter readings for this study were maintained at 9 lux for the white and 11 lux for the black compartment. Following previously used protocols (adopted from [52]), the lights in the experimental rooms were always switched off for all trials, as soon as the rats were put into the boxes, despite our apparatuses being opaque.

Room temperature of the third floor facility had to be carefully monitored and controlled since rats seemed to prefer the black chamber in colder conditions. Air temperature on all three compartments was checked and found to be equal around 24°C, a bias for the the black chamber could however be due to the greater immediate heat radiation from the dark walls. Heating the both rooms of the facility with an electrical heater before the trials resolved the bias.

For future experiments the outer walls of the CPP boxes should be repainted and dividing walls resealed early enough to allow for solvent fumes to vanish before the start of another experiment.

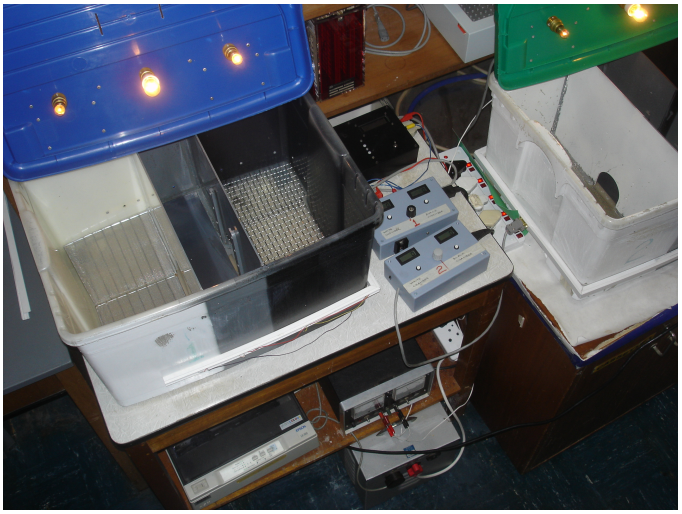


Figure 2.1: CPP Boxes with open lids, counters in between, the transformer for the DC lights and the printer below

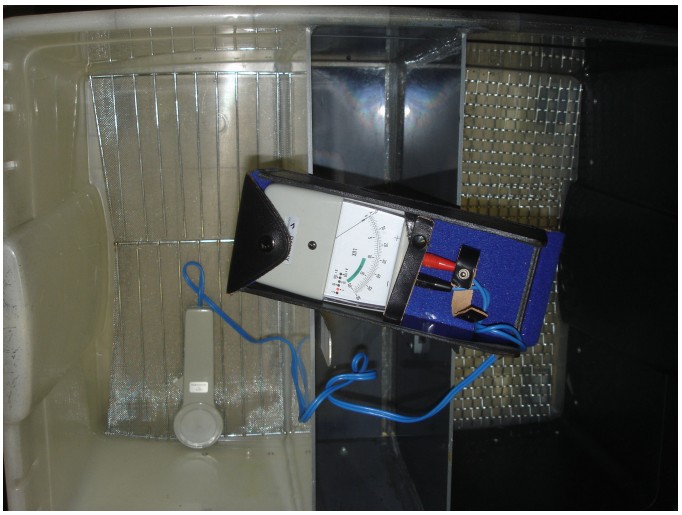


Figure 2.2: CPP Compartments showing sensor position to take the lux-meter reading

2.1.3 Intra-peritoneal injections

The employed i.p. injection was learned from Ms. Shula Johnson. The rats were held horizontally in the left hand, ventral side up, with at least index-finger and thumb having a firm grip on the neck and back skin fold and their tails tucked away under the little finger. Using a 0.5 ml insulin syringes with short needles made it possible to quickly insert the needle into the abdomen of the rat on its right hand side, making sure not to inject into gut nor liver. Despite being the generally more active strain, SHR were much easier to inject then WKY and SD. WKYs tried to wriggle out of the holding hand as soon as they were turned on their backs and vocalized occasionally. SD rats were hard to hold comfortably. They were generally bigger, their skin seemed much tighter and it was hard to get a good grip on them. Since they would try to bite when injected for the first time, they were consequently injected wearing gloves.

This difference in handling of different strains of rats was considered to have a minimal masking effect on behaviour.For future studies including SD rats, it should be considered to handle all rats with gloves.

Ketamine solutions were freshly prepared every week from Anaket-V (Centaur Labs, South Africa) dissolved in sterile saline to make adequate volumes of 12 and 20 mg/ml. The rats simply received injections of 0.1 ml per 100 g body-weight. Controls received saline injections only.

Rats that were only tested in the OF received injections on day three and two days prior to the testing day in order to give them a drug and injection history equal to that of the rats tested for CPP prior to the OF recording.

2.1.4 Open Field Recording

The Open Field (OF) consisted of a matt black square arena measuring 1 m x 1 m with 50 cm high walls. A white line on the floor demarcated a square inner zone of 66 cm x 66 cm, leaving a surrounding outer zone of ca. 34cm width.

On the day after the post-conditioning test, rats were brought up to the experimental room one hour before testing to acclimatize to the environment. During this time the recording computer was set up in the same room and the connection to the camera in the back-room was established and tested.

After partial data losses were discovered in July, a test recording was conducted on every testing day to make sure that the power connection from the low-voltage transformer to the extension lead and from there to the camera was well connected. However, even under optimal conditions, the Windows Media Player would only reproduce 80% of the actual recording time. This breakdown remained constant after it was discovered and was into account for the processing of the recorded files (section 2.2.1 on page 33).

Another peculiarity of the video surveillance system "Smart Guard" (Aver media), used in this study to record OF and EPM behaviour, is that it automatically creates a new video file on the hour, independent of the duration of the recording. For a smooth analysis of the data using the Ethovision software, it was found worthwhile to plan the recordings, so that every trial was finished before the hour changed on the internal clock of the recording computer. Also back-up copies of all recordings were made after every testing day, since Smart Guard deleted old files to empty memory for ongoing recordings. A minimum of 400MB memory must be available for every hour of recording.

For the recordings, rats were taken to the back room individually and placed into the top right corner of the OF (as seen through the recording camera) with their bodies parallel to the top wall and facing the left wall. For the ketamine study rats were injected i.p. (see 2.1.3 on the preceding page) immediately before being place into the OF.

Prior to placing the rats into the OF, a sheet of paper with the rat's name and treatment was held under the camera to label the video file. After each trial, the OF field was cleaned with ethanol (20% for the cross fostering study, 10% for the ketamine studie) and left to dry.

For future studies it is recommended that the environmental conditions are checked and controlled prior to the first experiments. In particular these are: repainting the OF to maintain a uniform wall colour after repeated cleaning, repairing the front door to allow it to shut and open more quietly, controlling the air ventilation to the room, which can greatly effect the room temperature, providing heating if the facility is used in winter and trying to keep clear of periods of high usage of the tutorial rooms on the same level since noises from the corridor are not sufficiently blocked off.

2.1.5 Elevated Plus Maze

This method was only applied to the rats of the cross fostering study.

The EPM was made from matt black plastic and had four arms of 100cm in length across and 10cm in width, all 50cm above floor-level. Two arms were walled with panels 40cm high, constituting the Closed Arms. The Open Arms were bordered by a fringe only 2-cm in height. The ends of all arms were open. The floor under the elevated plus was covered with black cardboard to increase the arena surface for the following video analysis and to dim the light reflected by the light coloured floor.

After the OF recordings rats were allowed to rest in their cages for two hours in the front room. Then they were individually taken back into the back room and carefully put into the square center zone at the crossing of closed and open arms and recorded for 5 minutes. Several SHR and SD rats fell of the end off the first arm they entered, were noted and put back onto the EPM for five minutes. Accounting for the additional stress of falling and being picked up again, these rats were not included in the study.

For future studies rats should be prevented from falling of the EPM by blocking the ends of all arms with the same kind of low fringe that borders the long sides of the open arms. Also the black cardboard under the apparatus could be removed in order to increase the contrast of the end of the elevated arms against the floor, possibly resulting in less accidentally falls. For the method of detection used in the video analysis the cardboard proved to be unnecessary if not even complicating the definition of the arena area on the computer screen (see A on page 83).

2.1.6 Cross-fostering

The cross fostering procedure described here was conducted by Fleur Howells [53].

Females of the SHR, WKY and SD strains were mated with one male per two dams, pregnant dams were housed individually and day of birth (postnatal day 0) noted. On postnatal day 2 (P2) litters were cross fostered, or stayed with their birth mother as controls. This study only used male rats.

Litters were reduced to eight pups, keeping females only in litters with less then eight males. Litters with less then five pups were not used for this study.

Pups stayed with the dams until weaning on P21 and then were housed in pairs of litter-mates in mouse cages (36 cm x 16 cm x12 cm). OF and EPM tests were conducted at the ratsage of P28 and P33 and occurred between 10.00h and 14.00h.

2.1.7 Oral Self Administration

This trial experiment adapted the limited access procedure employed by a parallel ethanol study in our lab (conducted by Heleen Soeters, [54]), and established to be a valid method of inducing high drug consumption and drug seeking behaviour. Rats were housed individually in mouse cages (see 2.1.6 on the preceding page) with standard rat chow and tap water ad libitum. In addition to the water bottle, the cages were equipped with another bottle (120 ml) containing an increasing concentration of ketamine in tap water. Starting on postnatal day 60 rats had access to 0,25 mg/ml Ketamine for 6 days followed by 0,5mg/ml Ketamine for 5 days and finally 0,75 mg/ml for another 5 days. Liquid consumption was determined every day in both water and ketamine bottle, by weighting the bottle on a laboratory scale noting the closed 0,1 g. After 16 days of continuous access to the ketamine solution, the limited access protocol started. Now the Ketamine bottle was only inserted into the cage for one hour daily (12.00h to 13.00h). This should trigger and show drug seeking behaviour in rats previously exposed and addicted to Ketamine with the increasing concentration schedule. The limited access procedure was continued for a minimum of 6 days during which water and Ketamine consumption was measured for the hour of access to both bottles. Water consumption during the past 23 hours was also measured prior to the insertion of the Ketamine bottle. After the OSA procedure, rats were humanely killed.

2.1.8 Transcardial Perfusion

The Method of Transcardial Perfusion was demonstrated by Mr. Musa Mabandla and supervised by Laurie Kellaway, University of Cape Town. Perfusions were conducted in Kellaway' s laboratory on the fourth level of the Anatomy Building. Rats were brought here one hour prior to the perfusion to reduce any stress effects of moving them into an unfamiliar surrounding.

Rats were ip injected with 12 or 20 mg/kg ketamine or saline-vehicle and after two hours individually deeply anaethetized in a perspex chamber saturated with halothane fumes. Unconsciousness of rats was tested by pinching their paws. When no reaction to the pinch was recognized, the rats legs were strapped with masking tape which then was pinned onto the cork surface of the perfusion plate (see Fig.: 2.3, page 32). The perfusion solutions were pressure injected from 60 ml syringes connected to a cannula with transparent rubber tubing and a T-piece to allow for continuous flow while changing from one solution to another. It is important to clear all air-bubbles and residual fixative from the tubing and cannula prior to perfusion since they would both lead to coagulation in the blood-vessles and thus prevention of flow of fixative to deeper tissue.

Once mounted, the rat's ventral skin was lifted using big forceps and a coronal cut below the chest was done to expose the diaphragm from the abdominal side. It was carefully cut open with fine scissors. The chest was opened by cutting up along one side of the sternum, exposing the lungs and heart. Both costal arches were bent outwards and held down by attaching a big clamp to them (see Fig.: 2.3, page 32). A 0.9 x38 mm cannula (Noels, Terumo Corp.) was inserted into the left ventricle and secured by a small clamp. It was slightly blunted by hand using a fine file to prevent accidentally perforating the opposite side of the ventricle. Through this needle 120 ml of physiological phosphate buffered saline (PBS) were slowly injected into the rat's circulation in order to flush out most blood. The right atrium was cut with fine scissors to allow the liquid to exit. Following the PBS, the rat was perfused with 300 ml 4% parafomaldehyde in PBS. The comprehensiveness of the fixative's delivery throughout the rats body could be anticipated by the quivering of the limbs, the stiffening of the neck and the leaking of fixative from the rat's nose.

The perfusion tray on which the rats were mounted was rinsed continuously with tap water. All liquids flowed into an adjacent sink. The whole setup was ventilated by a fume-hood which evactuated irritating paraformaldehyde fumes to the outside.

Figure 2.3: Set up for transcardially perfusing rats

After completed perfusion, the rats head was cut off with a pair of scissors. Skin and subcutaneous tissue was removed from the skull which was then carefully opened from the foramen magnum to the olfactory bulb using fine bone-pliers. The exposed brain was cleared from residual pieces of dura mater and carefully lifted with a blunt spatula starting at the ventral hind end. All cranial nerves and the olfactory bulb were cut in the process and the brain was transferred to 20 ml of 4% paraformaldehyde solution for additional post-fixation overnight at 4°C.

For cryoprotection the brains were then transferred into 20 ml of 30% sucrose in PBS until the sucrose penetrated the tissue. This was judged by the sinking of the brain into the solution on which it would first float.

Finally the brains were surrounded with Jung Tissue Freezing Medium (Leica Microsystems) and wrapped in a piece of Parafilm (Penchiney Plastic Packaging). The brain was secured and labeled with a piece of masking tape and quickly frozen in the fumes of liquid nitrogen. Care was taken for the brains not to touch the liquid nitrogen itself, otherwise they would break to pieces in the process despite the cryoptotection. Finally all brains were stored at -80°C.

All solutions were freshly prepared prior to the day of perfusions to allow for the paraformaldehyde to dissolve overnight at low temperature and stored in the fridge. Chemicals were supplied by Merck, South Africa.

2.2 Data Analysis

2.2.1 Video Analysis

The analysis of the behavioural recordings was done on the program EthoVision (Noldus Information Technology, Wageningen, The Netherlands). The program was licensed to Prof. Willie Daniels of the University of Stellenbosch, South Africa, and all analysis was done on a PC in his laboratory. Every batch of recordings was analyzed in a separate workspace-file, different experiments like EPM and OF were grouped in experiments in this workspace. Different experimental days were analyzed as different arena setups so that the apparatuses didn't have to be in exactly the same place on each recording day. The exact procedure is described "click-by-click" in the Appendix (see A on page 83).

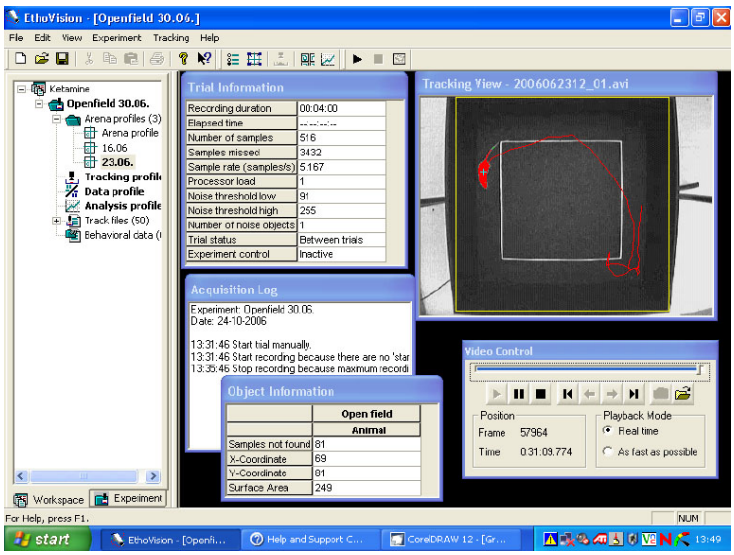


Figure 2.4: Screenshot of Ethovision while tracking an OF trail

2.2.2 Statistical Analysis

The collected data was put into spreadsheets using Microsoft Excel, licensed to Prof. Vivienne Russell.

Figures were edited on the Prism4 (GraphPad), licensed to Prof. Vivienne Russell.

All statistical analysis was done using Statistica 7, StatSoft, licensed to Vivienne Russell. Data from the Cross fostered Study was analyzed by Miss Fleur Howells. ANOVA test and post-hoc Newman-Keuls test were employed.

The data from the CPP experiments was analyzed with ANOVA. The resulting p-values were used to estimate an appropriate sample size using the STATA program at the UCT Health science learning center.

Levenes test was significant for the OF data of the ketamine experiments. Consequently they were treated as not normally distributed. Non-parametric analysis was performed on this data using the Kruskal-Wallis test. Correlations were tested using the Spearman Rank Order test.

Statistical Tables are presented in the Appendix (section B on page 87).

2.3 Immunohistochemistry

To further study the effects of subanaesthetic ketamine injections an attempt was made to quantify the c-fos expression in the prefrontal cortex (PFC) and the nucleus accumbens (NcA) (see 1.1.4 on page 20), in all three rat strains two hours after injection of 12 mg/kg or 20mg/kg Ketamine or vehicle only. Stainings were performed on the Cryostat sections of brains collected after transcardial perfusion (see 2.1.8 on page 31). Stainings were done with the Calbiochem anti-c-Fos (Ab-1) mouse mAb (2G9C3) (see data-sheet [2]), specified for immunofluorescence at a concentration of 2.5-5.0μg/ml. Visualization was done with a fluorescent Cy3 secondary antibody from JacksonImmunoResearch (see supplier's data-sheet [55]).

After some test series sections were cut at 20μm and collected in a 0.1M phosphate buffer (PB), containing 47,7g Na_2HPO_4 , 8,83g $NaH_2PO_4 \bullet H_2O$ and 10,2g NaCl in 2 l of distilled Water (dH_2O) and checked to have pH around 4,7.

Endogenous peroxidase was quenched by bathing the sections for 30 minutes in 0.05% H_2O_2 in dH_2O. A 30 minute incubation in 1% Milk-powder in PB was done to cover all nonspecific protein binding sites in the tissue. Sections were washed after every incubation step three times for 3 minutes in fresh PB. The washing and the thus far described incubations were done in ice-cube dishes perforated at the bottom and half submerged in a flat plastic container on a "Bellydancer"-shaker at low speed. The following incubations were done in 1ml liquid in sealable 2 ml Eppendorf tubes.

To increase permeability of the tissue and further reduce nonspecific binding, sections were incubated in a solution of 5% Bovine serum albumin (BSA), 5% Normal horse serum (NHS) and 0.05%TritonX in PB for 30 minutes.

The primary antibody was applied in a solution containing 0.5% BSA, 0.5% NHS and 0.05% TritonX in PB and left for 36 hours at 4°C on a Nutator-Mixer at low speed. To test the antibody and establish a protocol with maximum specific signal and minimal background, the antibody was applied in a range of concentrations (0,4 to 4μg/ml) for different staining series. After washing in PB, sections were incubated for 30 min at room temperature in the same solution now containing the secondary antibody also in different concentration. Finally sections were washed and mounted on gelati-coated cover slips and air-dried overnight. Entellen or glycerol with anti-fade was used to cover-slip the specimen. All steps from the application of the secondary antibody on were protected against direct light to protect the intensity of fluorescence. To better identify cellular structures, sections were counter-stained with 4',6-Diamidino-2-phenylindol (DAPI) (1:100, alliquoted antibody in PB; 30 minutes at room temperature). DAPI specifically stains the nuclei with a fluorescent light blue. To further test the primary antibody, stainings were conducted with an indirect avidin-biotin-peroxidase complex (ABC) technique, using a biotinylated secondary anti mouse antibody, the Elite-ABC kit from Vector Laboratories and 0.05% diaminobenzidine (DAB) in Tris buffer at pH4,9. This latter method was previously applied for staining thyrosinehydroxylase for the research of Fleur Howells and Musa Mabandla.

The staining protocol used here was mainly adapted from work previously done in the laboratory of Prof. B. Illing, University of Freiburg (see [56], closely discussed with Dr. Dirk Lang, University of Cape Town, and similar to many

other broadly applied ICC protocols [49] [57] [33] [58] [59] [48].

Microscopy and photo-documentation was done using a "Zeiss Axiovert 200" Microscope equipped with an "Axiocam HRm" digital camera, a fluorescence light-source "ebq100" and the AxioVision imaging program.

Chapter 3

Results

3.1 Behavioural Tests

3.1.1 Cross Fostering

Data from the cross fostering study is submitted for publication [53]. The behavioural tests OF and EPM with cross fosterd and control rats revealed significant differences within rat strains depending on the rearing mother and furthermore documented general strain differences unchanged by the rearing condition. From the 15 minutes open field recordings several parameters where analyzed with the NoldusEthovision program (see 2.2.1 on page 33): total distance(cm) travelled in the open field , latency to first enter the inner zone, numbers of entries into the inner zone and time spent in the inner zone.

From the EPM recordings the number of entries into the open arms and the time spent in open and closed arms were analyzed. The time spent in the open arms is not equal to the total time on the EPM minus the time spent in the closed arms due to the time that the rats spent in the center zone of the EPM where open and closed arms meet. This zone was not classified since it is not as protected as the closed arms but also not completely open due to the adjoining walls of the closed arms.

In the open field, all SHRs independent of their rearing background travelled a greater distance than WKY or SD rats ($p < 0.005$), SDs also travelled significantly further than WKYs ($p < 0.0005$) (see 3.1 on the following page). The rat strains

also revealed differences in first entering the inner zone. WKYs took significantly longer to first enter the inner zone than SHRs and SDs ($p < 0.0005$), SHRs showed a tendency to enter sooner than SDs ($p < 0.063$) (section 3.2 on the next page). SHRs entered the inner zone more often and spent more time there than WKYs and SDs ($p < 0.005$), while SDs entered more often and spent more time in the inner zone than WKYs ($p < 0.0005$). The number of *fecal boli* dropped in the OF during this 15 minute recording session was significantly different between SHRs and WKYs compared to SDs since the latter rats hardly defecated at all ($p < 0.005$)(section 3.3 on page 41).

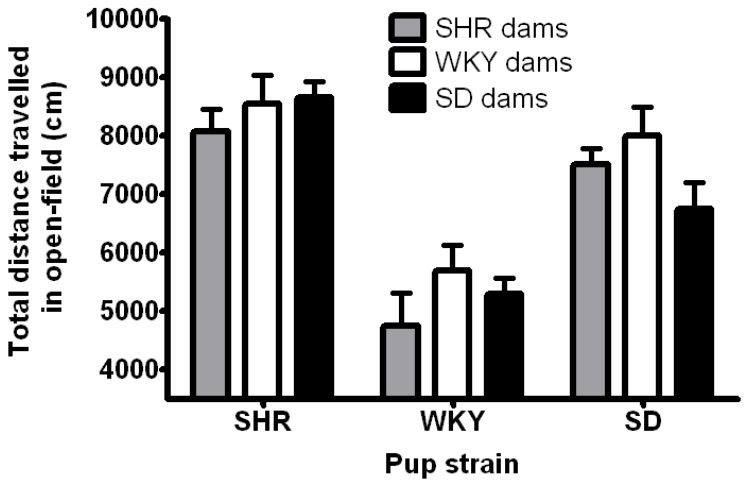


Figure 3.1: Cross fostering, OF: Total distance travelled in 15 minutes.
SHR travelled significantly further then WKY and SD ($p < 0.005$), SD travelled significantly further then WKY ($p < 0.0005$)

The cross fostering only resulted in significant differences in OF behaviour in the following cases. Control SD pups revealed a higher latency to enter the inner zone when compared with cross fostered SDs ($p < 0.05$) (Fig. 3.2 on the next page). Control SDs also spent significantly less time in the inner zone than cross fostered SDs and made fewer entries to the inner zone than SHR-reared SDs (see *d* and *c* in Fig. 3.3 on page 41, $p < 0.05$).

WKY pups reared by SD dams entered the inner zone more often than control

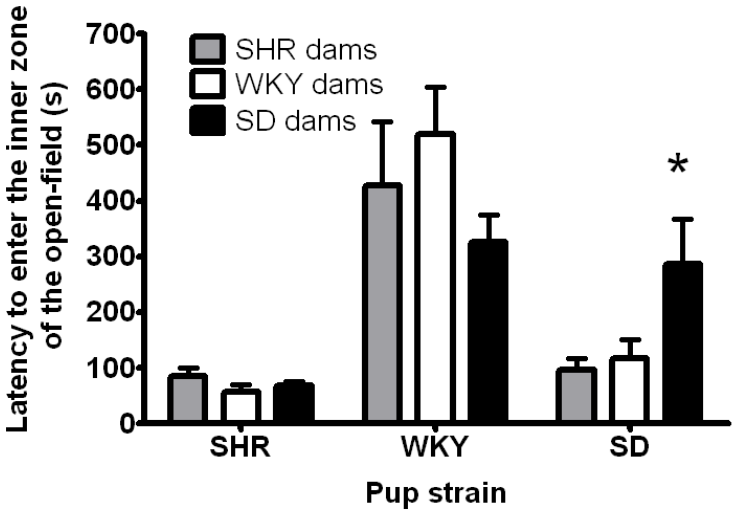


Figure 3.2: Cross fostering, OF: Latency of first entrance to the inner zone.

WKY took longer to enter the inner zone than SHR and SD ($p < 0.0005$), difference between SHR and SD only had a tendency to be different ($p = 0.063$), *SD controls significantly different from cross-fostered SDs ($p < 0.05$)

and SHR-reared WKYs and spent more time there than SHR-reared WKYs (see *a* and *b* in Fig. 3.3 on page 41, $p < 0.05$).

In the EPM the SHR pups performed significantly different from WKYs and SDs when pooling all rearing conditions. SHRs made more entries into the open arms (Fig. 3.1.1 on page 43, $p < 0.0005$), spent more time in the open arms ($p < 0.05$) and less time in the closed arms ($p < 0.0005$) than the other rat strains (Fig. 3.4 on page 42)

The effects of cross fostering were only observed in SDs pups. They made more entries into the open arms and spent less time in the closed arms when cross fostered onto SHR dams ($p < 0.05$, see *in Fig. 3.1.1 on page 43 and 3.4 on page 42).

Before being sacrificed for the superfusion experiments all rats from this study were weighed. Control SD rats were heavier than all other rats. Strain differences between SHR and WKY pups' weight were not apparent in this experiment (in contrast to data from OSA experiment in 3.3 on page 63) nor did the cross fostering influence their weight.

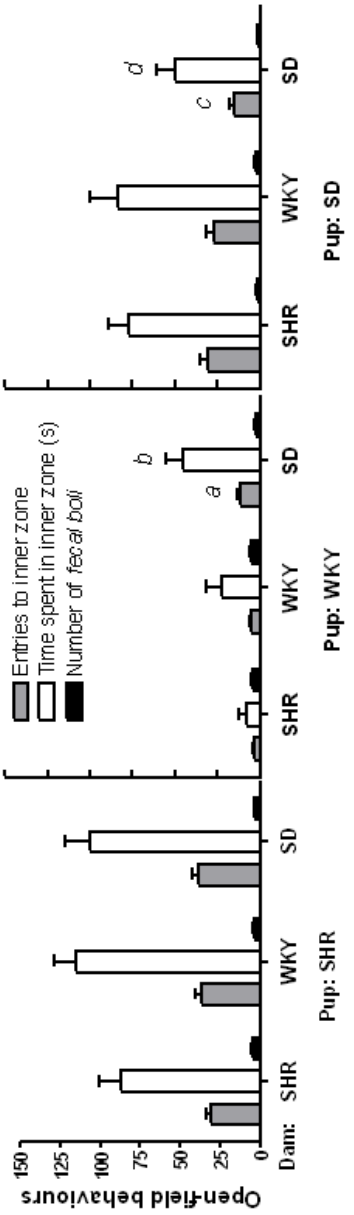


Figure 3.3: Cross fostering, OF: Number of entries to the inner zone, time(s) spent in the inner zone, number of *fecal boli* after 15 minutes.

For time spent in the inner zone and number of entries SHR are significantly higher than SDs and WKYs ($p < 0.005$). SDs score significantly higher in those two parameters than WKY ($p < 0.0005$) and defecated less than both other strains ($p < 0.005$). *a* WKY on SD dams enterd the inner zone more often than control WKY or WKY pups on SHR dams ($p < 0.05$), *b* WKYs on Sd dams stayed in the inner zone for significantly longer than WKYs fostered on SHR dams ($p < 0.05$), *c* control SDs made more entries to the inner zone than SD pups on SHR dams ($p < 0.05$) and *d*spent less time there than both cross-fostered SD groups ($p < 0.05$)

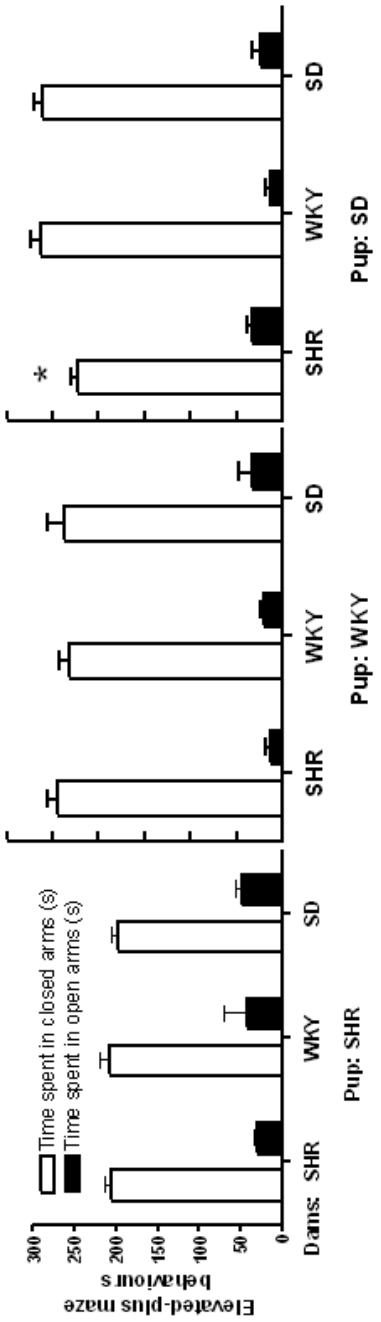
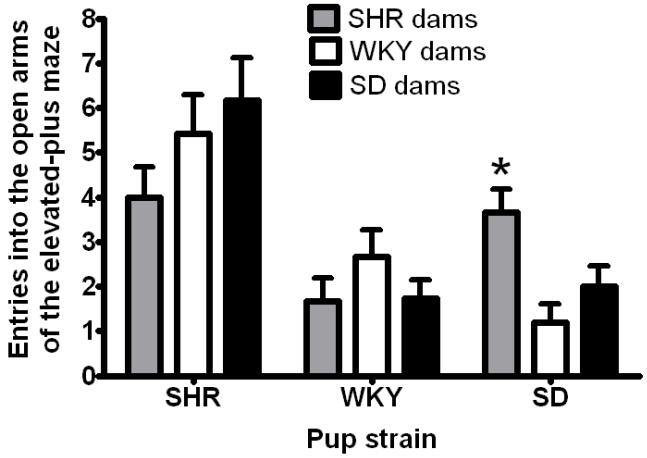


Figure 3.4: Cross fostering, EPM: Time spent in the open and closed arms.

SHRs spent generally more time in the open arms ($p < 0.05$) and less time in the closed arms ($p < 0.0005$) than SDs and WKYs. *SD pups on SHR dams spent significantly more time in the closed arms then SD pups on WKY dams and control SDs ($p < 0.05$)



captionCross fostering, EPM: Number of entries to the open arms. SHRs made more entries to the open arms than WKYs and SDs ($p < 0.0005$). *SD pups on SHR dams entered the open arm more often then control SDs and SDs reared by WKY dams ($p < 0.05$)

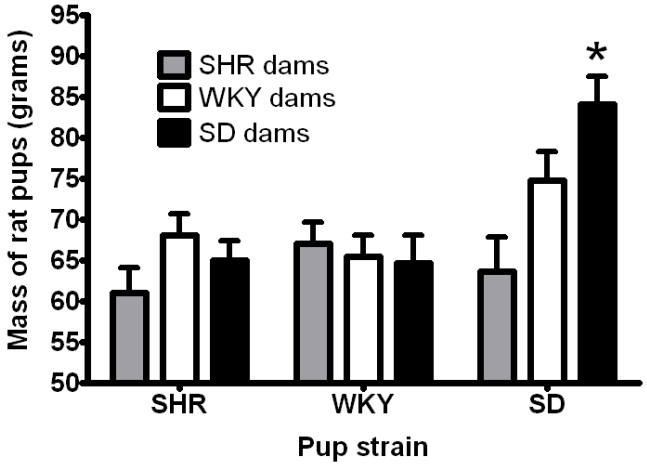


Figure 3.5: Cross fostering: Body mass

Two days after behavioural tests (P30 - P35). *Control SDs are significantly heavier then all other groups ($p < 0.05$)

Within the different parameters of the two behavioural tests (OF and EPM) and the results of glutamate stimulated nor-epinephrine release in the hippocampus and PFC (done by Ms. Fleur Howells [53]), many correlations were found. Only a few ostentatious clusters will be discussed here. The full table of correlations between behavioural parameters is presented in table 3.1 on the next page.

Over all, the SD groups show nearly twice as many correlations than the other two pup strains, with the control SDs exceeding all other experimental groups. This group represents the most unaltered group, not being inbred for special behavioural features and also not challenged with the environmental changes of cross-fostering (pups remained with their biological mother).

Also striking is the positive correlation in all groups between the total distance travelled in the open field and the numbers of entries to the inner zone and between and the number of entries to the inner zone and the time spent there and finally the necessarily negative correlation between the time spent in the open arms of the EPM and the time spent in the closed arms. This does not surprise since the two parameters are closely related. It could be argued that the parameters are then redundant measures of the same behaviour. However, in the EPM the correlation between the time spent in the closed and open arms both versus the number of entries to the open arms should also closely determine each other. But they conspicuously fail the correlate in particularly in to experimental groups: WKY fostered onto SD dams and the inverted case, SDs fostered onto WKY dams.

These two groups share other correlations that are not to be found in many other groups. For them the number of entries to the inner zone of the OF correlates with the number of to the entries to the open arms in the EPM, the total distance travelled in the OF positively correlates with the number of entries to the open arms and nor-epinephrin release in the PFC correlates negatively with the three main OF parameters being the distance travelled, the frequency of entering the inner zone and the time spent there.

Table 3.1: Significant correlations between cross-fostering parameters

		Pup		SHR				WKY				SD			
		Dam		SHR	WKY	SD	pooled SHR	SHR	WKY	SD	pooled WKY	SHR	WKY	SD	pooled SD
Open-field: Total distance covered	vs.	Open-field: Number of entries to the inner zone		0.81	0.79	0.58	*0.73*	0.67	0.63	0.74	*0.52*	0.54	0.89	0.84	*0.67*
Open-field: Total distance covered	vs.	Open-field: Time spent in the inner zone		0.84	0.62		*0.61*				*0.37*	0.51	0.69		*0.62*
Open-field: Total distance covered	vs.	Open-field: Time taken to enter the inner zone												-0.77	*-0.53*
Open-field: Total distance covered	vs.	Open-field: Number of *fecal boli* at the end of recording				-0.59									
Open-field: Total distance covered	vs.	Elevated-plus maze: Total time in the closed arms													*-0.40*
Open-field: Total distance covered	vs.	Elevated-plus maze: number of entries to open arms								0.77		0.53	0.70	0.65	*0.43*
Open-field: Number of entries to the inner zone	vs.	Open-field: Time spent in the inner zone		0.94	0.87	0.69	*0.82*	0.79	0.83	0.68	*0.80*	0.95	0.90	0.80	*0.89*
Open-field: Number of entries to the inner zone	vs.	Open-field: Time taken to enter inner zone				0.75								-0.78	*-0.47*
Open-field: Number of entries to the inner zone	vs.	Elevated-plus maze: Total time in the closed arms												-0.74	*-0.44*
Open-field: Number of entries to the inner zone	vs.	Elevated-plus maze: Total time in the open arms									*0.37*			0.66	
Open-field: Number of entries to the inner zone	vs.	Elevated-plus maze: number of entries to open arms								0.65			0.70	0.84	*0.43*
Open-field: Time spent in the inner zone	vs.	Open-field: Time taken to enter inner zone													*-0.35*
Open-field: Time spent in the inner zone	vs.	Elevated-plus maze: Total time in the closed arms								-0.71	*-0.38*			-0.84	
Open-field: Time spent in the inner zone	vs.	Elevated-plus maze: Total time in the open arms								0.79	*0.61*			0.89	*0.34*
Open-field: Time spent in the inner zone	vs.	Elevated-plus maze: number of entries to open arms												0.88	
Open-field: Time taken to enter inner zone	vs.	Elevated-plus maze: Total time in the closed arms			0.69		*0.48*								*0.40*
Open-field: Time taken to enter inner zone	vs.	Elevated-plus maze: Total time in the open arms			-0.62		*-0.43*								*-0.35*
Open-field: Time taken to enter inner zone	vs.	Elevated-plus maze: number of entries to open arms					*-0.35*							-0.61	*-0.40*
Open-field: Number of *fecal boli* at the end of recording	vs.	Elevated-plus maze: Total time in the closed arms							-0.70						
Open-field: Number of *fecal boli* at the end of recording	vs.	Elevated-plus maze: Total time in the open arms		0.58											
Open-field: Number of *fecal boli* at the end of recording	vs.	Elevated-plus maze: number of entries to open arms							0.65						
Elevated-plus maze: Total time in the closed arms	vs.	Elevated-plus maze: Total time in the open arms		-0.65	-0.90	-0.76	*-0.77*	-0.80	-0.87	-0.93	*-0.82*	-0.83	-0.90	-0.89	*-0.81*
Elevated-plus maze: Total time in the closed arms	vs.	Elevated-plus maze: number of entries to open arms			-0.85	-0.80	*-0.74*	-0.58	-0.76		*-0.58*	-0.79		-0.91	*-0.78*
Elevated-plus maze: Total time in the open arms	vs.	Elevated-plus maze: number of entries to open arms		0.81	0.84	0.77	*0.83*	0.82	0.87		*0.41*	0.78		0.94	*0.78*

3.1.2 Conditioned Place Preference

The CPP experiments were conducted with SHRs and WKYs only. Twelve SHRs and 10 WKYs were challenged with 12 mg/kg ketamine (SHR12, WKY12), 13 SHRs and 11 WKYs were challenged with 20 mg/kg ketamine (SHR20, WKY20). Only rats that were P60±, which represents the rats' adolescence, on the pre-conditioning day were included in the study (each group n=10).

No dose or strain effect could be found in any of the four experimental groups alone. However a general drug effect could be found when pooling the two WKY groups ($p < 0.01$). The two WKY groups alone showed a tendency to prefer the drug-associated compartment after conditioning (12 mg/kg p=0.058, 20 mg/kg p=0.056, see appendix Fig. B on page 95).

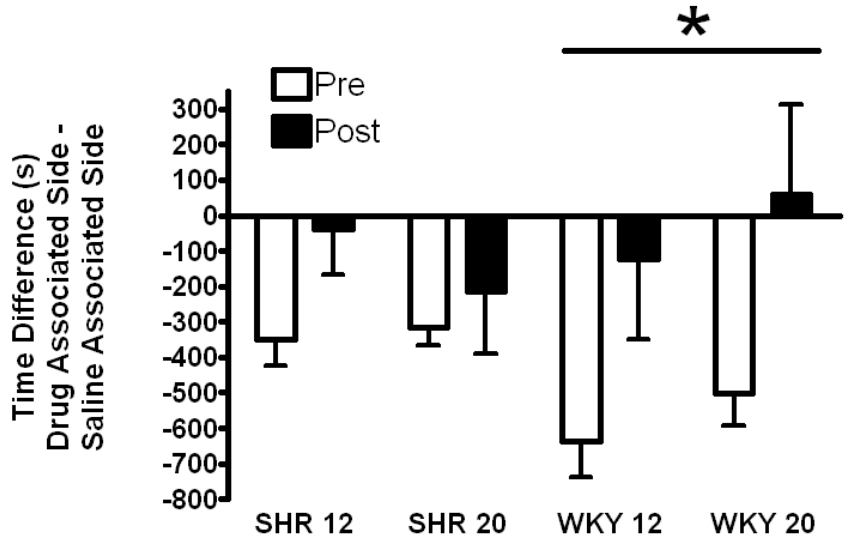


Figure 3.6: CPP: Effect of conditioning SHRs and WKYs at the age P60±1 with 12 and 20 mg/kg ketamine, n=10 in each group.

Significant shift to the drug-associated side after conditioning in: *both WKY rats pooled ($p < 0.01$)

Preliminary analyzes including the rats that were out of the P60 age-range showed a significant shift in preference for WKY20 and SHR12, consequently the sample size required to falsify our null-hypothesis in all P60 groups was estimated using the STATA program licensed to UCT. Using the mean values and standard deviations of the P60 groups the required minimum sample size was calculated to be 49. This number was considered to be too high to pursue in this study.

Since it found to be difficult to maintain even environmental conditions in the testing facility (see 2.1.2 on page 23) over the many weeks of testing an ANOVA test was conducted to see if for example the seasonal variations in room temperature had an effect on the compartment preferred in the pre conditioning tests. This proofed to be the case. Also the occurrence of an shift to the drug associated compartment after conditioning showed to be effected for a significant number of trials. Which of the two CPP-boxes was used didn't significantly influence both parameters. A Kruskal-Wallis rank ANOVA testing the differences in distance travelled in the OF during different testing weeks didn't show any significant effects either and was seen representative for all OF parameters. (see B on page 96).

3.1.3 OF behaviour after ketamine injection

In addition to the rats subjected to the CPP procedure 8 SHRs , 8 WKYs and 22 SDs were tested as controls in the OF. These SHRs and WKYs and 8 of the SD were injected with saline (1 ml/kg) before recording. Seven SD rats were challenged with 12 mg/kg ketamine and seven SDs with 20 mg/kg ketamine. Data is shown twice for every parameter analyzed: grouped by strains and grouped by injected dose.

Parameters analyzed with the Ethovision software are: total distance travelled (cm), meandering (mean degrees/cm, positive values representing more clockwise turns, negative values counter-clockwise turns), turns total (degrees). The two latter ones were included after qualitative observations of stereotyped circling on the spot after injecting the first SHR and WKY rats with ketamine. This behaviour seemed to be more pronounced with WKY (qualitative preliminary observation, validated after 20 mg/kg ketamine and saline injection, Fig. 3.10 on page 51). To further investigate the path shape as a measure of ketamine effected behaviour the total turns parameter was included.

One of the earliest qualitative observations during the CPP experiments was that especially SHRs made loud thumping noises in the chambers when injected with ketamine. While video recording the rats in the open field the source of this noise was resolved as being attempts at rearing hampered by ketamine induced ataxia. WKYs didnt produce such noises, and actually reared far less then SHR (Fig. 3.13) , suggesting that rearing could be a good parameter to distinguish different ketamine effects on the two rat strains. Rearing was scored manually while replaying the OF recording during Ethovision data acquisition. Defecation was scored as the number of *fecal boli* in the OF after 15 minutes.

For three parameters (total distance, meandering, and rearing) the 15 minutes recording time was furthermore broken up into three bins of 5 minutes each to study the temporal characteristics of the response to ketamine.

Ketamine at both dosages significantly increased the distance travelled by the SHRs ($p < 0.05$) only. Pooling data from all three treatments, WKYs travelled significantly less then both SHRs and SDs ($p < 0.0005$)(see Fig. 3.7 on the following page).

Under baseline conditions (saline injection) and after injection of 20 mg/kg ketamine WKYs also travel significantly less than both SHRs and SDs(both $p < 0.05$). Injected with 12 mg/kg ketamine WKYs were only different from SHRs ($p < 0.005$). Pooled data from all strains shows a difference between saline and 20 mg/kg injected rats ($p < 0.01$) (see Fig. 3.8 on the next page)

Ketamine altered the meandering patterns of SHR and WKY at both doses (both $p < 0.005$). Both strains turned more frequently in the opposed direction after ketamine administration. In SDs the animals injected with 12 mg/kg ketamine turned more pronouncedly counter-clockwise compared to the saline and 20 mg/kg groups ($p < 0.05$). Grouping all treatments revealed SDs to be different from both wistar strains ($p < 0.0005$), due to the maintained mean direction of turning (see Fig. 3.9 on page 51).

Comparing the strains showed that WKY turned more than the other strains after a saline injection ($p < 0.05$) and SDs were different from SHR and WKY strains after 12 mg/kg ketamine ($p < 0.05$). At 20 mg/kg all strains showed a different behaviour ($p < 0.05$). Pooling the strains revealed saline to be different from ketamine ($p < 0.00005$), (see Fig. 3.10 on page 51).

Ketamine decreased the number of total turns made by SHRs ($p < 0.005$), but not in WKYs and only at 12 mg/kg in SDs ($p < 0.05$). Pooling all testing conditions, revealed SDs to turn more than WKY and SHR ($p < 0.0005$). (see Fig. 3.11 on page 52)

At baseline WKYs turned least ($p < 0.005$), after 12 and 20 mg/kg ketamine SHRs reduced their total turns to the level of the unchanged WKYs, which made them significantly different from SDs that only reduced their turning at 12 mg/kg ($p < 0.05$ at 12 mg/kg and $p < 0.0005$ at 20 mg/kg). Both ketamine dosages had an overall reducing effect when pooling all three strains ($p < 0.0005$). (see Fig. 3.12 on page 52)

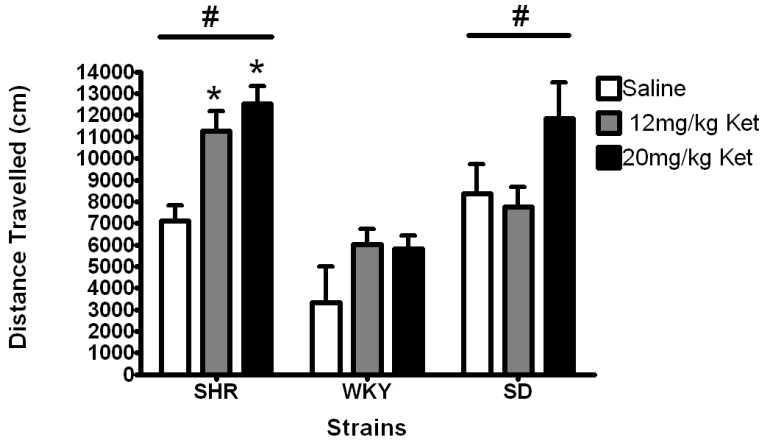


Figure 3.7: Total distance travelled in OF in 15 minutes, grouped by strains.

*significantly different from corresponding saline value ($p < 0.05$), # significantly different from WKY, $p < 0.0005$)

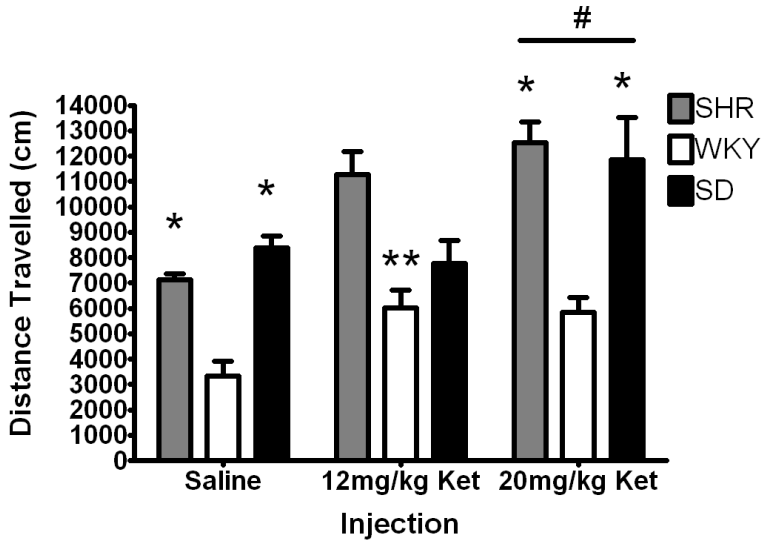


Figure 3.8: Total distance travelled in the OF in 15 minutes, grouped by injection.

(Data plotted in Fig. 3.7). *significantly different from WKY ($p < 0.05$), **significantly different from SHR ($p < 0.005$), # significantly different fromsaline ($p < 0.01$)

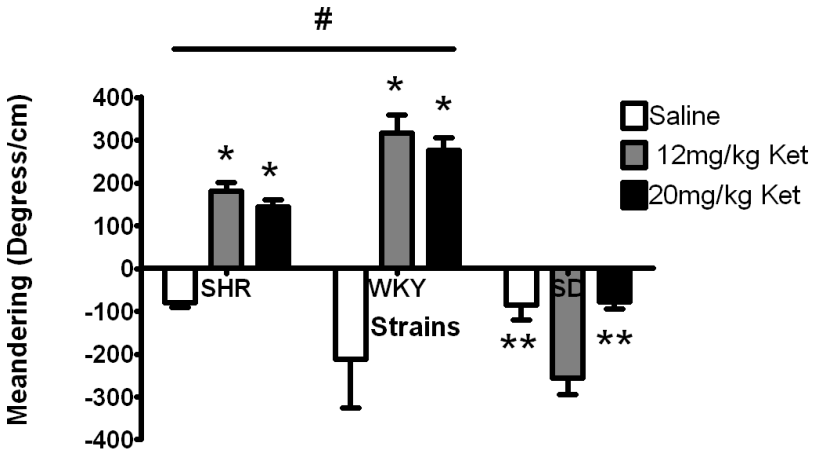


Figure 3.9: Meander scores for 15 minutes in the OF, grouped by strains.

*significantly different from saline ($p < 0.005$), **significantly different from 12 mg/kg ($p < 0.05$), # significantly different from SD ($p < 0.0005$)

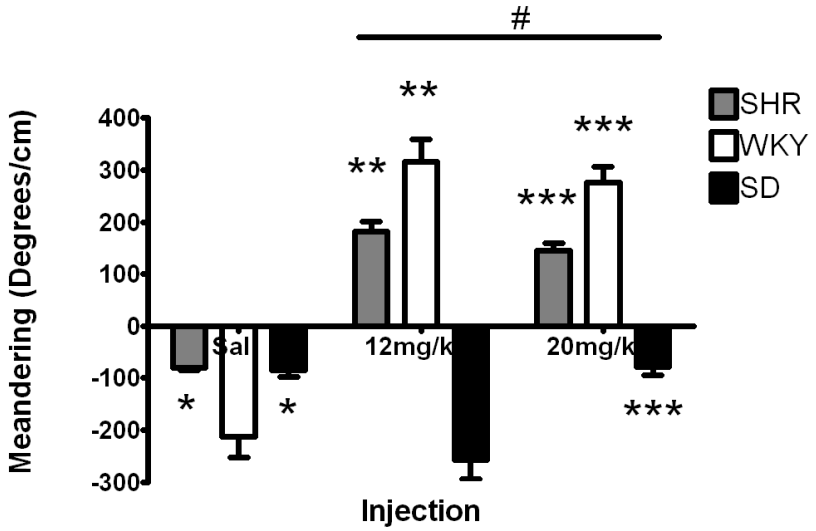


Figure 3.10: Meander scores for 15 minutes in the OF, grouped by injection.

(Data plotted in Fig. 3.9) *significantly different from WKY ($p < 0.05$), **significantly different from SD ($p < 0.05$), ***significantly different from both strains ($p < 0.05$ all comparisons but SD vs. WKY, here $p < 0.00001$), # significantly different from saline ($p < 0.00005$)

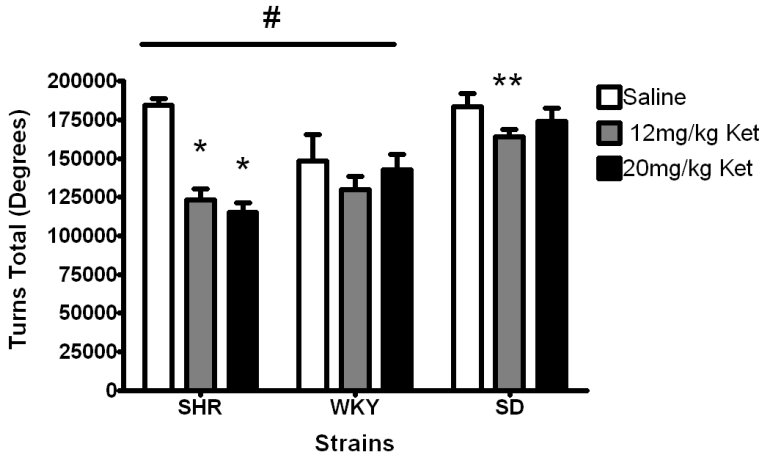


Figure 3.11: Turning in 15 minutes in the OF, grouped by strains.

*significantly different from saline ($p < 0.005$), **significantly different from saline ($p < 0.05$), # significantly different from SD ($p < 0.0005$)

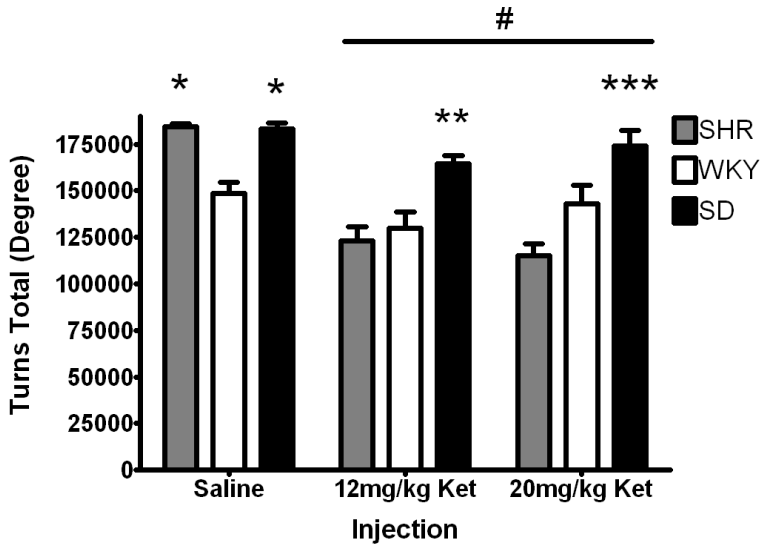


Figure 3.12: Turning in 15 minutes in the OF, grouped by injection.

(Data plotted in 3.11) *significantly different from WKY ($p < 0.005$), **significantly different from SHR ($p < 0.05$), ***significantly different from SHR ($p < 0.0005$), # significantly different from saline ($p < 0.0005$)

The number of rearings was different in all three strains, SHRs being the highest and WKY being the lowest ($p < 0.05$). Ketamine reduced rearing in all rat strains ($p < 0.005$ for grouped strains in Fig. 3.14 on the following page, $p < 0.05$ in SHRs and SDs alone, in Fig. 3.13 on the next page), in WKYs this reduction was only significant at 20 mg/kg ($p < 0.05$). Qualitative observations when screening the behavioural videos showed ataxia after ketamine injections especially in SD and SHR rats. WKY displayed the most pronounced head weaving behaviour after ketamine injections.

Comparing the strains at the different dosages shows that WKYs are different being the lowest at baseline conditions ($p < 0.05$) and SHRs being higher then both other strains at 12 mg/kg and higher then WKYs at 20 mg/kg ($p < 0.05$ and $p < 0.005$) (see Fig. 3.14 on the following page).

Defecation is different for all rats at baseline ($p < 0.005$, Fig. 3.16 on page 55). It is strongly reduced after ketamine injection in SHRs and WKYs ($p < 0.05$ and $p < 0.005$ in Fig.3.15). SDs hardly defecated at all ($p < 0.05$, Fig. 3.16).

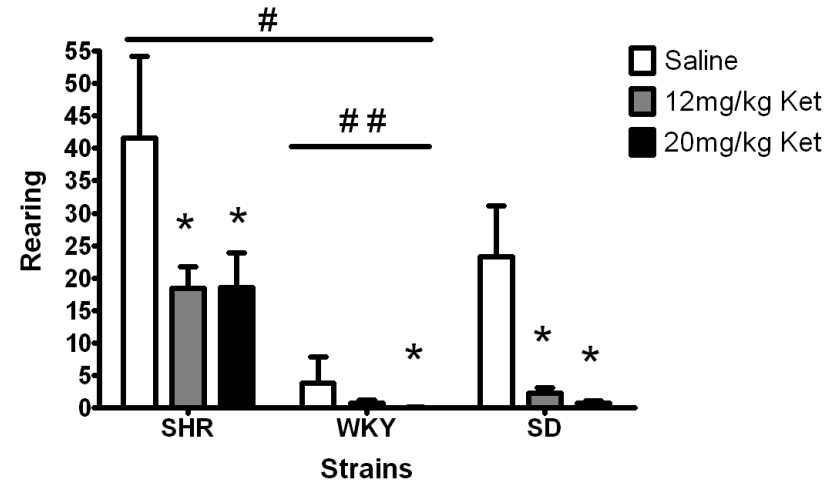


Figure 3.13: Rearing scores for 15 minutes in the OF, grouped by strains.

*significantly different from saline ($p < 0.05$), # significantly different from SD ($p < 0.05$), ## significantly different from SHR ($p < 0.000001$)

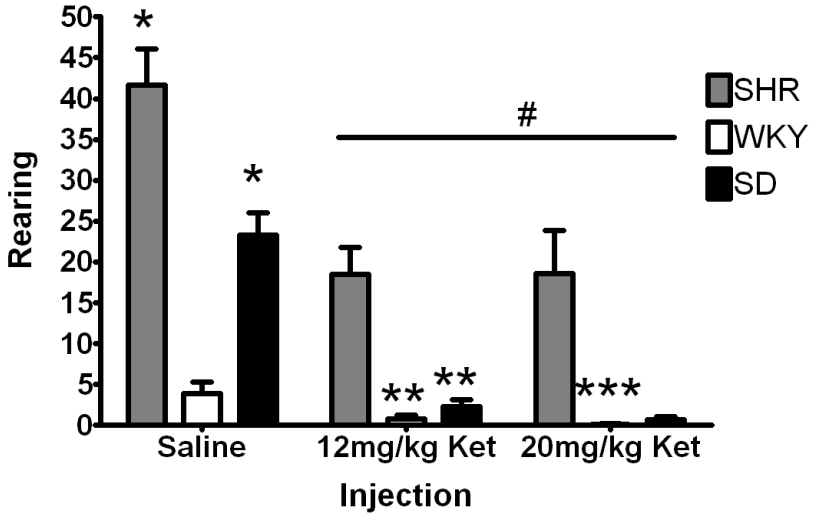


Figure 3.14: Rearing scores for 15 minutes in the OF, grouped by injection.

(Data plotted in Fig. 3.13) *significantly different from WKY ($p < 0.05$), **significantly different from SHR ($p < 0.05$), ***significantly different from SHR ($p < 0.005$), # significantly different from saline ($p < 0.005$)

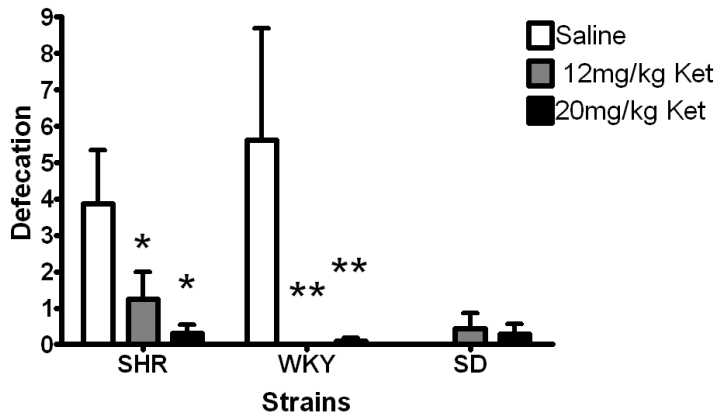


Figure 3.15: Defecation in 15 minutes in the OF, grouped by strains.

*significantly different from saline ($p < 0.05$), **significantly different from saline ($p < 0.005$)

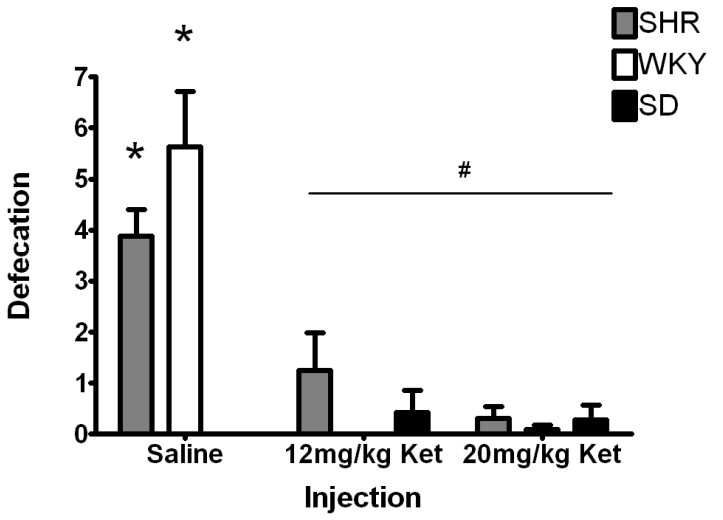


Figure 3.16: Defecation in 15 minutes in the OF, grouped by injection.

(Data plotted in Fig. 3.15) *significantly different from SD ($p < 0.05$), **significantly different from saline ($p < 0.005$)

Analysis of the distance travelled in five-minute bins, showed how the rats' activity gradually decreased during the 15 minutes in the open field. As described above, WKYs travelled less than SDs or SHRs after saline and 20 mg/kg injections but only less than SHRs after the 12 mg/kg dose of ketamine. This is due to the strong sedative effect of 12 mg/kg ketamine on SDs between 5 and 15 minutes after injection. After 10 minutes they actually travelled less than the wistar strains (siginficant only compared to SHRs, $p < 0.01$, see middle graph in Fig. 3.17 on the following page).After 15 minutes they were equal to WKYs and both were significantly lower then SHRs ($p < 0.002$). With an injection of 20 mg/kg that has an overall increasing effect on the locomotor activity of all rats (as described above) SDs increased their locomotor activity up to ten minutes after injection and then slowed down in the last five minutes of the experiment, ending beween SHRs and WKYs which were different from each other ($p < 0.002$).

The plotting of the meandering in five minute bins (see Fig. 3.18 on page 58) revealed an increase of turning per distance travelled in SHRs and WKYs after 12 mg/kg ketamine, while the activity of SDs decreased over time as it did in all rat strains after injection of saline.

At 20 mg/kg ketmamine the effect on SHRs and WKYs was not as pronounced as after 12 mg/kg and had actually returned to baseline in SDs (compare Fig. 3.18 and Fig. 3.9).

The SHR group displayed the greatest number of rearings in all time-bins at all dosages, with the most rearings occurring between five and ten minutes. This difference was always significant compared to WKYs (p values range from $p < 0.05$ after 15 minutes on each dosage to $p < 0.00001$ 5 minutes after saline injection). SDs had rearing scores similar to WKYs except for 15 minutes after the saline injection when they reared significantly more than WKYs ($p < 0.05$). (Fig. 3.19 on page 59)

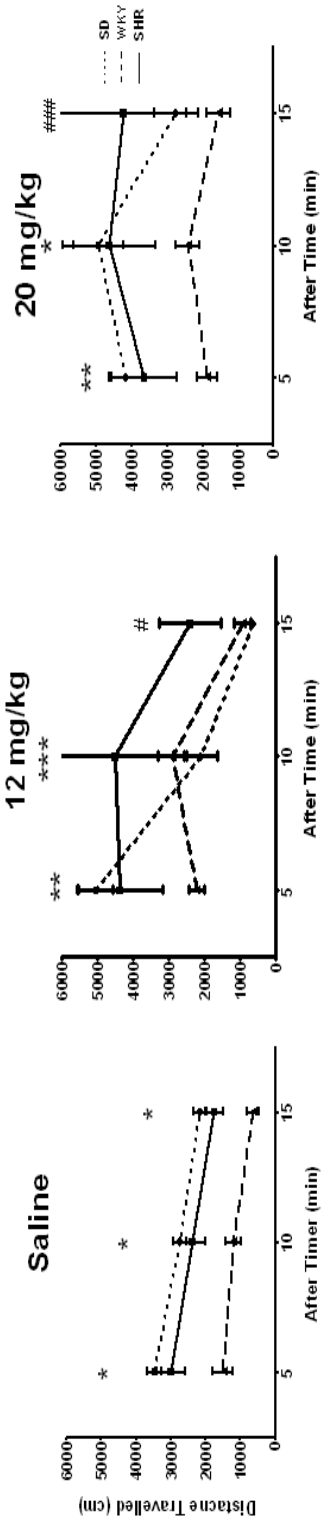


Figure 3.17: Total distance travelled data broken up into 5 minute bins.

*SD and SHR significantly different from WKY ($p < 0.05$), **SD and SHR significantly different from WKY ($p < 0.005$), ***SHR significantly different from WKY ($p < 0.01$), #SD and WKY significantly different from SHR ($p < 0.005$), ##SHR significantly different from WKY ($p < 0.002$)

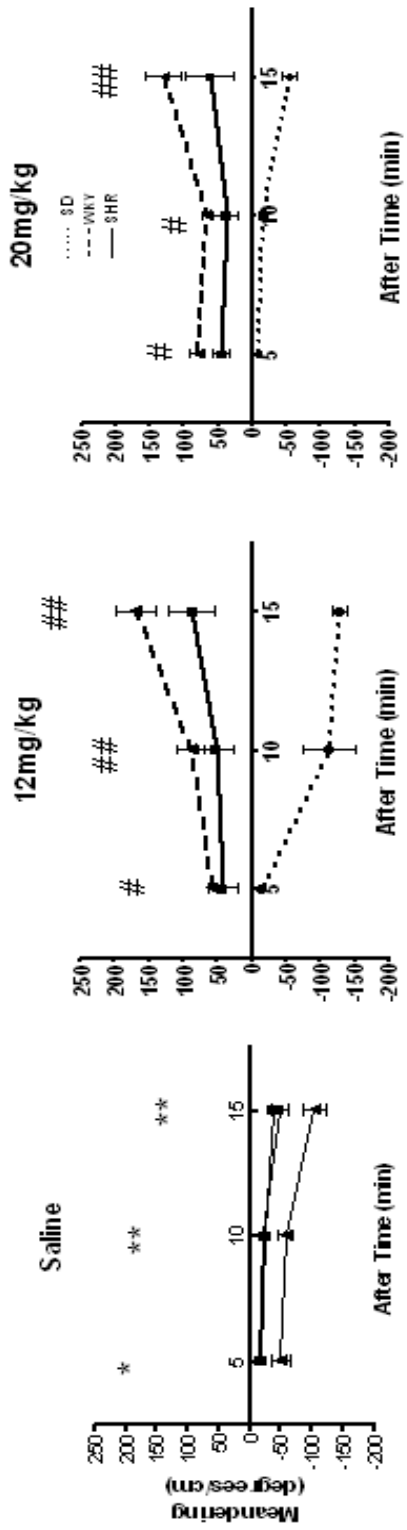


Figure 3.18: Meandering data broken up into 5 minute bins.

*SHR significantly different from WKY ($p < 0.0005$), **WKY significantly different from SHR and SD ($p < 0.05$), #SHR significantly different from WKY ($p < 0.05$), ##SHR significantly different from WKY and SD ($p < 0.001$), ###SHR significantly different from WKY and SD ($p < 0.05$)

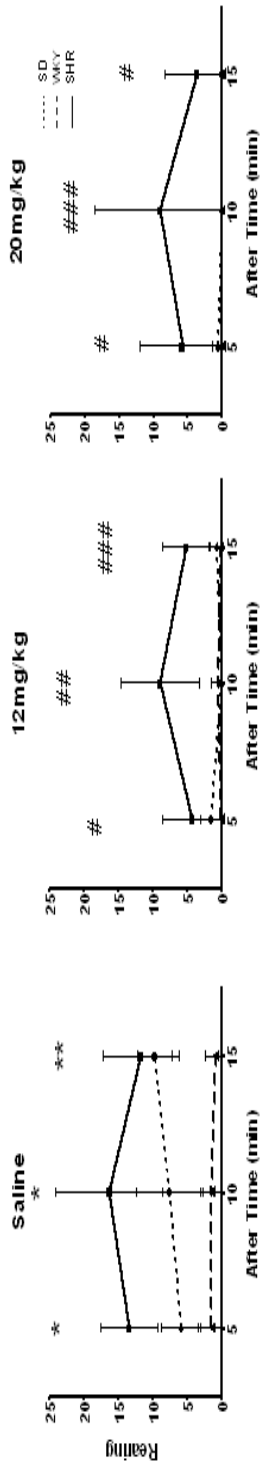


Figure 3.19: Rearing scores broken up into 5 minute time-bins.

*SD significantly different from WKY ($p < 0.01$), **WKY significantly different from SD and SHR ($p < 0.05$), #SD significantly different from SHR and WKY ($p < 0.05$), ##SD significantly different from SHR and WKY ($p < 0.01$)

Amongst the OF parameters only the total distance travelled correlated with the rearing scores ($p < 0.05$) and the meandering correlated with the total turns ($p < 0.05$). The latter correlation was seen as trivial since the two parameters are so closely related. No correlation was found between the rats' performance in any of the OF parameters and the CPP behaviour.

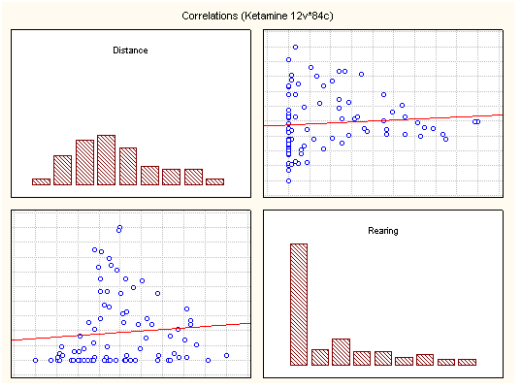


Figure 3.20: Correlation of Total distance travelled and rearings in the OF after injection of saline and ketamine

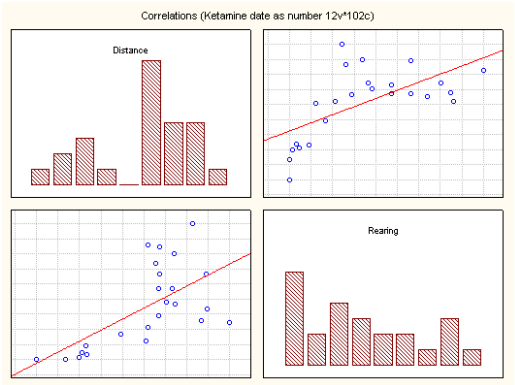


Figure 3.21: Correlation of Total distance travelled and rearings in the OF after saline injection only

3.2 Immunohistochemistry

Six test series with several concentration of primary and secondary antibody each were conducted using the protocol described in section 2.3. Primary antibody was used in concentrations of 1:50 to 1:500 (always stating antibody solution in incubation solution (section 2.3). This covered the range of concentrations suggested on the supplier's data-sheet [2] and below. The secondary antibody was applied in concentrations from 1:1000 to 1:2000 (amount of antibody alliquotted with 50% glycerol in incubation solution) as used in established protocols in the laboratory of Dr. Dirk Lang. In order to distinguish background from specific fluorescent signal, blocking solutions and mounting media were changed and modified and negative controls of all antibodies were included. None of the described conditions resulted in stainings that would have enabled quantification of c-fos expression in the brain tissue (section 1.1.4 on page 20). The Cy-3 secondary anitbody visualized round cellular structures. However, nuclei were not stained as expected from literature (e.g. in [57] [49]). DAPI counterstaining visualized the nuclei but did not co-localise with the c-fos staining (a and b in Fig. 3.22). The contrast between seemingly specific staining and Cy-3 background was to low for quantification, also after DAB staining (c and d in Fig. 3.22). Repeated consultations with the supplier of the primary antibody (Merck, Germany), revealed that the data-sheet which suggested it to be appropriate for the protocol used in this study has not been updated since Merck had bought up Calbiochem and it contained misleading descriptions ("Immunofluorescence" as opposed to "Free-floating Sections"). No recent references for the application of the product could be found to check the protocols. Merck apologized and offered a replacement product (Anti-c-Fos (AB-5)(4-17)Rabbit pAb, Catalog No. PC38 [47] or a credit note for further purchases. Since the time allocated to this part of the study was running out and the new primary antibody would also have required another secondary antibody, we decided that it was not feasible to continue with this part of the study.

The brains collected for this part of the study after transcardial perfusion remained at $-80°$C for future studies or teaching.

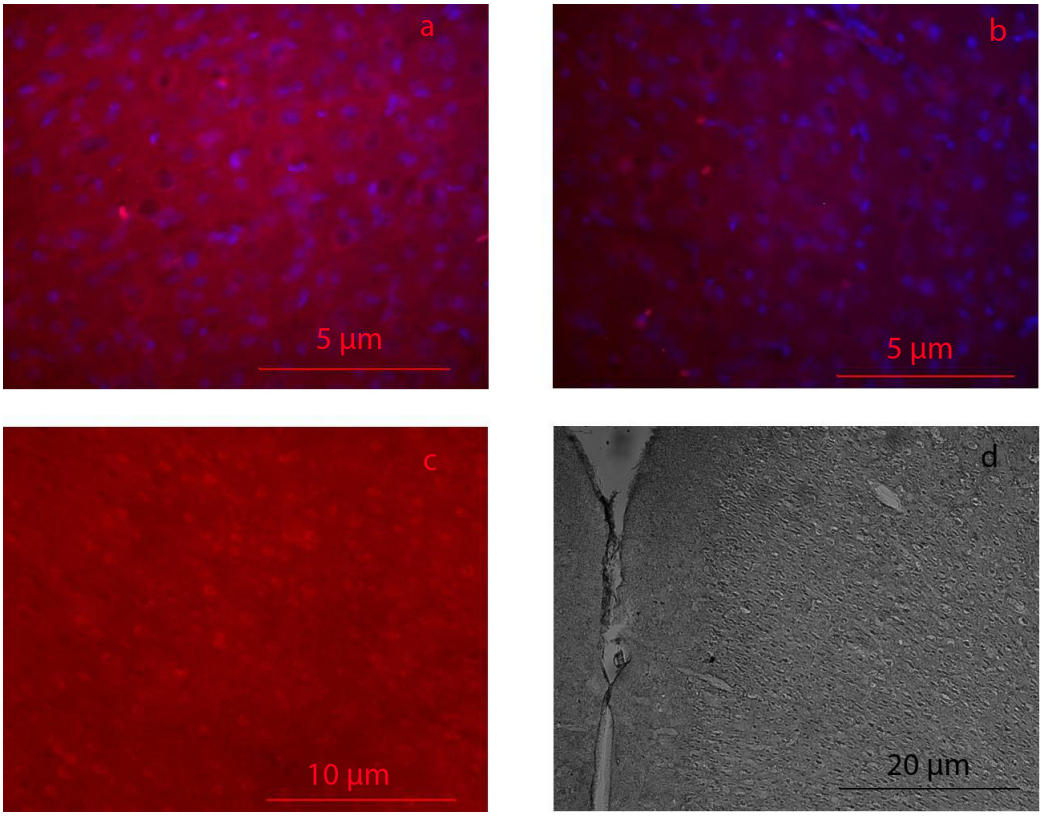


Figure 3.22: Microscopy results of free-floating c-Fos labeling with Merck OP17 [2]

All pictures taken in cingulate cortex, different antibody dilutions:
a: primary 1:100, secondary Cy-3 1:1000 and DAPI
b: primary 1:50, secondary Cy-3 1:2000 and DAPI
c: primary 1:50, secondary Cy-3 1:500, no DAPI
d: primary 1:100, biotinylated secondary 1:1000 with DAB staining

3.3 Oral Self Administration

The application of an oral self administration protocol established for alcohol did not result in sufficient uptake of Ketamine by the rats. With increasing concentration of Ketamine the rats drank less of the solution(Fig. 3.23). With the start of the limited access procedure (section 2.1.7 on page 30) there was hardly any consumption from the Ketamine bottle to be noticed . The difference in bottle weight before and after inserting the bottle into the cage could likely be accounted for by accidental spillage, evaporation, the rat touching the nozzle with its body while exploring or a combination of these factors. Even if the rats drank about two milliliter within the hour of access, the Ketamine received would not be expected to have effects comparable to those achieved by ip injections.

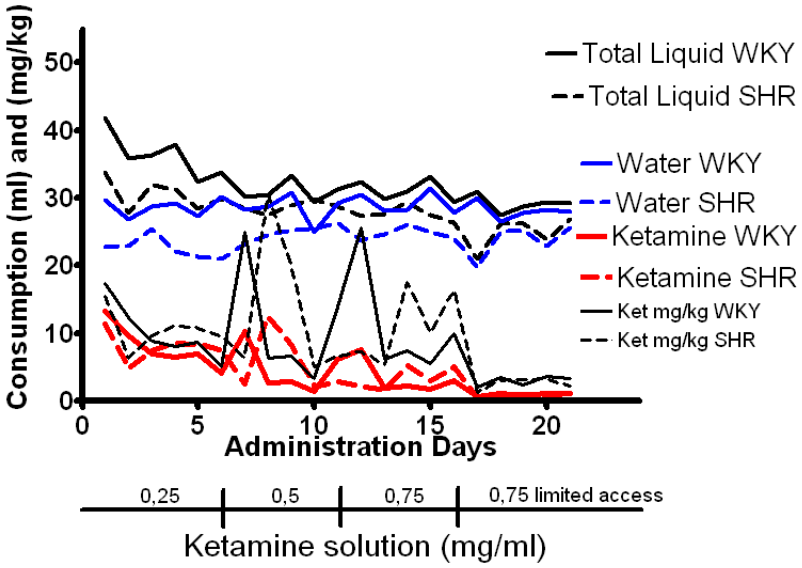


Figure 3.23: Average consumtion of Ketamine by WKY(n=12) and SHR (n=12)

Schedule of Ketamine availability: 0.25mg/ml from day 1 to 6; 0.5mg/ml from day 7 to day 11;0.75mg/ml from day 12 to 16 and limited access to 0.75mg/ml from day 17 to 21

The documentation of the rats' weight every day around the age relevant also to the other ketamine experiments showed a continuous gain in both rat strains (Fig. 3.24). This also shows that short term variations in weight observed in the first few weeks of the CPP experiments must have been due to environmental fluctuation in the animal facility (e.g. of the temperature or light/dark cycle, due to power failures) or in handling. These irregularities disappeared shortly into this study.

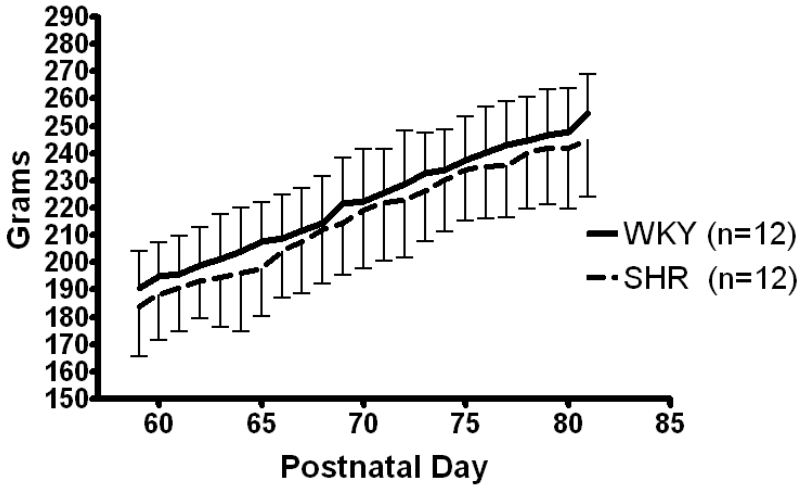


Figure 3.24: Average Bodyweigth of all rats in the oral self administration experiment

Chapter 4

Discussion

4.1 Cross Fostering

The behavioural tests on the cross fostered rats did not show any effects of cross-fostering on the SHR rats. In WKY pups the cross-fostered onto SD dams displayed increased exploratory behaviour in the OF. They entered the inner zone of the OF more often and spent more time in the inner zone than control WKY reared by WKY dams and WKY cross fostered onto SHR dams. This is confirms a similar result obtained by Cierpial et al. in 1998 [60], who showed no cross-fostering effects on WKY rats and mixed results in SHR in different age and sex groups.

The greatest effect of cross fostering was observed in the SD rats. First of all, cross fostering reduced the body weight of SD rats, which was also reported in the cross-fostering study by Cierpial et al. 1991 [61]. This alone can have many indirect effects on the rats vitality, physiology and behaviour. Secondly, WKY and SHR dams enhanced the SDs' exploratory behaviour in the OF. This was seen in the decreased latency to first enter the inner zone, the increased number of entries and increased time spent in the inner zone. Comparing this finding in SDs fostered onto WKY dams, one could expect the opposite effect in the reciprocal rearing condition, WKY rats cross-fostered onto SD dams. In that case the effect could be linked to the more caring nursing behaviour of WKY dams when rearing a cross-fostered litter as observed by Cierpial et al. in 1990 [66]. In

contrast to SHR dams, WKY mother were found to spent more time nursing the cross-fostered pups and less time away than they did with their own litters. Also WKY dams were reported to supply more milk to their own and cross-fostered litters when compared to SHR dams [62]. However, the reciprocal cross fostering of WKY and SD increased the number of entries to the open zone and the time spent therein in both groups when compared to their control conditions (Fig. 3.3 on page 41). This rather points to an general effect of separating the pups from their biological mothers than to effects of the strain specific rearing behaviour.

The other difference brought about by cross fostering of SD pups onto WKY or SHR dams concerns their anxiety related behaviour in the EPM. Being reared by SHR dams made SD rats spend less time in the closed arms and enter the open arms more often (Fig. 3.4 on page 42). In this anxiety related behaviour the SHR rearing mother seemed to have influenced the SD pups according to the general strain differences found in the OF and EPM behaviour.

When pooling all control and cross-fostered pups of the three strains, SHRs were found to be generally less anxious in the EPM, they spent significantly less time in the closed arms and more time in the open arms of the EPM then WKY and SD rats. In the OF all rats scored differently in the distance travelled, the number of entries into the inner zone and the time spent there. SHR was the most active and explorative strain followed by SDs. This is in line with other results on the behavioural characteristics of SHR as described earlier (see 1.1.2 on page 16), confirming this strain to be an appropriate model for AD/HD [9, 16]

In addition to the greater bodyweight of SD pups raised by SD dams, all SD rats stood out against the wistar strains in that they defecated less, which was also found in the OF test at P60 in the ketamine experiment reported in this study (see 4.4 on page 71).

Also SDs had a greater number of correlations between the different parameters studied in the OF and EPM (Fig. 3.1 on page 45). When pooling the rearing conditions within the three pup strains, there were twice as many correlations of between behavioural parameters found in SDs than in SHR and WKY. The control SDs reared by their biological mother showed the most correlations of

all nine experimental groups. They represents the least altered group, in that they are (a) not inbred for special behavioural features and (b) not challenged with the environmental changes of cross-fostering. The number of correlations between different parameters could be seen as a measure of the consistency of the overall behaviour of a certain strain. Few correlations meaning that only part of a strain's behaviour is altered compared to normal controls, many correlations showing the interdependence of most behavioural features expected in unaltered animals. The difference in number of correlations in this study being found between SDs and both wistar strains, puts the WKY close to the SHR strain in a general degree of alteration from normal controls, further challenging the use of WKY as a seemingly normal control for SHR.

Conspicuously the two reciprocal groups of WKY pups fostered onto SD dams and SD pups fostered onto WKY dams, that were discussed earlier, also shared correlation patterns between the different parameters.They show correlations that only share with the control SD group, namely between their number of entries to the open arms of the EPM and both the distance travelled in the OF and the numbers of entries to the inner zone of the OF. On the other hand they do both not correlate where all other groups do, namely in the correlations of the number of entries to the open arms and both the time spent in the open arms and the time spent in the closed arms. The paper by Howells et al. [53] that incorporates the findings from this behavioural study showed that the reciprocal cross fostered groups of SD and WKY are furthermore distinguished by a negative correlation between glutamate-stimulated release of NE in the PFC and all OF parameters. Also WKY dams showed to have an elevating effect on the NE-release in both strains in the hippocampus [53]. These findings remain to be further investigated and explained.

The behavioural methods applied to study the effects of the early postnatal environment were sensitive enough to pick up the few differences between reariing conditions in some groups. Parameters that correlated throughout all test groups, see for example the correlation between the total time in the open arms and the total time in the closed arms or the correlation between the number of entries

into the inner zone and the time spent therein (Fig. 3.1 on page 45), seem to be redundant. But since this was an exploratory study, it was deemed neccessary to look at every possible measure.

Another aspect that merits further comment is the fact that some of the SHR rats fell off the EPM. A first assumption is of course their higher locomotor activity and inattentiveness that could make them prone to just run along the arms of the EPM and right over the edge. It seemed, however, as if they slowed down before the edge and hung on it, exploring the environment below and then lost their grip and fell. An alternative explanation for this could possibly be impairments in eyesight and spacial perception, leading them to first misjudge their own approach to the edge and then the elevation from the floor. Albino rats are known to have impaired vision when compared to pigmented rats [63], different albino strains score differently in visual tasks ([64] in [63]) and SHRs were shown to have defects in light intensity discrimination when compared to WKY [65]. Future studies with the EPM should take note of these findings and adjust the setup accordingly (see suggestions in 2.1.5).

4.2 Conditioned place preference

Ketamine at doses of 12 and 20 mg/kg ketamine was shown to have rewarding effects on rats when tested in the CPP apparatus. This is a novel finding in rats (for review see [36]). However, positive results of ketamine CPP in mice were reported by Suzuki et al. 2000 [34] which supports the finding of ketamine CPP in rats.

The hypothesis that SHR would be more susceptable to the rewarding effects of drugs of abuse, in line with the comorbidity of AD/HD and SUD [3], was not confirmed in this study. On the contrary, CPP could only be found in WKY and not in SHR at either of the two dosages. In WKY it was more pronounced at 20 mg/kg ketamine (see 3.6 on page 46). This confirms and extends data from an earlier CPP study in our lab which showed that SHR are less susceptible to the rewarding effects of cocaine [27]. In the present study there seemed to be a tendency for SHR to display CPP, but the statistcal power test, estimating the required sample size from preliminary results and

standard deviations, revealed that about four times the number of rats would have to be tested in order to show preference for the drug associated side in SHR.

Considering the findings that ketamine only stimulated locomotor activity in the SHR (see 4.4 on page 71) and that ketamine evoked noticeable ataxia only in SHR and SD but not in WKY (see 3.1.3 on page 48), leads to the speculation that negative physical experiences (hyperactivity paired with ataxia) might have masked the rewarding properties of ketamine only in SHR. Future studies should investigate this for example by using lower dosages that might not effect the rats physically and by scoring ataxia and stereotyped behaviour according to Sams-Dodd 1998 [45].

With regard to a predisposition towards SUD in adolescence described for children with AD/HD [4] , this study does not support the notion of SHR being an ideal model for AD/HD because they did not express CPP to ketamine as found in WKY.

4.3 Oral self administration

If the continious administration days of the OSA experiment had resulted in a ketamine addiction and consequently drug seeking behaviour during the limited access period, this part of the study would have been continued. Unfortunately, the rats drank very little from the ketamine bottle especially during the limited access period ($<$1.2 ml, see 3.23 on page 63).

Plotting the consumed ketamine in mg/kg showed that the rats consumed up to 30 mg/kg in one day, conspicuously peaking one day after changing the ketamine concentration in WKYs and two or three days after changing the ketamine concentration to 0.5 and 0.75 mg/ml respectively in SHRs. As also found in the OSA experiment in an ethanol study conducted in our lab [54] rats seemed to maintain their drinking behaviour over the change of concentrations and consequently increase the actual ketamine intake. However, with the start of the limited access period, ketamine uptake (mg/kg) was below the doses injected i.p. in the OF and CPP experiments (12 and 20 mg/kg). Since only minimal

weight loss of the ketamine bottles was registered at this stage ($<$1.5 g), these results are also prone to be confounded or possibly accounted for by accidental spillage from the ketamine bottle when the rat touches it with its body while moving about in its cage.

A follow-up experiment should use much lower concentrations of ketamine during the continuous and limited access procedure, requiring the rats to drink more liquid to uptake equal amounts of ketamine. This would guard against the confounding errors of accidental spillage. In other studies a high and controlled uptake of ketamine during the continuous access period was achieved by adding a sweet substance to the drug solution ([39][40], for review [38]). This was not done in this study not to introduce confounding factors of food reward. In addition to the use of sweet substances, Silvestre et al. 2002 [40] food deprived the rats to 80% of their normal bodyweight, this resulted in a consumption of about 64 ml of a 0,28 mg/ml ketamine solution containing 10% w/v glucose within one hour of limited access. This ingestion of around 18 mg/kg ketamine did not result in any behavioural changes in OF behaviour (distance travelled, rearing and defecation) and only prolonged time spent in the open arms of an EPM directly after the limited access hour [40]. A different deprivation element could be introduced in future studies making use of the average liquid consumption documented in this study. Rats could be supplied with only one drinking bottle containing a limited daily amount of water close to or just above the reported consumption. This water could be carefully mixed with increasing concentrations of substances, without leaving the rats the choice to uptake the drug or not. In case the substance used has a strongly aversive effect, care has to be taken not to dehydrate the rats. To prevent this, a bottle of pure drinking water could be provided for a limited time per day. Once the rats are habituated to the uptake and the possibly bad taste, which might have been the reason of minimal ketamine consumption in this experiment, a free choice paradigm could follow. Addiction to and craving for the presented substance could subsequently be tested with a free choice and limited access protocol.

4.4 Open field behaviour

The OF test after ketamine injections showed several results contrasting and complimenting the CPP and corss-fostering experiments. As in the CPP ketamine had a different effect on SHR and WYK. The stimulatory effect of subanaestheitc dosages descibed by Imre et al. 2006 [43] was only found in the SHR (Fig. 3.7 on page 50). Imre et al. found increased locomotion untill twenty minutes after injection in wistar rats, in this study the total distance travelled was elevated mostly in the first ten minutes only (Fig. 3.17 on page 57).

At the testing age of P60 (adolescence) SHR only travelled more then WKY. This points to them not being an ideal model for AD/HD at that age as reported by Bergh et al. 2005 [21].

The analysis of turning parameters in this study showed that turning was reduced after ketamine injection in SHR. However, meandering expressed as the average degrees turned per distance travelled, showed a difference between SD and both Wistar strains (Fig. 3.11 on page 52). SHR and WKY changed their average turning direction from counter-clockwise to clockwise after ketamine injection, while SD continued to turn to the left. Dose dependent increase in circling was described by Sams-Dodd 1998 [45] as one of the stereotyped behaviours after NMDA receptor antagonist injection. The apparently unilateral effect on the wistar rats resulting in a change of their average turning direction has not been descibed previously and remains to be investigated. The side of i.p. injections could be a candidate reason, since rats were injected on the right hand side of the body mid-line and ketamine could have had a peripheral paralyzing effect primarily at the injection side. SD might have been less affected because of their greater body mass.

Ketamine had a similar lowering effect on the rearing behaviour of all rat strains. After saline injection and when pooling all dosages, SHR reared more than WKY and SD, and SD reared more then WKY. In contrast to the similarity in the distance travelled at this age (P60), SHR was markedly different from SD in this parameter. Defecation was lowered in both wistar strains after ketamine injections, SDs hardly defecated in the OF.

4.5 Conclusion

This study looked at the effects of ketamine and cross-fostering on the best validated animal model for AD/HD, the SHR, using its normotensive progenitor strain WKY and the unrelated SD strain as controls. All behavioural tests showed differences between SHR and WKY. Differences between SHR and SD were age dependent.

Findings challenging the SHR as a universal model for AD/HD were twofold: Susceptibility to the rewarding effects of ketamine were lower in adolescent SHRs compared to WKY rats, which does not mimic the higher rate of SUD in humans with AD/HD compared to non-sufferers.
SHRs were not more active than SD rats in adolescence, at that age only the WKY rats stood out for their lower locomotor activity.

However, a different picture emerged when SHR were studied at pre-puberty age. At this age equivalent to the stage at which symptoms of AD/HD are most pronounced in humans, SHR was significantly different from WKY and SD in terms of increased locomotor activity.

Challenging SHR as a model for AD/HD also questions wether WKY are the appropriate control. Being the SHRs progenitor strain makes WKY likely to be genetically similar to SHR in most features but those leading to the peculiar characteristics of SHR. However, qualitatively WKY do not appear to be a representative of normal rodents, being very inactive and even being suggested to be a model for depression. This was highlighted in the present study where WKYs were significantly different from SHRs and SDs in terms of distance travelled in the OF at P60. They seemed to represent an extreme in the continuous range of behaviour, opposite to the SHR but equally far from being normal (see. SD rats on the other hand can not easily substitute the WKY as a control for the SHR since its general physical condition is quite different from them, which might be a main factor for different behavioural performance. Using WKY and a second outbred strain as a complimentary control is a widely used paradigm in recent studies and proved to be an appropriate strategy in the present study.

Unfortunately c-fos immunocytochemistry did not result in quantifiable results. Questions remain concerning the neural correlates of the differential effect

of ketamine in SHR and WKY. Looking at strain specific stimulation in candidate structures as the PFC and NAc will merit further investigations.

In the cross-fostering experiment, SHR proved to be a very stable genetic model since no changes in its behaviour could be achieved by cross-fostering SHR pups onto dams of other strains. WKY showed a change in the OF behaviour when fostered onto SD dams. Conspicuously SD rats showed the most susceptibility to environmental conditions. Being an outbred rat seems to give them greater variability in their possible range of behaviour and greater flexibility to adapt to their surroundings. WKY behaviour and even more so SHR seemed to be largely determined by their genetic disposition. This apparent restriction in gene-environment interaction in inbred rats must be born in mind when using them to model human developmental diseases such as AD/HD.

References

[1] E. J. S. Sonuga-Barke. Causal models of attention-deficit/hyperactivity disorder: from common simple deficits to multiple developmental pathways. *Biological Psychiatry*, 57:1231–1238, 2005.

[2] Calbiochem. Anti-c-Fos (Ab-1) mouse mAb (2G9C3) Cat. No. OP17. Data Sheet, October 05.

[3] T.E.Wilens, S.V. Faraone, and J. Biederman. Attention deficit/hyperactivity disorder in adults. *Journal of the American Medical Association*, 292:619–623, 2004.

[4] T.E.Wilens, M.C. Monuteaux, L.E. Snyder, and et al. The clinical dilemma of using medication in substance-abusing adolescents and adults with attention deficit/hyperactivity disorder: What does literature tell us? *Journal of child and adolescent psychopharmachology*, 15(5):787–798, 2005.

[5] R.A. Barkley, M. Fischer, L. Smallish, and K. Flethcer. Does the treatment of attention deficit/hyperactivity disorder with stimulant drugs contribute to drug use/abuse? a 13-year prospective study. *Pediatrics*, 111(1):97–109, 2003.

[6] V.A. Russell, D. Lam, T. Sagvolden, and E.B. Johansen. *Attention deficit/Hyperactivity disorder (AD/HD) and the hyperkinetic syndrome (HKS): Current ideas and ways forward*, chapter 5. Nova Science, New York, 2006.

[7] A. Thapar, K. Langley, and M. Gill. Gene-environment interplay in attention deficit/hyperactivity disorder and the importance of a developmental perspective. *British journal of Psychiatry*, 190:1–3, 2007.

[8] J. Kuntsi, B.M.Neale, W. Chen, S.V. Faraone, and P. Asherson. The image project: methodological issues for the molecular genetic analysis of attention deficit hyperactivity disorder. *Behavioural and brain research*, 2(27), 2006.

[9] V.A. Russell. Neurobiology of animal models of attention-deficit hyperactivity disorder. *Neuroscience Methods*, in print, 2007.

[10] D. Pozzi, C. Verderio, L. Patti, C. Grumelli, and et al. Snap-25 modulation of calcium dynamics underlies differences in gabaergic and glutamatergic responsiveness to depolarization. *Neuron*, 41(4):599–610, 2004.

[11] V.A. Russell, T. Sagvolden, and E.B. Johansen. Animal models of attention-deficit hyperactivity disorder. *Behavioural and Brain Functions*, 1(9), 2005.

[12] A.F.T. Arnsten. Fundamentals of attention-deficit/hyperactivity disorder: Circuits and pathways. *Journal of Clinical Psychiatry*, 67:7–12, 2006.

[13] T. Sagvolden, H. Aase, P. Zeiner, and D. Berger. Altered reinforcement mechanism in attention-deficit hyperactivity disorder. *Behaviourla brain research*, 94, 61-71 1998.

[14] V.A.Russell, R.D.Oades, R.Tannock, P.R. Killen, J.G.Auerbach, E.B. Johansen, and T.Sagvolden. Response variability in attention-deficit/hyperactivity disorder: a neuronal and glial energetics hypothesis. *Behavioural and brain research*, 2(30), 2006.

[15] M.Lehohla, L. Kellaway, and V.A. Russell. NMDA receptor function in the prefrontal cortex of a rat model for attention-deficit hyperactivity disorder. *Metabolic Brain Disease*, 19(1,2), 2001.

[16] T. Sagvolden. Behaviourla validation of the spontaniously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder. *Neuroscience Biobehavioural Review*, 24:31–39, 2000.

[17] K.Okamoto and K. Aoki. Development of a strain of spontaneoulsy hypertensive rats. *Japanese Circulation Journal*, 27:282–293, 1963.

[18] S. Knardahl and T. Sagvolden. Open-Field behaviour of spontaneously hypertensive rats. *Behavioural and Neural Biology*, 27:187–200, 1979.

[19] E. Bull, C. Reavill, J. Hagan, P. Overend, and D.N.C. Jones. Evaluation of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder: acquisition and performance of the DRL-60s test. *Behavioural Brain Research*, 109:27–35, 2000.

[20] M. Calzavara, G.B. Lopez, V.C. Abilio, R.H.Silva, and R. Frussa-Filho. Role of anxiety levels in memory performance of spontaneously hypertensive rats. *Behavioural Pharmacology*, 15(8):545–553, 2004.

[21] F.S. van den Bergh, E. Bloemarts, J.S.W. Chan, L. Groenink, B. Olivier, and R.S.Oosting. Spontaneously hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder. *Pharmacology Biochemistry Behaviour*, 83:380–439, 2006.

[22] K.I. Ueno, H.Togashi, K. Mori, M. Matsumoto, S. Ohashi, A. Hoshino, T. Fujita, H. Saito, M. Minami, and M. Yoshioka. Behavioural and pharmacological relevance of stroke-prone spontaneously hypertensive rats as an animal model of a developmental disorder. *Behavioural Pharmacology*, 13:1–13, 2002.

[23] M. van den Buuse and W. de Jong. Open-field behaviour and blood preassure in spontaneously hypertensive rats. *Clinical experimental Hypertension*, 10(4):667–684, 1988.

[24] T. Sagvolden, E.D.Hendley, and S. Knardahl. Behaviour of hypertensive and hyperactive rat strains: Hyperactivity is not unitarely determined. *Physiology and Behaviour*, 52(1):49–57, 1992.

[25] T. Sagvolden. *The attention deficit disorder might be a reinforcement deficit disorder*. Number 131-143. Hogrefe und Huber, 1996.

[26] O. Malkesman, Y. Braw, O. Zagoory-Sharon, O. Golan, and et al. Reward and anxiety in genetic animal models of childhood depression. *Behavioural Brain Research*, 164:1–10, 2005.

[27] G.S. Lelaka. Study of the effects of methylphenidate in an animal model for attention deficit hyperactivity disorder. Master's thesis, Biomedical Science, University of Cape Town, 2004.

[28] C.L. Littlewood, D.Cash, A.L. Dixon, S.L.Dix, C.T. White, and et al. Using BOLD MR signal to differentiate the stereoisomers of ketamine in the rat. *NeuroImage*, 32(4):1733–1746, 2006.

[29] C.L. Littlewood, N. Jones, M.J. O'Neil, S.N. Mitchell, and et al. Mapping the central effects of ketamine in the rat using pharmacological MRI. *Psychopharmacology*, 186(1):64–81, 2005.

[30] P.M. Gahlinger. Club drugs: MDMA, gamma-hydroxybutyrate (GHB), rohypnol, and ketamine. *American family physician*, 6(11):2619–2626, 2004.

[31] C.A. Zarate, J.B. Singh, P.J.Carlson, N.J. Brutsche, and E. Ameli. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of General Psychiatry*, 63(8):856–864, 2006.

[32] S. Aalto, J. Ihlainen, J. Hirvonen, and J. Kajandera. Cortical glutamate-dopamine interaction and ketamine-induced psychotic symptoms in man. *Psychopharmacology*, 182:375–383, 2005.

[33] G. Keilhoff, A. Becker, G. Grecksch, G. Wolf, and H.G. Bernstein. 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, 2004.

[34] T. Suzuki, H. Kato, T. Aoki, M.Tsuda, M. Narita, and M. Misawa. Effects of the non-competitive NMDA receptor antagonist ketmamine on morphine-induced place preference in mice. *Life Sciences*, 67:383–389, 2000.

[35] J.M.Boyce-Rustay and C.L. Cunningham. The role of NMDA receptor binding sites in ethanol place conditioning. *Behavioural neuroscience*, 118(4):822–834, 2004.

[36] T.M.Tzschentke. Measuring reward with the conditioned place prefernce paradigm: a comprehensive review of drug effects, recent progress and new issues. *Progress in Neurobiology*, 56:613–672, 1998.

[37] W.A. Carlezon and R.A. Wise. Rewarding actions of phencyclidine and related drugs in nucleus accumbens shell and frontal cortex. *Journal of Neuroscience*, 16(9):3112–3122, 1996.

[38] R.A.Meisch. Oral drug self administration: an overview of laboratory animal studies. *Alcohol*, 24:117–128, 2001.

[39] M.E.Carroll and D.C. Stotz. Oral d-amphetamine and ketamine self-administration by rhesus monkeys: effects of food deprivation. *The Journal of pharmacology and experimental therapeutics*, 227(1):28–34, 1983.

[40] J.S.Slivestre, M. Pallares, R. Nadal, and N. Ferre. Opposite effects of ethanol and ketamnie in the elevated plus-maze test in wistar rats undergoing a chronic oral voluntary consumption procedure. *Psychopharmacology*, 16(4):305–312, 2002.

[41] F.Razoux, R.Garcia, and I.Léna. Ketamine, at a dose that disrupts motor behavior and latent inhibition, enhances prefrontal cortex synaptic efficacy and glutamate release in the nucleus accumbens. *Neurophsychopharmacology*, pages 1–9, 2006.

[42] M.J.Hunt, B. Raynaud, and R. Garcia. Ketamine dose-dependently induces high-frequency oscillations in the nucleus accumbens in freely moving rats. *Biological Psychiatry*, 60(11):1206–1214, 2006.

[43] G.Imre, D.S.Fokkema, J.A.DenBoer, and G.J.T.Horst. Dose-response characteristics of ketamine effect on locomotion, cognitive function and central euronal activitiy. *Brain Research Bulletin*, 69:338–345, 2006.

[44] E. De Leonibus, A.Mele, A. Oliverio, and A. Pert. Locomotor activity induced by the non-competitive NMDA antagonist, MK-801: role of nucleus accumbens efferent pathways. *Neuroscience*, 104(1):105–116, 2001.

[45] F. Sams-Dodd. Effects of continuous d-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. *Neuropsychopharmacology*, 19(1):18–25, 1998.

[46] R.D.Heijtz and F.X. Castellanos. Differential effect of a selective dopamine D1-like receptor agonist on motor activity and c-fos expression in the frontal-striatal circuitry of SHR and Wistar-Kyoto rats. *Behavioural and Brain Research*, 2(18), 2006.

[47] Calbiochem. Anti-c-Fos (Ab-5) (4-17) rabbit pAb Cat. No. PC38. Data Sheet, June 2006.

[48] B.K.Tolliver, M.W.Sganga, and F.R.Sharp. Suppression of c-fos induction in the nucleus accumbens prevents acquisition but not expression of morphine-conditioned place preference. *European Journal of Neuroscience*, 12:3399–3406, 2000.

[49] Michelle M. Ostrandera, Neil M. Richtand, and James P. Hermana. Stress and amphetamine induce fos expression in medial prefrontal cortex neurons containing glucocorticoid receptors. *Brain Research*, 990:209– 214, 2003.

[50] G.E. Duncan, S.S. Moy, D.J. Knapp, R.A. Mueller, and G.R.Breese. Metabolic mapping of the rat brain after subanesthetic doses of ketamine: potential relevance to schizophrenia. *Brain Research*, 787:181–190, 1998.

[51] M. Papa, J.A. Sergeant, and A.G. Sadile. Differential expression of transcription factors in the accumbens of an animal model of attention deficit/hyperactivity disorder. *Neuroreport*, 8:1607–1612, 1997.

[52] W.A. Carlzon. Place conditioning to study drug reward and aversion. *Methods mol. med.*, 84:243–249, 2003.

[53] F.M. Howells, L. Bindewald, and V.A. Russell. Changes in early postnatal environment alter the development of coping strategies in. *Behavioural Brain Research*, 2007 unpublished.

[54] H.Soeters, V.A. Russell, and F.M. Howells. Does methaphenidate reduce the susceptibility to drug addiction in a rat model for attention-deficit hyperactivity disorder ? Master's thesis, Biomedical Science, University of Cape Town, 2006.

[55] Jackson ImmunoResearch. Cy3-conjugated affinipure F(ab)2 fragment donkey anti-mouse IgG(H+L). DataSheet.

[56] K.S. Kraus and R.-B. Illing. Cell death or survival: Molecular and connectional conditions for olivocochlear neurons after axotomy. *Neuroscience*, 134(2):467–481, 2005.

[57] N. Hussain, B.A. Flumerfelt, and N. Rajakumar. Glutamatergic regulation of halperidol-induced c-fos expression in the rat striatum and nucleus accumbens. *Neuroscience*, 102(2):391–399, 2001.

[58] N. Nishizawa, S. Nakao, A. Nagata, T. Hirose, and et al. The effect of ketamine isomers on both mice behavioural response and c-fos expression in the posterior cingulate and retrosplenial cortices. *Brain Research*, 857:188–192, 2000.

[59] K.Wang, S.E.F.Guldenaar, and J.T.McCabe. Fos and jun expression in rat supraoptic nucleus neurons after acute vs. repeated osmotic stimulation. *Brain Research*, 746:117–125, 1997.

[60] M.A. Cierpial, D.E. Shasby, C.A. Murphy, and A.H. Borom. Open field behaviour of Spontaneously Hypertensive and Wistar Kyoto normotensive rats:effects of reciprocal cross-fostering. *Behavioural and Neural Biology*, 51:203–210, 1989.

[61] M.A. Cierpial and R. McCarty. Adult blood pressure reduction in spontaneously hypertensive rats reared by normotensive sprague-dawley mothers. *Behavioural Neural Biology*, 56:262–270, 1991.

[62] J.L. Rose and R. McCarty. Maternal influences on milk intake in SHR and WKY pups. *Physiology and Behaviour*, 56:901–906, 1994.

[63] K.T. Harker and I.Q. Whishaw. Place and matching-to-place learning affected by rat inbreeding(Dark Agouti, Fisher 344) and albinism (Wistar, Sprague–Dawley) but not domestication (wild rat vs. Long-Evans, Fisher–Norway). *Behavioural Brain Research*, 134:467, 2002.

[64] G.T. Prusky, K.T. Harker, and R.M. Douglas. Variation in visual acuity within pigmented, and between pigmented and albino rat strains. *Behavioural Brain Research*, 136(2):339–48, 2002.

[65] L.J. Rogers, S.W. Bolden, A.S. Patrech, and D. Ehrlich. Visual dysfunction in the Spontaneously Hypertensive Rat. *Physiology and Behaviour*, 54(5):903–907, 1993.

[66] M.A. Cierpial, C.A. Murphy, and R. McCarty. Maternal behaviour of Spontaneously Hypertensive and Wistar-Kyoto normotensive rats: Effects of reciprocal cross-fostering of litters. *Behavioural and Neural Biology*, 54:90–96, 1990.

[67] P. N. Augustyniak, S. Kourrich, S. M. Rezazadeh, J. Stewart, and A. Arvanitogiannis. Differential behavioral and neurochemical effects of cocaine after early exposure to methylphenidate in an animal model of attention deficit hyperactivity disorder. *Behavioural Brain Research*, 167:379–382, 2005.

[68] J.L. Horn, P.K. Janicki, and J.J. Franks. Diminished brain synaptic plasma membrane Ca(2+)-atpase activity in spontaneously hypertensive rats: association with reduced anesthetic requirements. *Life Sciences*, 56(L):427–432, 1995.

[69] Thapar A., O'Donovan M., and Owen M.J. The genetics of attention deficit hyperactivity disorder. *Human Molecular Genetics*, 14(2):275–282, 2005.

[70] M.Papa, L.Diewald, M.P.Carey, F.J.Espito, U.A.G. Carnevale, and A.G.Sadile. A rosto-caudal dissociation in the dorsal and ventral striatum of

the juvenile SHR suggests an anterior hypo- and posterior hyperfunctioning mesocorticolimbic system. *Behavioural Brain Research*, 130:171–179, 2002.

[71] O.Viltart, J.Mairesse, M.Darnaudery, H.Louvart, C.Vanbesien-Mailliot, A.Catalani, and S.Maccari. Prenatal stress alters fos expression in hippocampus and locus coeruleus stress-related brain structures. *Psychoneuroendocrinology*, 31:769–780, 2006.

Appendix A

Video-Analysis on Ethovision

A.1 Setting up

- Start Program
    - Scroll-down Menu File
        - choose New Workspace
            - choose New Experiment

- Scroll-down menu "File", New Arena profile.
  Once a arena profile existes, it can siply be copy by right-clicking on it and pasting it onto Arena Profiles
    - Right-click on new Arena profile and choose Rename
        - Choose Open Arena Profile in the "File" Scroll-down menu and select the new Arena profile
            - Choose the right video file in the pop up box
              (Rather choose a later file, since the background image sometimes cant be refreshed in case the position of the apparatus changes later in the file)

- Choose "Arena Definition" in the Scroll-down menu "Experiment"
    - Choose "Refresh Background Image" in the "View" Scroll-down menu
        - Previoulsy chosen file shows, click Snap-shot button (Camera icon)
            - Create or adjust arena outlines on the Green "Arena" sheet and the Acquisition Zones on the blue "Acquisition ZoneDef" sheet (botton right).
              Use the Square outline icons whenever possible, the point-to-point outlines are neverthelss neccesary for zones that appear at an angle.
              Calibration is done or adjusted on the grey sheet on the botton left.

A.2 Tracking the videos

- Choose "Acquire Data" in the "Experiment" scroll-down menu
    - Play video and find a frame with an full view of an empty aparatus
        - Choose "Processing" in the "Tracking" scroll-down menu
            - Choose "Detection Method" and tick "Substraction" and set the noise removal minimum object size to 24 (depending on size of rats and brightnes of the recording)
- Also in the "Tracking" scroll-down menu choose "Update detection variables" and set the lower limit to 91 (leaving the upper limit at maximum)
    - Also in the "Tracking" scroll-down menu set the tracking time for the next trail under "Trail Protocol" - "Recording Duration"
        - Wait till everything is out of the view of the arena and press F5 to start Recording
          (write down the rat name and treatment and the prospective track number, that being the present track number plus one)
            - After recording time passed choose "Yes" when prompted if you want to add the acquired track to the experiment
- If not breaking the recording of each rat up into several bins, choose "No" when promted if you want to continue with the next trial.
    - If the playing video file ends before the end of the tracking duration (e.g. when Smart Guard made the hourly change in the middle of a trail), save this track (click "Yes", then"No") noting the time that has allready been analyzed in this track, open the next file with the "Open file" icon on the video control panel, find an frame with no rat in the arena on this file, repeat the "Detection Method: Subtraction" step in the tracking menu and set the recording duration for the exact time missing on this trail. Then go back to the beginning of this new file and press F5 to start the tracking for the rest of the trial.

The values from these two tracks have to be added in the end when creating the result spreadsheets.

- Repeat these steps untill all trails screened by Ethovision with corresponding number of tracks have been saved.

Should samples be lost/missed during the tracking of the animals, it will not effect some parameters as the distance travelled since it only happens when the animal sits still in a rather dark part of the arena. Other parameters such as "time in zone" will be affected, however, so it is important to try to find the smallest minimum object size that will suit all animals used and not be of similar size to white areas (contrast noise) by light reflections on smooth surfaces.

A.3 Analysing the tracks

- Scroll Down Menu "Experiment": Choose "Analyse Data": Choose "Select Tracks"
  - Scroll Down Menu "Data": Choose Nesting : Choose "Zones"
    - Scroll Down Menu "Analysis": Choose "Add Parameters"
      - Select Parameters According to Experiment eg. "In Zone" and "Distance Moved" from the Distance&Time Package. Parameter output can be modified by right clicking on the parameter in the calculation table.
- Scroll Down Menu "Analyse"
  - Click "Calculate", this will give you the output of statistical data!
    - Scroll Down Menu "File": Choose "Export", choose "Statistics"
      - Export data table as ".cqd" or ".csv" (comma seperated values) both are recognized by excel but layout might be different depending on version used!

Exported files will be saved under the path:
My Documents/UCT Viv/(Project name)/(workspace name & experiment no.)/
Export - if not specified to save directly on external medium (disc or flashdrive)

Appendix B

Statistical Tables

Table B.1: Spreadsheet to cross-fostering data in 3.1.1 on page 37

	1 Name	2 Dam	3 Pup	4 Total (cm)	5 Inner(freq)	6 Inner(s)	7 latency Inner (s)	8 OF(def)	9 Closed(s)	10 Open(s)	11 Open(freq)
1	Control SHR	SHR	SHR	5446.31	20	50.87417	50.539004	5	199.63277	35.65278	8
2	Control SHR	SHR	SHR	6552.83	22	46.76332	83.616771	4	165.06268	49.27188	6
3	Control SHR	SHR	SHR	7583.28	32	90.51176	167.7	4	238.22971	22.79347	3
4	Control SHR	SHR	SHR	7173.2	14	17.6625	103.55	9	176.375	60	7
5	Control SHR	SHR	SHR	7077.84	26	52.7375	39.9125	3	237.3375	12.3375	1
6	Control SHR	SHR	SHR	9781.2	42	161.125	37.5	2	187.75	15.2375	1
7	Control SHR	SHR	SHR	8416.83	23	66.775	181.2125	2	242.6625	7.0125	1
8	Control SHR	SHR	SHR	8856.6	36	85.1625	11.85	5	155.8	29.5125	4
9	Control SHR	SHR	SHR	7778.76	27	81.775	121.9375	8	226.925	20.325	3
10	Control SHR	SHR	SHR	10180.97	50	177.3375	95.075	5	195.9625	51.5375	5
11	Control SHR	SHR	SHR	9099.45	33	106.9375	73.0625	0	215.075	22.0125	5
12	Control SHR	SHR	SHR	8373.2	42	110.8	58.7875	5	221.85	31.9375	4
13	SHR pups onto VWKY dams	VWKY	SHR	8288.72	22	79.58824	41.664706	5	215.43624	44.12435	8
14	SHR pups onto VWKY dams	VWKY	SHR	10650.21	44	120.9176	13.323529	5	189.69862	36.01368	7
15	SHR pups onto VWKY dams	VWKY	SHR	8331.02	22	84.37059	76.9	4	222.40724	27.94099	5
16	SHR pups onto VWKY dams	VWKY	SHR	8072.23	36	177.6	6.4941176	2	196.30872	34.93099	5
17	SHR pups onto VWKY dams	VWKY	SHR	7842.53	29	98.36471	99.741176	8	265.42991	11.75763	3
18	SHR pups onto VWKY dams	VWKY	SHR	7582.39	41	146.4296	3.5279855	0	194.00849	48.24742	4
19	SHR pups onto VWKY dams	VWKY	SHR	4985.14	7	4.8375	138.15	4	234.1875	18.6375	4
20	SHR pups onto VWKY dams	VWKY	SHR	5320.6	28	88.0625	10.8875	2	208.3125	46.6875	3
21	SHR pups onto VWKY dams	VWKY	SHR	9794.67	44	131.125	73.3125	5	247.7375	28.55	2
22	SHR pups onto VWKY dams	VWKY	SHR	8852.37	48	143.225	26.375	3	184.8375	66.2875	6
23	SHR pups onto VWKY dams	VWKY	SHR	8597.12	28	61.2125	54.1875	6	194.0375	65.8125	6
24	SHR pups onto VWKY dams	VWKY	SHR	9657.62	55	183.875	21.5375	3	127.2625	108.8625	14
25	SHR pups onto VWKY dams	VWKY	SHR	10817.02	55	148.0625	164.525	4	283.3	5.075	1
26	SHR pups onto VWKY dams	VWKY	SHR	10845.94	56	149.275	65.075	2	151.6875	58.8	8
27	SHR pups onto SD dams	SD	SHR	6920.75	17	43.7875	50.325	5	235.1625	27.825	3
28	SHR pups onto SD dams	SD	SHR	7894.73	29	72.575	54.925	5	127.2625	108.8625	14
29	SHR pups onto SD dams	SD	SHR	8334.09	15	27.575	58.7875	2	227.6625	42.825	5
30	SHR pups onto SD dams	SD	SHR	10073	44	101.375	77.425	0	235.8875	33.875	2
31	SHR pups onto SD dams	SD	SHR	8832.86	25	50.075	40.65	6	211.45	55.4125	6
32	SHR pups onto SD dams	SD	SHR	8298.01	41	83.225	44.7625	0	186.5375	70.6375	6
33	SHR pups onto SD dams	SD	SHR	8964.16	61	145.1625	127.75	5	200.075	23.95	3
34	SHR pups onto SD dams	SD	SHR	8348.71	47	198.875	78.375	6	182.9125	71.375	8
35	SHR pups onto SD dams	SD	SHR	9539.78	56	110.8	103.55	2	183.1375	36.775	5
36	SHR pups onto SD dams	SD	SHR	7502.96	35	136.9375	54.1875	5	193.55	33.625	10
37	SHR pups onto SD dams	SD	SHR	9671.06	38	181.9375	64.1125	0	179.5125	43.3125	5
38	SHR pups onto SD dams	SD	SHR	9418.59	48	122.9	56.125	2	202.5	42.825	7
39	Control VWKY	VWKY	VWKY	4703.88	2	6.494118	555.37059	7	198.89198	44.10536	7
40	Control VWKY	VWKY	VWKY	6641.04	14	112.3765	374.01176	4	223.16703	31.24604	3
41	Control VWKY	VWKY	VWKY	5573.54	1	4.445403	797.57933	8	200.73446	26.09852	4
42	Control VWKY	VWKY	VWKY	5589.32	9	29.3599	624.22853	7	182.70862	43.74446	5
43	Control VWKY	VWKY	VWKY	4230.5	0	0	900	5	204.4194	29.76346	3
44	Control VWKY	VWKY	VWKY	5542.26	6	43.04118	790.35882	10	199.27188	25.73762	3
45	Control VWKY	VWKY	VWKY	4296.53	0	0	0	8	227.93466	6.989996	3
46	Control VWKY	VWKY	VWKY	5033.29	0	0	0	7	236.76713	15.44257	1
47	Control VWKY	VWKY	VWKY	4277.69	2	2.4125	609.675	0	295.8875	0	0
48	Control VWKY	VWKY	VWKY	6474.32	10	27.575	743.225	0	250.4	0	0
49	Control VWKY	VWKY	VWKY	6025.94	7	31.9375	544.6	5	289.1125	2.425	2
50	Control VWKY	VWKY	VWKY	9819.95	8	9.675	287.425	3	280.65	5.5625	1
51	VWKY pups onto SHR dams	SHR	VWKY	5483.44	1	0.688235	882.22941	4	240.79397	0	0
52	VWKY pups onto SHR dams	SHR	VWKY	4495.63	0	0	0	8	224.62961	0	0
53	VWKY pups onto SHR dams	SHR	VWKY	5640.26	0	0	0	0	232.72129	9.193365	3
54	VWKY pups onto SHR dams	SHR	VWKY	5350.81	0	0	0	2	248.16386	0	0
55	VWKY pups onto SHR dams	SHR	VWKY	7059.32	8	21.51176	433.78235	13	259.5416	6.610105	3
56	VWKY pups onto SHR dams	SHR	VWKY	843.63	0	0	0	4	300	0	0
57	VWKY pups onto SHR dams	SHR	VWKY	2863.64	2	4.219034	873.24914	0	148.25349	53.86901	4
58	VWKY pups onto SHR dams	SHR	VWKY	3253.46	1	4.219034	886.26995	3	233.46877	33.45664	5
59	VWKY pups onto SHR dams	SHR	VWKY	6660.76	9	50.68297	32.024651	0	194.37235	36.25834	3
60	VWKY pups onto SHR dams	SHR	VWKY	3528.84	2	1.45	529.1125	5	281.375	0	0
61	VWKY pups onto SHR dams	SHR	VWKY	4559.96	0	0	900	8	284.2625	5.325	1
62	VWKY pups onto SHR dams	SHR	VWKY	7295.62	12	17.425	588.625	0	281.125	8.4625	1
63	VWKY pups onto SD dams	SD	VWKY	4781.14	10	108.6176	418.41176	3	118.55949	160.592	1
64	VWKY pups onto SD dams	SD	VWKY	6772.45	11	66.6	332.68235	3	268.22408	12.97517	3
65	VWKY pups onto SD dams	SD	VWKY	5414.8	11	28.00588	231.56471	2	294.86289	0	0
66	VWKY pups onto SD dams	SD	VWKY	4647.55	2	2.047059	658.18235	3	281.88773	0.688478	1
67	VWKY pups onto SD dams	SD	VWKY	4078.18	4	9.229412	329.94706	4	272.31964	4.448629	1
68	VWKY pups onto SD dams	SD	VWKY	5371.62	10	31.2125	286.45	4	221.8625	19.6	3
69	VWKY pups onto SD dams	SD	VWKY	7079.55	30	94.1125	180.725	0	159.1875	89.275	5
70	VWKY pups onto SD dams	SD	VWKY	5322.56	12	58.55	242.175	8	244.1125	10.65	1
71	VWKY pups onto SD dams	SD	VWKY	4948.99	15	46.9375	309.9125	0	274.1125	8.4625	1
72	VWKY pups onto SD dams	SD	VWKY	4989.63	6	10.4	58.0625	0	211.2	30.725	2
73	VWKY pups onto SD dams	SD	VWKY	4807.42	12	41.6125	531.2875	3	271.6875	25.1625	1
74	Control SD pups	SD	SD	3300.92	0	0	900	0	300	0	0
75	Control SD pups	SD	SD	7987.23	16	33.625	215.325	0	287.175	11.375	2
76	Control SD pups	SD	SD	8597.45	28	56.125	2.425	0	250.8875	19.5875	3
77	Control SD pups	SD	SD	6882.11	20	52.5	149.275	8	259.8375	22.7375	2
78	Control SD pups	SD	SD	6614.63	8	33.875	290.075	4	285.2375	8.225	1
79	Control SD pups	SD	SD	7264	13	25.1625	157.0125	0	240.9625	23.95	2
80	Control SD pups	SD	SD	8291.9	29	139.1125	97.2625	0	193.3	115.4125	6
81	Control SD pups	SD	SD	5762.78	14	54.1875	113.95	0	250.1625	20.325	2
82	Control SD pups	SD	SD	5814.04	14	51.5375	361.9375	0	258.8625	15.725	2
83	Control SD pups	SD	SD	6981.13	17	63.875	193.0625	0	272.175	7.7375	1
84	Control SD pups	SD	SD	6780.42	12	43.7875	667.2625	0	273.15	7.9875	1
85	SD pups onto VWKY dams	VWKY	SD	7702.46	37	144.4765	68.311765	0	268.54184	10.92739	1
86	SD pups onto VWKY dams	VWKY	SD	8971.92	38	144.0793	168.8435	6	294.19207	0	0
87	SD pups onto VWKY dams	VWKY	SD	10842.06	56	181.7118	57.723529	5	223.1405	32.2137	4
88	SD pups onto VWKY dams	VWKY	SD	8107.21	21	39.97059	185.11765	5	261.03919	0	0
89	SD pups onto VWKY dams	VWKY	SD	8257.01	19	34.5	45.423529	0	182.79981	41.00859	1
90	SD pups onto VWKY dams	VWKY	SD	7416.63	18	59.5125	103.55	4	248.4625	30.725	1
91	SD pups onto VWKY dams	VWKY	SD	5940.91	7	43.0625	364.1125	0	291.775	0	0
92	SD pups onto VWKY dams	VWKY	SD	5987.18	13	39.5125	22.575	0	296.6125	2.6625	1
93	SD pups onto VWKY dams	VWKY	SD	9476.04	37	83.95	135.725	0	266.6125	10.4	3
94	SD pups onto VWKY dams	VWKY	SD	7403.04	28	64.1125	20.8125	4	287.9	7.0125	1
95	SD pups onto SHR dams	SHR	SD	7358.03	30	88.55	266.85	1	182.6625	34.8375	4
96	SD pups onto SHR dams	SHR	SD	6419.74	26	82.7375	180	0	242.6625	16.6875	1
97	SD pups onto SHR dams	SHR	SD	5137.71	1	0.725	41.375	0	296.125	0	0
98	SD pups onto SHR dams	SHR	SD	6979.03	14	69.1875	250.65	3	266.375	5.075	1
99	SD pups onto SHR dams	SHR	SD	7119.01	20	31.2125	63.875	2	216.2875	27.825	4
00	SD pups onto SHR dams	SHR	SD	7872.72	35	74.7625	11.125	6	193.0625	46.95	7
01	SD pups onto SHR dams	SHR	SD	7686.65	28	54.1875	51.05	1	212.6625	37.975	4
02	SD pups onto SHR dams	SHR	SD	6114.33	16	31.6875	90.2375	4	210.2375	44.5125	6
03	SD pups onto SHR dams	SHR	SD	7336.27	41	108.15	29.275	0	166.6875	81.525	5
04	SD pups onto SHR dams	SHR	SD	7937.31	33	91.2125	27.3375	4	214.5875	59.275	4
05	SD pups onto SHR dams	SHR	SD	8337.31	31	76.2125	87.825	0	250.65	30.725	4
06	SD pups onto SHR dams	SHR	SD	9065.2	76	182.9125	40.4	4	212.6625	39.675	5
07	SD pups onto SHR dams	SHR	SD	7277.52	61	145.4	78.3875	0	237.1	7.9875	1
08	SD pups onto SHR dams	SHR	SD	9246.94	18	53.95	201.525	0	202.7375	40.6375	5
09	SD pups onto SHR dams	SHR	SD	8616.64	34	74.7625	12.1	0	223.0625	24.675	4

Table B.2: ANOVA and post hoc tests on WKY rats in 3.1.1 on page 37

Analysis of Variance (WKY spreadsheet 14 december)
Marked effects are significant at p < .05000

Variable	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Hippocampus	0	2	0	0	32	0	4.684436	0.016429
Prefrontal Cortex	0	2	0	0	32	0	0.052872	0.948584
Total (cm)	5244121	2	2622060	74006995	32	2312719	1.133757	0.334409
Inner(freq)	422	2	211	977	32	31	6.909764	0.003203
Inner(s)	7906	2	3953	25791	32	806	4.904820	0.013867
latency Inner (s)	214830	2	107415	3016018	32	94251	1.139676	0.332566
OF(def)	39	2	20	346	32	11	1.814238	0.179326
Closed(s)	803	2	401	67727	32	2116	0.189672	0.828154
Open(s)	2420	2	1210	30690	32	959	1.261645	0.296896
Open(freq)	7	2	4	104	32	3	1.152492	0.328612

ANOVA WKY

Newman-Keuls test; variable Inner(freq) (WKY spreadsheet 14 december)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 30.546, df = 32.000

Cell No.	Name	{1} 4.9167	{2} 2.9167	{3} 11.182
1	Control WKY		0.389187	0.010209
2	WKY pups onto SHR dams	0.389187		0.002991
3	WKY pups onto SD dams	0.010209	0.002991	

Newman-Keul on "number of entries to inner zone"

Newman-Keuls test; variable Inner(s) (WKY spreadsheet 14 december)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 805.98, df = 32.000

Cell No.	Name	{1} 22.276	{2} 8.3497	{3} 45.211
1	Control WKY		0.245320	0.060170
2	WKY pups onto SHR dams	0.245320		0.010129
3	WKY pups onto SD dams	0.060170	0.010129	

Newman-Keuls on "time spent in inner zone"

Table B.3: ANOVA and post hoc tests on SD rats in 3.1.1 on page 37

Variable	Analysis of Variance (SD spreadsheet 14 december) Marked effects are significant at p < .05000 SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Hippocampus	0	2	0	1	33	0	2.646265	0.085921
Prefrontal Cortex	0	2	0	2	33	0	0.430726	0.653639
Total (cm)	8365081	2	4182541	59193899	33	1793755	2.331724	0.112927
Inner(freq)	1567	2	783	7412	33	225	3.487610	0.042260
Inner(s)	6928	2	3464	66049	33	2001	1.730781	0.192840
latency Inner (s)	255803	2	127901	925082	33	28033	4.562564	0.017805
OF(def)	9	2	4	185	33	6	0.803941	0.456134
Closed(s)	13814	2	6907	35255	33	1068	6.465360	0.004273
Open(s)	2377	2	1188	18444	33	559	2.126299	0.135331
Open(freq)	40	2	20	97	33	3	6.815423	0.003329

ANOVA on SD

Cell No.	Newman-Keuls test; variable Inner(freq) (SD spreadsheet 14 december) Approximate Probabilities for Post Hoc Tests Error: Between MS = 224.61, df = 33.000 Name	{1} 15.545	{2} 27.400	{3} 30.933
1	Control SD pups		0.065132	0.047508
2	SD pups onto WKY dams	0.065132		0.573376
3	SD pups onto SHR dams	0.047508	0.573376	

Newman-Keul on "entries to inner zone"

Cell No.	Newman-Keuls test; variable latency Inner (s)(SD spreadsheet 14 december) Approximate Probabilities for Post Hoc Tests Error: Between MS = 28033., df = 33.000 Name	{1} 286.14	{2} 117.22	{3} 95.467
1	Control SD pups		0.020591	0.025548
2	SD pups onto WKY dams	0.020591		0.755992
3	SD pups onto SHR dams	0.025548	0.755992	

Newman-Keuls on "lat. to first enter inner zone"

Cell No.	Newman-Keuls test; variable Closed(s) (SD spreadsheet 14 december) Approximate Probabilities for Post Hoc Tests Error: Between MS = 1068.3, df = 33.000 Name	{1} 261.07	{2} 262.11	{3} 221.84
1	Control SD pups		0.939383	0.006783
2	SD pups onto WKY dams	0.939383		0.014818
3	SD pups onto SHR dams	0.006783	0.014818	

Newman-Keuls on "time in closed arms"

Cell No.	Newman-Keuls test; variable Open(freq) (SD spreadsheet 14 december) Approximate Probabilities for Post Hoc Tests Error: Between MS = 2.9374, df = 33.000 Name	{1} 2.0000	{2} 1.2000	{3} 3.6667
1	Control SD pups		0.268238	0.025207
2	SD pups onto WKY dams	0.268238		0.004155
3	SD pups onto SHR dams	0.025207	0.004155	

Newman-Keuls on "entries to open arms"

Table B.4: Correlations in SHR rats in Fig. 3.1 on page 45

Variables	Within-Group Correlations (All rats with def parameters) Group: Name:Control SHR Marked correlations are significant at p < .05000 Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	-0.294801	-0.144829	0.097919	0.013203	0.094366	-0.041022	-0.306321	0.288602	0.056720
Prefrontal Cortex	-0.294801	1.000000	-0.048726	-0.212280	-0.195385	-0.166688	-0.069008	-0.378123	0.200100	0.422508
Total (cm)	-0.144829	-0.048726	1.000000	0.810346	0.838917	-0.044409	-0.323199	-0.029011	-0.172150	-0.441398
Inner(freq)	0.097919	-0.212280	0.810346	1.000000	0.944369	-0.255737	-0.290549	-0.003834	-0.100055	-0.305911
Inner(s)	0.013203	-0.195385	0.838917	0.944369	1.000000	-0.136320	-0.347746	0.033765	-0.143459	-0.316757
latency Inner (s)	0.094366	-0.166688	-0.044409	-0.255737	-0.136320	1.000000	0.077614	0.543752	-0.109127	-0.163184
OF(def)	-0.041022	-0.069008	-0.323199	-0.290549	-0.347746	0.077614	1.000000	-0.265628	0.582007	0.420644
Closed(s)	-0.306321	-0.378123	-0.029011	-0.003834	0.033765	0.543752	-0.265628	1.000000	-0.647421	-0.524402
Open(s)	0.288602	0.200100	-0.172150	-0.100055	-0.143459	-0.109127	0.582007	-0.647421	1.000000	0.814631
Open(freq)	0.056720	0.422508	-0.441398	-0.305911	-0.316757	-0.163184	0.420644	-0.524402	0.814631	1.000000

Correlations control SHR

Variables	Within-Group Correlations (All rats with def parameters) Group: Name:SHR pups onto WKY dams Marked correlations are significant at p < .05000 Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	0.020858	-0.123307	-0.322514	-0.277693	-0.226257	0.006788	0.033118	-0.292650	-0.061948
Prefrontal Cortex	0.020858	1.000000	-0.091513	-0.082725	-0.221599	0.007085	0.006821	-0.121067	0.006839	-0.164281
Total (cm)	-0.123307	-0.091513	1.000000	0.796600	0.620324	0.039560	0.118933	-0.168817	0.155353	0.264670
Inner(freq)	-0.322514	-0.082725	0.796600	1.000000	0.870594	-0.174597	-0.298337	-0.346591	0.369936	0.254244
Inner(s)	-0.277693	-0.221599	0.620324	0.870594	1.000000	-0.397539	-0.429096	-0.380225	0.362750	0.282386
latency Inner (s)	-0.226257	0.007085	0.039560	-0.174597	-0.397539	1.000000	0.443213	0.692398	-0.615329	-0.453826
OF(def)	0.006788	0.006821	0.118933	-0.298337	-0.429096	0.443213	1.000000	0.484168	-0.334365	-0.126310
Closed(s)	0.033118	-0.121067	-0.168817	-0.346591	-0.380225	0.692398	0.484168	1.000000	-0.898471	-0.849292
Open(s)	-0.292650	0.006839	0.155353	0.369936	0.362750	-0.615329	-0.334365	-0.898471	1.000000	0.838198
Open(freq)	-0.061948	-0.164281	0.264670	0.254244	0.282386	-0.453826	-0.126310	-0.849292	0.838198	1.000000

Correlations SHR onto WKY

Variables	Within-Group Correlations (All rats with def parameters) Group: Name:SHR pups onto SD dams Marked correlations are significant at p < .05000 Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	-0.525048	-0.022774	-0.114257	-0.594080	-0.001959	0.322394	-0.032100	0.279299	0.187949
Prefrontal Cortex	-0.525048	1.000000	-0.374360	-0.412440	0.033808	-0.568464	-0.050842	0.230728	-0.158567	0.004180
Total (cm)	-0.022774	-0.374360	1.000000	0.580209	0.344138	0.413641	-0.585649	0.070493	-0.212127	-0.391669
Inner(freq)	-0.114257	-0.412440	0.580209	1.000000	0.691361	0.752284	-0.166958	-0.231926	-0.174888	-0.152856
Inner(s)	-0.594080	0.033808	0.344138	0.691361	1.000000	0.448790	-0.020774	-0.326579	-0.066791	0.072882
latency Inner (s)	-0.001959	-0.568464	0.413641	0.752284	0.448790	1.000000	0.027891	-0.027858	-0.376887	-0.358761
OF(def)	0.322394	-0.050842	-0.585649	-0.166958	-0.020774	0.027891	1.000000	-0.149349	0.163717	0.382524
Closed(s)	-0.032100	0.230728	0.070493	-0.231926	-0.326579	-0.027858	-0.149349	1.000000	-0.756467	-0.798146
Open(s)	0.279299	-0.158567	-0.212127	-0.174888	-0.066791	-0.376887	0.163717	-0.756467	1.000000	0.769622
Open(freq)	0.187949	0.004180	-0.391669	-0.152856	0.072882	-0.358761	0.382524	-0.798146	0.769622	1.000000

Correlations SHR onto SD

Variables	Within-Group Correlations (SHR spreadsheet 14 december) Group: Pup:SHR Marked correlations are significant at p < .05000 Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	-0.247663	-0.131141	-0.134757	-0.253799	0.015128	0.060350	-0.115242	0.001932	0.011316
Prefrontal Cortex	-0.247663	1.000000	-0.126296	-0.205835	-0.082398	-0.186465	-0.017887	-0.045257	-0.016476	0.011459
Total (cm)	-0.131141	-0.126296	1.000000	0.734223	0.605771	0.008270	-0.210527	-0.096600	0.063873	-0.000105
Inner(freq)	-0.134757	-0.205835	0.734223	1.000000	0.820775	-0.060851	-0.275489	-0.240430	0.165100	0.064165
Inner(s)	-0.253799	-0.082398	0.605771	0.820775	1.000000	-0.177375	-0.272588	-0.240311	0.153266	0.114835
latency Inner (s)	0.015128	-0.186465	0.008270	-0.060851	-0.177375	1.000000	0.218013	0.484188	-0.427709	-0.354091
OF(def)	0.060350	-0.017887	-0.210527	-0.275489	-0.272588	0.218013	1.000000	0.087080	0.001175	0.134630
Closed(s)	-0.115242	-0.045257	-0.096600	-0.240430	-0.240311	0.484188	0.087080	1.000000	-0.772496	-0.737760
Open(s)	0.001932	-0.016476	0.063873	0.165100	0.153266	-0.427709	0.001175	-0.772496	1.000000	0.825286
Open(freq)	0.011316	0.011459	-0.000105	0.064165	0.114835	-0.354091	0.134630	-0.737760	0.825286	1.000000

Correlations all SHR

Table B.5: Correlations in SHR rats in Fig. 3.1 on page 45

Variables	Within-Group Correlations (WKY spreadsheet 14 december) Group: Name:Control WKY Marked correlations are significant at p < .05000 Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	0.055843	0.237889	0.135619	-0.051261	-0.081170	0.123125	-0.199297	-0.026482	-0.135680
Prefrontal Cortex	0.055843	1.000000	-0.062015	-0.586226	-0.549257	0.309720	0.287037	-0.263760	0.100064	0.187078
Total (cm)	0.237889	-0.062015	1.000000	0.626517	0.295691	-0.128183	-0.277327	0.341949	-0.227904	-0.276956
Inner(freq)	0.135619	-0.586226	0.626517	1.000000	0.828684	0.106715	-0.335093	0.104668	0.029399	-0.115363
Inner(s)	-0.051261	-0.549257	0.295691	0.828684	1.000000	0.047071	-0.063555	-0.100839	0.223290	0.050881
latency Inner (s)	-0.081170	0.309720	-0.128183	0.106715	0.047071	1.000000	-0.105081	-0.262953	0.268928	0.172133
OF(def)	0.123125	0.287037	-0.277327	-0.335093	-0.063555	-0.105081	1.000000	-0.692167	0.557708	0.650642
Closed(s)	-0.199297	-0.263760	0.341949	0.104668	-0.100839	-0.262953	-0.692167	1.000000	-0.866461	-0.764838
Open(s)	-0.026482	0.100064	-0.227904	0.029399	0.223290	0.268928	0.557708	-0.866461	1.000000	0.871289
Open(freq)	-0.135680	0.187078	-0.276956	-0.115363	0.050881	0.172133	0.650642	-0.764838	0.871289	1.000000

Correlations control WKY

Variables	Within-Group Correlations (WKY spreadsheet 14 december) Group: Name:WKY pups onto SHR dams Marked correlations are significant at p < .05000 Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	-0.033625	-0.137656	0.121671	-0.067170	-0.409470	0.104771	0.416139	-0.315031	-0.117033
Prefrontal Cortex	-0.033625	1.000000	0.029878	0.079492	0.083775	0.295469	0.106879	0.497899	-0.096360	0.172841
Total (cm)	-0.137656	0.029878	1.000000	0.667962	0.550859	-0.071322	0.049711	-0.050458	-0.102572	0.068043
Inner(freq)	0.121671	0.079492	0.667962	1.000000	0.794245	0.023960	-0.084744	-0.004711	0.207818	0.207911
Inner(s)	-0.067170	0.083775	0.550859	0.794245	1.000000	-0.191186	-0.132302	-0.271548	0.402161	0.344242
latency Inner (s)	-0.409470	0.295469	-0.071322	0.023960	-0.191186	1.000000	0.089010	-0.069462	0.314338	0.282120
OF(def)	0.104771	0.106879	0.049711	-0.084744	-0.132302	0.089010	1.000000	0.387080	-0.456670	-0.199440
Closed(s)	0.416139	0.497899	-0.050458	-0.004711	-0.271548	-0.069462	0.387080	1.000000	-0.798441	-0.578003
Open(s)	-0.315031	-0.096360	-0.102572	0.207818	0.402161	0.314338	-0.456670	-0.798441	1.000000	0.817674
Open(freq)	-0.117033	0.172841	0.068043	0.207911	0.344242	0.282120	-0.199440	-0.578003	0.817674	1.000000

Corrrelations WKY onto SHR

Variables	Within-Group Correlations (WKY spreadsheet 14 december) Group: Name:WKY pups onto SD dams Marked correlations are significant at p < .05000 Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	0.382707	-0.434681	-0.540945	-0.222502	0.209085	-0.160955	0.094474	0.058046	-0.450891
Prefrontal Cortex	0.382707	1.000000	-0.727112	-0.695432	-0.713587	0.478274	0.390498	0.517791	-0.472131	-0.495129
Total (cm)	-0.434681	-0.727112	1.000000	0.738886	0.540709	-0.370321	-0.217553	-0.239778	0.155486	0.774563
Inner(freq)	-0.540945	-0.695432	0.738886	1.000000	0.676912	-0.367381	-0.331388	-0.410784	0.369511	0.649652
Inner(s)	-0.222502	-0.713587	0.540709	0.676912	1.000000	-0.104718	-0.016832	-0.710817	0.787263	0.392965
latency Inner (s)	0.209085	0.478274	-0.370321	-0.367381	-0.104718	1.000000	0.241274	0.256363	-0.030238	-0.348242
OF(def)	-0.160955	0.390498	-0.217553	-0.331388	-0.016832	0.241274	1.000000	0.154163	-0.182912	-0.296910
Closed(s)	0.094474	0.517791	-0.239778	-0.410784	-0.710817	0.256363	0.154163	1.000000	-0.931682	-0.467350
Open(s)	0.058046	-0.472131	0.155486	0.369511	0.787263	-0.030238	-0.182912	-0.931682	1.000000	0.271161
Open(freq)	-0.450891	-0.495129	0.774563	0.649652	0.392965	-0.348242	-0.296910	-0.467350	0.271161	1.000000

Correlations WKY onto SD

Variables	Within-Group Correlations (WKY spreadsheet 14 december) Group: Pup:WKY Marked correlations are significant at p < .05000 Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	0.028041	0.082553	-0.002294	0.009303	-0.130255	0.147019	0.062771	-0.015651	-0.039970
Prefrontal Cortex	0.028041	1.000000	-0.111674	-0.288511	-0.338077	0.297289	0.183828	0.262692	-0.166729	0.040388
Total (cm)	0.082553	-0.111674	1.000000	0.519173	0.365682	-0.085163	-0.047081	-0.002062	0.007378	0.086403
Inner(freq)	-0.002294	-0.288511	0.519173	1.000000	0.794764	-0.138572	-0.291812	-0.166607	0.372488	0.154275
Inner(s)	0.009303	-0.338077	0.365682	0.794764	1.000000	-0.119408	-0.134314	-0.375621	0.605588	0.165082
latency Inner (s)	-0.130255	0.297289	-0.085163	-0.138572	-0.119408	1.000000	0.122826	-0.061582	0.066567	0.193151
OF(def)	0.147019	0.183828	-0.047081	-0.291812	-0.134314	0.122826	1.000000	-0.015488	-0.126743	0.169197
Closed(s)	0.062771	0.262692	-0.002062	-0.166607	-0.375621	-0.061582	-0.015488	1.000000	-0.816078	-0.582321
Open(s)	-0.015651	-0.166729	0.007378	0.372488	0.605588	0.066567	-0.126743	-0.816078	1.000000	0.413255
Open(freq)	-0.039970	0.040388	0.086403	0.154275	0.165082	0.193151	0.169197	-0.582321	0.413255	1.000000

Correlations all WKY

Table B.6: Correlations in SHR rats in Fig. 3.1 on page 45

Within-Group Correlations (SD spreadsheet 14 december)
Group: Name:Control SD pups
Marked correlations are significant at p < .05000

Variables	Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	0.387673	0.555622	0.645972	0.255187	-0.553984	0.632340	-0.282410	0.120053	0.308005
Prefrontal Cortex	0.387673	1.000000	0.542538	0.488409	0.154980	-0.532601	-0.179070	-0.334313	0.116321	0.342289
Total (cm)	0.555622	0.542538	1.000000	0.839993	0.571087	-0.766517	0.012847	-0.530272	0.452722	0.652142
Inner(freq)	0.645972	0.488409	0.839993	1.000000	0.799823	-0.779865	0.025558	-0.747816	0.655945	0.837127
Inner(s)	0.255187	0.154980	0.571087	0.799823	1.000000	-0.534909	-0.054548	-0.840979	0.885384	0.882164
latency Inner (s)	-0.553984	-0.532601	-0.766517	-0.779865	-0.534909	1.000000	-0.155196	0.593205	-0.404624	-0.614413
OF(def)	0.632340	-0.179070	0.012847	0.025558	-0.054548	-0.155196	1.000000	0.116685	-0.075076	-0.099819
Closed(s)	-0.282410	-0.334313	-0.530272	-0.747816	-0.840979	0.593205	0.116685	1.000000	-0.893351	-0.912023
Open(s)	0.120053	0.116321	0.452722	0.655945	0.885384	-0.404624	-0.075076	-0.893351	1.000000	0.935611
Open(freq)	0.308005	0.342289	0.652142	0.837127	0.882164	-0.614413	-0.099819	-0.912023	0.935611	1.000000

Correlations control SD

Within-Group Correlations (SD spreadsheet 14 december)
Group: Name:SD pups onto WKY dams
Marked correlations are significant at p < .05000

Variables	Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	-0.320050	0.175957	0.334611	0.462194	0.231380	0.100779	-0.095012	-0.027350	-0.114213
Prefrontal Cortex	-0.320050	1.000000	-0.637139	-0.644540	-0.671544	0.365352	-0.679335	0.429093	-0.434911	-0.214273
Total (cm)	0.175957	-0.637139	1.000000	0.888020	0.688636	-0.255848	0.447312	-0.476722	0.412335	0.697507
Inner(freq)	0.334611	-0.644540	0.888020	1.000000	0.904542	-0.353945	0.407401	-0.199423	0.225917	0.696977
Inner(s)	0.462194	-0.671544	0.688636	0.904542	1.000000	-0.173773	0.371056	-0.025547	0.112867	0.502245
latency Inner (s)	0.231380	0.365352	-0.255848	-0.353945	-0.173773	1.000000	-0.036378	0.326635	-0.453596	-0.410560
OF(def)	0.100779	-0.679335	0.447312	0.407401	0.371056	-0.036378	1.000000	0.048422	-0.033098	-0.026062
Closed(s)	-0.095012	0.429093	-0.476722	-0.199423	-0.025547	0.326635	0.048422	1.000000	-0.904917	-0.392531
Open(s)	-0.027350	-0.434911	0.412335	0.225917	0.112867	-0.453596	-0.033098	-0.904917	1.000000	0.496656
Open(freq)	-0.114213	-0.214273	0.697507	0.696977	0.502245	-0.410560	-0.026062	-0.392531	0.496656	1.000000

Correltations SD onto WKY

Within-Group Correlations (SD spreadsheet 14 december)
Group: Name:SD pups onto SHR dams
Marked correlations are significant at p < .05000

Variables	Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	0.727015	0.025988	0.124805	-0.038444	-0.519053	0.682600	-0.337323	0.484358	0.544114
Prefrontal Cortex	0.727015	1.000000	-0.049852	-0.115317	-0.221561	-0.366731	0.443862	-0.620792	0.710079	0.594824
Total (cm)	0.025988	-0.049852	1.000000	0.542429	0.518643	-0.078675	0.077402	-0.452489	0.376157	0.525930
Inner(freq)	0.124805	-0.115317	0.542429	1.000000	0.950655	-0.304444	0.146252	-0.372202	0.259520	0.228281
Inner(s)	-0.038444	-0.221561	0.518643	0.950655	1.000000	-0.073978	0.111286	-0.319921	0.220231	0.099161
latency Inner (s)	-0.519053	-0.366731	-0.078675	-0.304444	-0.073978	1.000000	-0.200613	0.076760	-0.323321	-0.298699
OF(def)	0.682600	0.443862	0.077402	0.146252	0.111286	-0.200613	1.000000	-0.225912	0.279169	0.511628
Closed(s)	-0.337323	-0.620792	-0.452489	-0.372202	-0.319921	0.076760	-0.225912	1.000000	-0.833917	-0.785241
Open(s)	0.484358	0.710079	0.376157	0.259520	0.220231	-0.323321	0.279169	-0.833917	1.000000	0.775729
Open(freq)	0.544114	0.594824	0.525930	0.228281	0.099161	-0.298699	0.511628	-0.785241	0.775729	1.000000

Correlation SD onto SHR

Within-Group Correlations (SD spreadsheet 14 december)
Group: Pup:SD
Marked correlations are significant at p < .05000

Variables	Hippocampus	Prefrontal Cortex	Total (cm)	Inner(freq)	Inner(s)	latency Inner (s)	OF(def)	Closed(s)	Open(s)	Open(freq)
Hippocampus	1.000000	0.504766	0.306104	0.265333	0.193579	-0.400776	0.549399	-0.146599	0.150048	0.201273
Prefrontal Cortex	0.504766	1.000000	-0.023408	-0.103474	-0.226479	-0.249464	0.011979	-0.245661	0.266100	0.321120
Total (cm)	0.306104	-0.023408	1.000000	0.674138	0.623673	-0.530515	0.238180	-0.404116	0.317929	0.429155
Inner(freq)	0.265333	-0.103474	0.674138	1.000000	0.890643	-0.474612	0.226571	-0.439449	0.312010	0.426292
Inner(s)	0.193579	-0.226479	0.623673	0.890643	1.000000	-0.350303	0.207301	-0.321836	0.340288	0.311538
latency Inner (s)	-0.400776	-0.249464	-0.530515	-0.474612	-0.350303	1.000000	-0.183222	0.404842	-0.350804	-0.398193
OF(def)	0.549399	0.011979	0.238180	0.226571	0.207301	-0.183222	1.000000	-0.019191	0.012024	0.111719
Closed(s)	-0.146599	-0.245661	-0.404116	-0.439449	-0.321836	0.404842	-0.019191	1.000000	-0.813848	-0.780376
Open(s)	0.150048	0.266100	0.317929	0.312010	0.340288	-0.350804	0.012024	-0.813848	1.000000	0.781883
Open(freq)	0.201273	0.321120	0.429155	0.426292	0.311538	-0.398193	0.111719	-0.780376	0.781883	1.000000

Correlation all SD

Table B.7: Spreadsheet to CPP data in 3.6 on page 46

	1 Strain	2 P	3 Ket	4 Day	5 Box	6 PrePref	7 SS	8 DS	9 DS-SS	10 DS%-SS%	11 PostPref	12 New	13 Post SS	14 Post DS	15 Post DS-SS	16 Post%	17 Shift Post-Pre	18 Shift Post%-Pre%
1	SHR	57	12	522	1	white	1805	57	-1736.026	3.06122449	white	no	1395	209	-1149.23	13.02993	586.7922	9.9687007
2	SHR	57	12	522	2	white	1031	628	-400.2394	37.854129	white	no	858	739	-118.185	46.27426	282.0546	8.42013525
3	SHR	60	12	529	1	black	1054	505	-531.981	32.3925593	white	yes	454	1059	586.245	69.99339	1118.226	37.6008313
4	SHR	60	12	529	2	black	1012	526	-482.6709	34.2002601	black	no	968	550	-415.137	36.23188	67.5342	2.03162398
5	SHR	60	12	619	1	black	829	724	-101.745	46.6194462	black	no	1000	571	-415.701	36.34628	-313.956	-10.27317
6	SHR	60	12	619	2	black	795	604	-189.6916	43.1736955	black	no	1018	454	-560.137	30.84239	-370.445	-12.331304
7	SHR	60	12	626	1	black	756	592	-158.916	43.9169139	white	yes	387	445	56.202	53.48558	215.118	9.56866298
8	SHR	60	12	626	2	black	1187	333	-848.1501	21.9078947	black	no	676	654	-21.8493	49.17293	826.3008	27.2650376
9	SHR	60	12	710	1	white	910	610	-290.7	40.1315789	black	yes	592	968	364.344	62.05128	655.044	21.9197031
10	SHR	60	12	710	2	black	963	444	-515.4448	31.5565032	white	yes	213	286	72.49995	57.31463	587.9448	25.7581261
11	SHR	60	12	717	1	white	892	629	-254.847	41.3543721	black	yes	542	953	398.259	63.74582	653.106	22.3914473
12	SHR	60	12	717	2	white	837	721	-115.2054	46.2772786	white	no	1005	544	-457.842	35.11943	-342.637	-11.157847
13	SHR	60	20	529	1	white	747	630	-113.373	45.751634	black	yes	552	858	296.514	60.85106	409.887	15.0994298
14	SHR	60	20	529	2	black	956	536	-417.123	35.924933	black	no	579	511	-67.5342	46.88073	349.5888	10.955801
15	SHR	60	20	619	1	black	869	578	-281.979	39.9447132	black	no	868	524	-333.336	37.64368	-51.357	-2.301035
16	SHR	64	20	619	2	black	799	733	-65.5479	47.845953	black	no	902	680	-220.479	42.98357	-154.931	-4.8623879
17	SHR	64	20	626	1	white	784	777	-6.783	49.7757848	white	no	824	694	-125.97	45.71805	-119.187	-4.0577347
18	SHR	65	20	626	1	white	491	362	-125.001	42.4384525	black	yes	418	439	20.349	51.2252	145.35	8.78675168
19	SHR	60	20	710	2	black	946	580	-363.4929	38.0078637	white	yes	191	1153	955.4103	85.78869	1318.903	47.7808268
20	SHR	60	20	717	1	black	984	669	-305.235	40.4718693	black	no	924	582	-331.398	38.64542	-26.163	-1.826451
21	SHR	60	20	717	2	black	1082	437	-640.5817	28.7689269	black	no	1100	401	-694.212	26.71552	-53.6301	-2.0534039
22	SHR	60	20	717	1	black	925	522	-390.507	36.0746372	black	no	1070	551	-502.911	33.99136	-112.404	-2.0832738
23	SHR	60	20	605	1	black	870	657	-206.397	43.0255403	black	no	1179	304	-847.875	20.49899	-641.478	-22.526552
24	SHR	60	20	605	2	white	708	620	-87.3972	46.686747	black	yes	640	748	107.2602	53.89049	194.6574	7.20374293
25	SHR	60	20	605	2	white	917	549	-365.4792	37.4488404	white	no	1098	358	-734.931	24.58791	-369.452	-12.860928
26	WKY	60	12	612	2	black	1430	145	-1276.198	9.20634921	black	no	871	473	-395.274	35.19345	880.9241	25.9871032
27	WKY	60	12	612	1	black	1057	599	-443.802	36.1714976	black	no	1040	588	-437.988	36.11794	5.814	-0.0535615
28	WKY	60	12	619	2	black	1193	240	-946.4719	16.7480809	black	no	1094	395	-694.212	26.52787	252.2601	9.77979011
29	WKY	60	12	619	1	black	1135	440	-673.455	27.9365079	black	no	1083	406	-656.013	27.26662	17.442	-0.669886
30	WKY	60	12	626	2	black	1259	346	-906.7459	21.5576324	white	yes	240	1525	1276.198	86.40227	2182.944	64.8446339
31	WKY	60	12	626	1	black	1103	571	-515.508	34.1099164	black	no	1233	517	-693.804	29.54286	-178.296	-4.5670592
32	WKY	60	12	710	2	white	949	550	-396.2668	36.6911274	white	no	1026	580	-442.945	36.11457	-46.678	-0.5765571
33	WKY	60	12	710	1	black	1022	380	-622.098	27.1041369	white	yes	552	1105	535.857	66.66678	1157.955	39.5826464
34	WKY	60	12	710	1	black	893	600	-283.917	40.1875419	white	yes	344	1183	812.991	77.47217	1096.908	37.2846258
35	WKY	60	12	717	2	black	940	615	-322.7738	39.5498392	black	no	1073	540	-529.349	33.47799	-206.575	-6.0718479
36	WKY	60	20	605	2	white	827	709	-117.1917	46.1586542	black	yes	363	1122	753.8008	75.55556	870.9925	29.3967014
37	WKY	60	20	605	1	white	746	556	-184.11	42.703533	white	no	800	575	-218.025	41.81818	-33.915	-0.8853512
38	WKY	60	20	605	2	white	787	714	-72.49995	47.5682878	black	yes	540	956	413.1504	63.90374	485.6504	16.3354555
39	WKY	60	20	612	1	white	1302	395	-878.883	23.2763701	black	yes	294	1305	979.659	81.61351	1858.542	58.3371384
40	WKY	60	20	612	2	black	1189	419	-764.7255	26.0572139	white	yes	379	1320	934.5541	77.69276	1699.28	51.6355465
41	WKY	60	20	619	2	black	1076	434	-637.6023	28.7417219	black	no	1266	307	-952.431	19.51685	-314.829	-9.2248751
42	WKY	57	20	619	1	black	757	668	-86.241	46.877193	black	no	489	1010	504.849	67.37825	591.09	20.5010592
43	WKY	60	20	710	1	black	983	473	-494.19	32.4862637	white	yes	327	441	110.466	57.42188	604.656	24.9356113
44	WKY	60	20	710	2	black	1125	518	-602.842	31.5276932	white	yes	479	1009	526.3695	67.80914	1129.212	36.2814465
45	WKY	60	20	717	1	black	1076	523	-535.857	32.7079425	black	no	1364	223	-1105.63	14.05167	-569.772	-18.656273
46	WKY	60	20	717	2	white	1209	465	-738.9036	27.7777778	white	no	1206	355	-845.171	22.74183	-106.267	-5.0359456

Table B.8: Statistica output for repeated meassures ANOVA on CPP data in 3.6 on page 46

Repeated Measures Analysis of Variance (CPP P60 only in %)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	136342.0	1	136342.0	524.7275	0.000000
Strain	106.3	1	106.3	0.4093	0.526381
Ketamine	49.3	1	49.3	0.1899	0.665588
Strain*Ketamine	366.8	1	366.8	1.4117	0.242561
Error	9354.0	36	259.8		
CPP	3110.3	1	3110.3	12.6254	0.001085
CPP*Strain	492.5	1	492.5	1.9993	0.165966
CPP*Ketamine	41.8	1	41.8	0.1695	0.682955
CPP*Strain*Ketamine	108.0	1	108.0	0.4385	0.512054
Error	8868.6	36	246.4		

WKY and SHR, Ketamine 12 and 20

Table B.9: Statistica output for t-tests on CPP data in 3.6 on page 46

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	35.04761	8.93122						
Post%	47.51813	20.42908	40	-12.4705	22.08484	-3.57125	39	0.000963

WKY and SHR, Ketamine 12 and 20

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000
Include cases: 21:30

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	28.92626	10.40893						
Post%	45.48025	22.40531	10	-16.5540	24.15435	-2.16724	9	0.058375

WKY, 12 mg/kg Ketamine

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000
Include cases: 21:40

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	31.41341	9.62711						
Post%	48.84638	23.74431	20	-17.4330	24.61382	-3.16743	19	0.005071

WKY, Ketamine 12 and 20 mg/kg

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000
Include cases: 31:40

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	33.90057	8.57718						
Post%	52.21251	25.74977	10	-18.3119	26.34097	-2.19838	9	0.055487

WKY, 20 mg/kg Ketamine

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000
Exclude cases: 21:40

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	38.68181	6.57613						
Post%	46.18987	17.00451	20	-7.50806	18.53284	-1.81176	19	0.085860

SHR, Ketamine 12 and 20 mg/kg

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000
Include cases: 1:30
Exclude cases: 11:20

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	33.53966	10.17552						
Post%	47.45531	18.29213	20	-13.9156	21.02461	-2.95999	19	0.008043

WKY and SHR, 12 mg/kg Ketamine

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000
Include cases: 1:10

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	38.15305	7.93377						
Post%	49.43036	13.98983	10	-11.2773	18.28339	-1.95051	9	0.082898

SHR, 12 mg/kg Ketamine

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000
Include cases: 11:40
Exclude cases: 21:30

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	36.55557	7.44334						
Post%	47.58095	22.84841	20	-11.0254	23.55249	-2.09349	19	0.049953

WKY and SHR, 20 mg/kg Ketamine

T-test for Dependent Samples (CPP P60 only in %)
Marked differences are significant at p < .05000
Include cases: 11:20

Variable	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
Pre%	39.21057	5.26593						
Post%	42.94939	19.78345	10	-3.73882	18.95366	-0.623794	9	0.548245

SHR, 20 mg/kg Ketamine

Table B.10: Statistica output for repeated ANOVA on CPP testing conditions (page 46)

Univariate Tests of Significance for PrePref (CPP All in % in Workbook5)
Sigma-restricted parameterization
Effective hypothesis decomposition
Include condition: P=60

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	380252.5	1	380252.5	2198248	0.000000
Day	2.7	6	0.4	3	0.036001
Error	5.7	33	0.2		

Prefered compartment on different testing days

Univariate Tests of Significance for Shift Post-Pre (CPP All in % in Workbook5)
Sigma-restricted parameterization
Effective hypothesis decomposition
Include condition: P=60

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	6888755	1	6888755	22.50143	0.000039
Day	8158191	6	1359698	4.44132	0.002131
Error	10102866	33	306147		

Preference shift occuring on different testing days

Univariate Tests of Significance for PrePref (CPP All in %)
Sigma-restricted parameterization
Effective hypothesis decomposition
Include condition: P=60

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	412695.5	1	412695.5	1877938	0.000000
Box	0.0	1	0.0	0	0.639068
Error	8.4	38	0.2		

Prefered compartment in different boxes

Univariate Tests of Significance for Shift Post-Pre (CPP All in %)
Sigma-restricted parameterization
Effective hypothesis decomposition
Include condition: P=60

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	5447165	1	5447165	11.42743	0.001686
Box	147417	1	147417	0.30926	0.581394
Error	18113640	38	476675		

Prefernce shift occurring in different boxes

Kruskal-Wallis ANOVA by Ranks; Distance (Ketamine date as number)
Independent (grouping) variable: Day
Kruskal-Wallis test: H (7, N= 46) =1.072387 p =.9936
Include cases: 1:46

Depend.: Distance	Code	Valid N	Sum of Ranks
522	522	2	43.0000
529	529	4	104.0000
605	605	6	148.0000
612	612	4	89.0000
619	619	8	170.0000
626	626	6	154.0000
710	710	8	204.0000
717	717	8	169.0000

Distance travelled in the OF on different testing days

Table B.11: Spreadsheet for OF data after 15 minutes in 3.1.3 on page 48

	1 Strain	2 Ket	3 Day	4 Distance	5 TurnsTotal	6 Meander	7 Rear	8 Defac	9 CPP
1	SHR	12	522	7540.31	119526.48	244.32	6	0	586.7922
2	SHR	12	522	8795.04	127568.29	187.66	30	2	282.0546
3	SHR	12	529	14153.19	99257.01	128.25	14	0	1118.226
4	SHR	12	529	13778.61	90732.47	102.74	3	0	67.5342
5	SHR	12	619	8458.27	159464.24	287.33	41	1	-313.956
6	SHR	12	619	11157.93	133364.04	173.47	10	0	-370.445
7	SHR	12	626	9986.35	113884.95	160.45	33	9	215.118
8	SHR	12	626	14957.86	88619.34	100.48	23	0	826.3008
9	SHR	12	710	5799.82	172573.05	313.42	17	2	655.044
10	SHR	12	710	11702.49	132277.85	185.13	16	1	587.9448
11	SHR	12	717	15371.77	116974.01	115.2	18	0	653.106
12	SHR	12	717	13430.14	124882.4	173.71	11	0	-342.637
13	WKY	12	529	7486.29	96912.65	140.09	0	0	880.9241
14	WKY	12	529	5372.35	106769.18	265.47	0	0	5.814
15	WKY	12	619	4981.99	171293.76	302.8	0	0	252.2601
16	WKY	12	619	2832.14	177233.87	471.13	0	0	17.442
17	WKY	12	626	6349.94	107882.98	283.14	1	0	2182.944
18	WKY	12	626	3017.97	103323.06	562.89	3	0	-178.296
19	WKY	12	710	7773.33	129788.03	215.13	0	0	-46.6781
20	WKY	12	717	4883.65	123570.81	466.61	4	0	1157.955
21	WKY	12	717	9222.1	135547.29	233.98	0	0	1096.908
22	WKY	12	717	8366.07	145427.58	215.52	0	0	-206.575
23	SHR	20	605	9003	116065.83	204.01	21	0	409.887
24	SHR	20	605	15930.86	76114.32	112.02	7	0	349.5888
25	SHR	20	605	7908.99	129005	228.55	43	1	-51.357
26	SHR	20	612	13261.83	100613.12	99.05	0	0	-154.931
27	SHR	20	612	12286.77	88803.47	100.23	0	0	-119.187
28	SHR	20	619	14287.82	132620.25	116.13	1	0	145.35
29	SHR	20	619	17030.49	120835.3	173.87	0	0	1318.903
30	SHR	20	626	11239.91	95475.73	115.54	19	0	-26.163
31	SHR	20	626	12238.08	96512.47	96.85	30	0	-53.6301
32	SHR	20	710	10806.97	142642.04	189.96	36	0	-112.404
33	SHR	20	710	15376.39	119613.76	99.55	16	0	-641.478
34	SHR	20	710	14779.02	124614.63	111.21	9	0	194.6574
35	SHR	20	717	8670.63	153625.95	236.39	60	3	-369.452
36	WKY	20	605	4786.34	112923.55	312.74	0	0	870.9926
37	WKY	20	605	9706.93	97162.47	144.06	0	1	-33.915
38	WKY	20	605	8380.61	121481.35	147.27	0	0	485.6504
39	WKY	20	612	4199.13	132517.11	221.67	0	0	1858.542
40	WKY	20	612	6037.73	102933.49	453.57	0	0	1699.28
41	WKY	20	619	4182.83	188831.22	272.04	0	0	-314.829
42	WKY	20	619	7457.63	144531.63	276.49	1	0	591.09
43	WKY	20	710	6269.65	171278.76	214.48	0	0	604.656
44	WKY	20	710	5718.55	170019.31	228.35	0	0	1129.212
45	WKY	20	717	3045.72	188360.55	358.05	0	0	-569.772
46	WKY	20	717	4343.95	141226.47	407.95	0	0	-106.267
47	SHR	0	807	6925.36	189536.07	-76.18	37	2	
48	SHR	0	807	6902.34	182168.72	-76.33	31	5	
49	SHR	0	807	7677.57	190186.36	-78.36	46	4	
50	SHR	0	807	6879.42	179414.6	-82.2	19	6	
51	SHR	0	810	6341.33	185811.35	-91.44	50	4	
52	SHR	0	810	6957.51	184631.46	-100.84	49	3	
53	SHR	0	810	6731.59	179143.62	-71.32	42	5	
54	SHR	0	810	8595.6	184598.72	-68.05	59	2	
55	WKY	0	806	2200.42	135357.87	-216.67	0	8	
56	WKY	0	806	2917.55	140212.4	-201.15	1	3	
57	WKY	0	806	3223.21	125296.9	-196.28	6	6	
58	WKY	0	806	3057.77	163951.48	-233.02	3	0	
59	WKY	0	810	767.61	140242.19	-463	0	10	
60	WKY	0	810	3303.68	157120.95	-183.85	2	6	
61	WKY	0	810	6256.94	177181.92	-98.54	8	7	
62	WKY	0	810	5008.36	148812.39	-104.08	11	5	
63	SD	0	815	7557.55	193228.02	-104.11	31	0	
64	SD	0	815	9284.51	190870.24	-113.19	37	0	
65	SD	0	815	10452.52	174829.71	-56.58	16	0	
66	SD	0	819	6373.7	190708.34	-153.28	14	0	
67	SD	0	819	7284.42	190337.11	-69.45	25	0	
68	SD	0	821	9338.07	179247.95	-52.28	22	0	
69	SD	0	821	7695.72	174995.76	-69.33	24	0	
70	SD	0	821	9027.11	172561.67	-61.88	17	0	
71	SD	12	815	4402.7	171507.47	-250.52	4	0	
72	SD	12	815	9157.28	170071.8	-231.83	0	0	
73	SD	12	815	6203.9	166579.08	-288.04	0	3	
74	SD	12	819	5347.36	181058.2	-231.49	5	0	
75	SD	12	819	10631.46	160913.38	-140.53	5	0	
76	SD	12	821	9290.95	157998.71	-456.51	0	0	
77	SD	12	821	9348.89	142023.84	-199.14	2	0	
78	SD	20	815	14947.94	150340.89	-80.68	1	0	
79	SD	20	815	10158.15	190132.73	-60.85	0	0	
80	SD	20	815	18710.76	168554.06	-28.35	2	2	
81	SD	20	819	12322.1	146279.13	-66.27	2	0	
82	SD	20	819	13408.65	167180.39	-42.85	0	0	
83	SD	20	821	6203.3	189944.74	-154.36	0	0	
84	SD	20	821	7239.98	205796.29	-117.59	0	0	

Table B.12: Statistics to Fig. 3.7 on page 50: non-parametric ANOVA by ranks of total distance travelled in 15 min. OF, grouped by strain

Multiple Comparisons p values (2-tailed); Distance (Ketamine date as number)
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 33) =14.67297 p =.0007
Exclude condition: Strain='WKY'
Include cases: 1:54

Depend.: Distance	0 R:6.0000	12 R:18.500	20 R:22.385
0		0.013868	0.000488
12	0.013868		0.946802
20	0.000488	0.946802	

within SHR

Multiple Comparisons p values (2-tailed); Distance (Ketamine)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 84) =36.62217 p =.0000

Depend.: Distance	SHR R:57.182	WKY R:20.759	SD R:49.136
SHR		0.000000	0.692357
WKY	0.000000		0.000116
SD	0.692357	0.000116	

Strain effect

Table B.13: Statistics to Fig. 3.8 on page 50: non-parametric ANOVA by ranks of total distance travelled in 15 min. OF, grouped by dose

Multiple Comparisons p values (2-tailed); Distance (Ketamine)
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 84) =9.730002 p =.0077

Depend.: Distance	0 R:30.083	12 R:44.172	20 R:50.548
0		0.109020	0.006091
12	0.109020		0.934909
20	0.006091	0.934909	

Dose effect, all strains

Multiple Comparisons p values (2-tailed); Distance (Ketamine)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =17.36000 p =.0002
Include cases: 47:70

Depend.: Distance	SHR R:14.000	WKY R:4.5000	SD R:19.000
SHR		0.021629	0.471898
WKY	0.021629		0.000123
SD	0.471898	0.000123	

All strains, Saline

Multiple Comparisons p values (2-tailed); Distance (Ketamine date as number)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =16.86184 p =.0002
Include cases: 23:84
Exclude cases: 47:77

Depend.: Distance	SHR R:21.538	WKY R:7.0000	SD R:19.857
SHR		0.000285	1.000000
WKY	0.000285		0.010342
SD	1.000000	0.010342	

All strains, 12 mg/kg

Multiple Comparisons p values (2-tailed); Distance (Ketamine date as number)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =12.28144 p =.0022
Include cases: 1:77
Exclude cases: 23:70

Depend.: Distance	SHR R:21.167	WKY R:8.5000	SD R:13.714
SHR		0.001536	0.197172
WKY	0.001536		0.641986
SD	0.197172	0.641986	

All strains, 20 mg/kg

Table B.14: Statistics to Fig. 3.9 on page 51: non-parametric ANOVA by ranks of meandering in 15 min. OF, grouped by strain

	Multiple Comparisons p values (2-tailed); Meander (Ketamir Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 84) =25.22474 p =.0000		
Depend.: Meander	SHR R:47.121	WKY R:53.862	SD R:20.591
SHR		0.832812	0.000233
WKY	0.832812		0.000004
SD	0.000233	0.000004	

Strain effect

	Multiple Comparisons p values (2-tailed); Meander (Keta Independent (grouping) variable: Ketamine Kruskal-Wallis test: H (2, N= 33) =18.71829 p =.0001 Exclude condition: Strain='WKY' Include cases: 1:54		
Depend.: Meander	0 R:4.5000	12 R:23.083	20 R:19.077
0		0.000076	0.002383
12	0.000076		0.902002
20	0.002383	0.902002	

within SHR

	Multiple Comparisons p values (2-tailed); Meander (Keta Independent (grouping) variable: Ketamine Kruskal-Wallis test: H (2, N= 29) =16.96853 p =.0002 Exclude condition: Strain='SHR' Include cases: 13:62		
Depend.: Meander	0 R:4.5000	12 R:19.800	20 R:18.273
0		0.000455	0.001498
12	0.000455		1.000000
20	0.001498	1.000000	

within WKY

	Multiple Comparisons p values (2-tailed); Meander (Keta Independent (grouping) variable: Ketamine Kruskal-Wallis test: H (2, N= 22) =12.72671 p =.0017 Include cases: 63:84		
Depend.: Meander	0 R:14.500	12 R:4.2857	20 R:15.286
0		0.007114	1.000000
12	0.007114		0.004587
20	1.000000	0.004587	

within SD

Table B.15: Statistics to Fig. 3.10 on page 51: non-parametric ANOVA by ranks of meandering in 15 min. OF, grouped by dose

	Multiple Comparisons p values (2-tailed); Meander (Ketamine) Independent (grouping) variable: Ketamine Kruskal-Wallis test: H (2, N= 84) =26.62347 p =.0000		
Depend.: Meander	0 R:20.833	12 R:50.069	20 R:52.194
0		0.000042	0.000007
12	0.000042		1.000000
20	0.000007	1.000000	

Dose effect, all strains

	Multiple Comparisons p values (2-tailed); Meander (Ketamine) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 24) =12.24500 p =.0022 Include cases: 47:70		
Depend.: Meander	SHR R:15.625	WKY R:5.3750	SD R:16.500
SHR		0.011226	1.000000
WKY	0.011226		0.004955
SD	1.000000	0.004955	

All strains, saline

	Multiple Comparisons p values (2-tailed); Meander (Keta Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 29) =19.05149 p =.0001 Include cases: 1:77 Exclude cases: 23:70		
Depend.: Meander	SHR R:15.333	WKY R:22.300	SD R:4.0000
SHR		0.168057	0.015394
WKY	0.168057		0.000039
SD	0.015394	0.000039	

all strains, 12mg/kg

	Multiple Comparisons p values (2-tailed); Meander (Keta Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 31) =22.00361 p =.0000 Include cases: 23:84 Exclude cases: 47:77		
Depend.: Meander	SHR R:15.231	WKY R:24.545	SD R:4.0000
SHR		0.037182	0.025255
WKY	0.037182		0.000009
SD	0.025255	0.000009	

all strains, 20mg/kg

Table B.16: Statistics to Fig. 3.11 on page 52: non-parametric ANOVA by ranks of turning in 15 min. OF, grouped by strain

	Multiple Comparisons p values (2-tailed); TurnsTotal (Ketamine) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 84) =21.15104 p =.0000		
Depend.: TurnsTotal	SHR R:33.970	WKY R:36.690	SD R:62.955
SHR		1.000000	0.000047
WKY	1.000000		0.000420
SD	0.000047	0.000420	

strain effect

	Multiple Comparisons p values (2-tailed); TurnsTotal (K Independent (grouping) variable: Ketamine Kruskal-Wallis test: H (2, N= 33) =17.85445 p =.0001 Exclude condition: Strain='WKY' Include cases: 1:54		
Depend.: TurnsTotal	0 R:29.500	12 R:13.917	20 R:12.154
0		0.001243	0.000196
12	0.001243		1.000000
20	0.000196	1.000000	

within SHR

	Multiple Comparisons p values (2-tailed); TurnsTotal (K Independent (grouping) variable: Ketamine Kruskal-Wallis test: H (2, N= 22) =7.095991 p =.0288 Include cases: 63:84		
Depend.: TurnsTotal	0 R:16.000	12 R:7.1429	20 R:10.714
0		0.025207	0.347311
12	0.025207		0.910519
20	0.347311	0.910519	

within SD

Table B.17: Statistics to Fig. 3.12 on page 52: non-parametric ANOVA by ranks of turning in 15 min. OF, grouped by dose

	Multiple Comparisons p values (2-tailed); TurnsTotal (Ketamine) Independent (grouping) variable: Ketamine Kruskal-Wallis test: H (2, N= 84) =22.02435 p =.0000		
Depend.: TurnsTotal	0 R:62.167	12 R:33.241	20 R:35.935
0		0.000052	0.000229
12	0.000052		1.000000
20	0.000229	1.000000	

Dose effect, all strains

	Multiple Comparisons p values (2-tailed); TurnsTotal (Ketamine) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 24) =13.95500 p =.0009 Include cases: 47:70		
Depend.: TurnsTotal	SHR R:16.375	WKY R:4.8750	SD R:16.250
SHR		0.003430	1.000000
WKY	0.003430		0.003882
SD	1.000000	0.003882	

all strains, saline

	Multiple Comparisons p values (2-tailed); TurnsTotal (K Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 29) =8.739901 p =.0127 Include cases: 1:77 Exclude cases: 23:70		
Depend.: TurnsTotal	SHR R:11.500	WKY R:13.500	SD R:23.143
SHR		1.000000	0.012117
WKY	1.000000		0.064675
SD	0.012117	0.064675	

all strains, 12mg/kg

	Multiple Comparisons p values (2-tailed); TurnsTotal (K Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 31) =13.52088 p =.0012 Include cases: 23:84 Exclude cases: 47:77		
Depend.: TurnsTotal	SHR R:9.9231	WKY R:17.182	SD R:25.429
SHR		0.153972	0.000825
WKY	0.153972		0.181976
SD	0.000825	0.181976	

all strains, 20mg/kg

Table B.18: Statistics to Fig. 3.13 on page 54: non-parametric ANOVA by ranks of rearing in 15 min. OF, grouped by strain

Multiple Comparisons p values (2-tailed); Rearing (Ketamin
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 84) =36.14174 p =.0000

Depend.: Rearing	SHR R:60.182	WKY R:23.724	SD R:40.727
SHR		0.000000	0.011278
WKY	0.000000		0.041053
SD	0.011278	0.041053	

Strain effect

Multiple Comparisons p values (2-tailed); Rearing (Ketan
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 33) =10.36334 p =.0056
Exclude condition: Strain='WKY'
Include cases: 1:54

Depend.: Rearing	0 R:26.563	12 R:14.250	20 R:13.654
0		0.015826	0.008909
12	0.015826		1.000000
20	0.008909	1.000000	

within SHR

Multiple Comparisons p values (2-tailed); Rearing (Ketan
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 29) =10.13871 p =.0063
Exclude condition: Strain='SHR'
Include cases: 13:62

Depend.: Rearing	0 R:21.563	12 R:14.150	20 R:11.000
0		0.199389	0.022776
12	0.199389		1.000000
20	0.022776	1.000000	

within WKY

Multiple Comparisons p values (2-tailed); Rearing (Ketan
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 22) =15.63684 p =.0004
Include cases: 63:84

within SD

0		0.010782	0.000836
12	0.010782		1.000000
20	0.000836	1.000000	

Table B.19: Statistics to Fig. 3.8 on page 50: non-parametric ANOVA by ranks of rearing in 15 min. OF, grouped by dose

Multiple Comparisons p values (2-tailed); Rearing (Ketamine)
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 84) =17.37100 p =.0002

Depend.: Rearing	0 R:59.083	12 R:39.310	20 R:32.645
0		0.009926	0.000201
12	0.009926		0.870592
20	0.000201	0.870592	

Dose effect, all strains

Multiple Comparisons p values (2-tailed); Rearing (Ketamine)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =18.50914 p =.0001
Include cases: 47:70

Depend.: Rearing	SHR R:19.625	WKY R:4.5000	SD R:13.375
SHR		0.000057	0.231300
WKY	0.000057		0.036196
SD	0.231300	0.036196	

All strains, saline

Multiple Comparisons p values (2-tailed); Rearing (Ketan
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =20.14365 p =.0000
Include cases: 1:77
Exclude cases: 23:70

Depend.: Rearing	SHR R:23.125	WKY R:7.8500	SD R:11.286
SHR		0.000084	0.010380
WKY	0.000084		1.000000
SD	0.010380	1.000000	

all strains, 12mg/kg

Multiple Comparisons p values (2-tailed); Rearing (Ketan
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =13.63783 p =.0011
Include cases: 23:84
Exclude cases: 47:77

Depend.: Rearing	SHR R:22.231	WKY R:9.9091	SD R:14.000
SHR		0.002819	0.160451
WKY	0.002819		1.000000
SD	0.160451	1.000000	

all strains, 20mg/kg

Table B.20: Statistics to Fig. 3.15 on page 55: non-parametric ANOVA by ranks of defecation in 15 min. OF, grouped by strain

Multiple Comparisons p values (2-tailed); Defecation (H
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 33) =17.40825 p =.0002
Exclude condition: Strain='WKY'
Include cases: 1:54

Depend.: Defecation	0 R:27.938	12 R:15.542	20 R:11.615
0		0.014927	0.000517
12	0.014927		0.931314
20	0.000517	0.931314	

within SHR

Multiple Comparisons p values (2-tailed); Defecation (H
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 29) =20.56649 p =.0000
Exclude condition: Strain='SHR'
Include cases: 13:62

Depend.: Defecation	0 R:24.125	12 R:11.000	20 R:12.000
0		0.003466	0.006538
12	0.003466		1.000000
20	0.006538	1.000000	

within WKY

Table B.21: Statistics to Fig. 3.16 on page 55: non-parametric ANOVA by ranks of defecation in 15 min. OF, grouped by dose

Multiple Comparisons p values (2-tailed); Defecation (Ketamine)
Independent (grouping) variable: Ketamine
Kruskal-Wallis test: H (2, N= 84) =22.34747 p =.0000

Depend.: Defecation	0 R:58.417	12 R:37.879	20 R:34.500
0		0.006841	0.000932
12	0.006841		1.000000
20	0.000932	1.000000	

Dose effect, all strains

Multiple Comparisons p values (2-tailed); Defecation (Ketamine)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =15.28164 p =.0005
Include cases: 47:70

Depend.: Defecation	SHR R:14.563	WKY R:17.938	SD R:5.0000
SHR		1.000000	0.020511
WKY	1.000000		0.000759
SD	0.020511	0.000759	

all strains, saline

Table B.22: Spreadsheet for OF data in 5 minutes bins in 3.1.3

	1 Strain	2 Ket	3 Bin	4 Time	5 Distance	6 TurnTotal	7 Meander	8 Rearing
1	SHR	12	1	168	3237.75	36153.91	52.65	3
2	SHR	12	2	168	2775.33	39878.76	79.8	3
3	SHR	12	3	168	1527.23	43493.81	111.87	0
4	SHR	12	1	168	4148.64	39168.09	39.72	8
5	SHR	12	2	168	2622.37	44183.56	65.96	12
6	SHR	12	3	168	2024.03	44216.64	81.98	10
7	SHR	12	1	180	5762.38	31212.07	28.23	3
8	SHR	12	2	180	5965.41	28095.64	27.87	5
9	SHR	12	3	180	2425.4	39946.9	72.15	6
10	SHR	12	1	180	3751.45	34040.23	38.86	0
11	SHR	12	2	180	5512.17	25756.66	25.04	1
12	SHR	12	3	180	4514.99	30935.58	38.84	2
13	SHR	12	1	240	3176.04	47117	51.8	14
14	SHR	12	2	240	3422.55	56800.12	68.16	19
15	SHR	12	3	240.19	1859.68	55547.12	167.37	8
16	SHR	12	1	240	4668.59	39902.01	31.53	6
17	SHR	12	2	240	4359.04	41836.91	47.19	5
18	SHR	12	3	240	2130.3	51625.12	94.75	5
19	SHR	12	1	177.87	4042.4	33698.82	40.78	6
20	SHR	12	2	177.87	3359.29	41712.31	59.15	19
21	SHR	12	3	177.87	2584.66	38473.82	60.52	8
22	SHR	12	1	177.87	5840.92	24152.21	17.37	8
23	SHR	12	2	177.87	6705.02	25031.87	23.35	12
24	SHR	12	3	177.87	2411.92	39435.26	59.76	3
25	SHR	12	1	240	2013.79	53972.08	103.31	6
26	SHR	12	2	240	2108.73	57206.44	104.05	9
27	SHR	12	3	240	1677.3	61394.53	106.06	2
28	SHR	12	1	240	4719.22	40498.06	42.29	3
29	SHR	12	2	240	4884.31	44361.1	53.77	7
30	SHR	12	3	240	2098.96	47418.69	89.07	6
31	SHR	12	1	240	4962.52	37329.8	33.93	0
32	SHR	12	2	240	6779.65	33257.59	26.45	8
33	SHR	12	3	240	3629.6	46386.62	54.82	10
34	SHR	12	1	240	6013.38	38615.92	32.04	1
35	SHR	12	2	240	5513.4	40073.04	40.46	8
36	SHR	12	3	240	1903.36	46193.44	101.21	2
37	VMKY	12	1	180	1674.51	36239.34	54.18	0
38	VMKY	12	2	180	3060.04	30286.3	39.28	0
39	VMKY	12	3	180	2751.74	30387.01	46.63	0
40	VMKY	12	1	180	2462.1	39649.65	42.97	0
41	VMKY	12	2	180	2386.98	33223.53	55.29	0
42	VMKY	12	3	180	523.27	33896	167.21	0
43	VMKY	12	1	240	1651.01	67661.12	76.14	0
44	VMKY	12	2	240	2843	52992.81	62.09	0
45	VMKY	12	3	240	487.98	50639.83	164.57	0
46	VMKY	12	1	240	1436.44	65681.41	90.31	0
47	VMKY	12	2	240.78	877.97	61038.26	243.2	0
48	VMKY	12	3	240	517.73	50514.2	147.62	0
49	VMKY	12	1	177.87	2385	38099.57	49.1	0
50	VMKY	12	2	177.87	3235.7	34249.24	90.16	0
51	VMKY	12	3	177.87	729.24	35534.17	143.88	1
52	VMKY	12	1	177.87	1527.98	31698.15	65.73	0
53	VMKY	12	2	177.87	1092.5	43812.87	111.28	2
54	VMKY	12	3	177.68	397.49	27802.05	385.88	1
55	VMKY	12	1	240	2788.94	46929.66	52.16	0
56	VMKY	12	2	240	3561.35	39082.89	52.4	0
57	VMKY	12	3	240	1423.04	43775.48	110.57	0
58	VMKY	12	1	240	1993.05	42590.04	63.35	1
59	VMKY	12	2	239.81	2743.97	46664.2	148.37	3
60	VMKY	12	3	240	146.63	34316.57	254.89	0
61	VMKY	12	1	240	3152.09	48961.09	50.24	0
62	VMKY	12	2	240	5044.81	39536.38	33.8	0
63	VMKY	12	3	240	1025.4	47049.82	149.94	0
64	VMKY	12	1	240	3045.29	49320.81	44.18	0
65	VMKY	12	2	240	4036.17	48072.71	48.23	0
66	VMKY	12	3	240	1284.61	48034.06	123.11	0
67	SHR	20	1	170.9	3614.36	36802.93	51.27	10
68	SHR	20	2	170.9	3068.86	37141.28	66.99	5
69	SHR	20	3	170.9	2319.78	42121.62	78.75	6
70	SHR	20	1	170.9	4410.35	30007.35	27.17	5
71	SHR	20	2	170.9	5604.09	20713.29	13.92	2
72	SHR	20	3	171.68	5916.42	25393.68	70.93	0
73	SHR	20	1	170.9	3510.31	37848.79	51.67	10
74	SHR	20	2	170.9	3391.55	44890.44	59.36	26
75	SHR	20	3	170.9	1007.13	46265.77	117.52	7
76	SHR	20	1	170.9	2678.67	38080.27	48.44	0
77	SHR	20	2	170.9	5008.7	31721.09	23.91	0
78	SHR	20	3	170.9	5574.46	30811.76	26.7	0
79	SHR	20	1	170.9	2719.82	30388.33	38.01	0
80	SHR	20	2	170.9	4424.66	28650.57	25.51	0
81	SHR	20	3	170.9	5142.29	29764.57	36.71	0
82	SHR	20	1	240	2926.74	54569.39	51.92	0
83	SHR	20	2	240	5347.85	42147.79	33.02	1
84	SHR	20	3	240	6013.23	35903.07	31.19	0
85	SHR	20	1	240	5386.06	41391.71	33.26	0
86	SHR	20	2	240	7361.52	35840.98	25.74	0
87	SHR	20	3	240.2	4282.91	43602.61	114.87	0
88	SHR	20	1	177.87	2205.95	38578.65	68.28	1
89	SHR	20	2	177.87	3415.83	30763.71	25.96	12
90	SHR	20	3	177.87	5618.13	26133.37	21.3	6
91	SHR	20	1	177.87	3186.43	34325.92	38.4	8
92	SHR	20	2	177.87	3809.41	33369.05	29.91	18
93	SHR	20	3	177.87	5242.24	28817.5	28.54	4
94	SHR	20	1	240	4169.7	43181.84	43.6	12
95	SHR	20	2	240	4334.42	51336.74	64.26	18
96	SHR	20	3	240	2302.85	48123.46	82.1	6
97	SHR	20	1	240	3874.87	43823.57	33.08	5
98	SHR	20	2	240	5317.04	37893.44	32.67	9
99	SHR	20	3	240	6184.48	37896.75	33.8	2
00	SHR	20	1	240	5003.29	43286.77	27.95	5
01	SHR	20	2	240	6159.55	34159.74	28.67	3
02	SHR	20	3	240	3616.18	47168.12	54.59	1
03	SHR	20	1	240	3734.42	49497.28	57.64	20
04	SHR	20	2	240	3028.3	51206.42	85.28	24
05	SHR	20	3	240	1907.91	52922.25	113.47	16

	1 Strain	2 Ket	3 Bin	4 Time	5 Distance	6 TurnTotal	7 Meander	8 Rearing
1	VMKY	20	1	170.9	1620.9	42081.89	77.55	0
2	VMKY	20	2	170.9	2790.36	37922.07	54.59	0
3	VMKY	20	3	170.9	375.08	32919.59	180.6	0
4	VMKY	20	1	170.9	3529.52	32092.87	34.22	0
5	VMKY	20	2	170.9	4520.23	25183.15	25.38	0
6	VMKY	20	3	170.9	1657.18	39886.45	84.46	0
7	VMKY	20	1	170.9	2008.91	48494.81	55.18	0
8	VMKY	20	2	170.9	2996.29	37249.86	43.46	0
9	VMKY	20	3	170.9	3375.41	36736.68	48.63	0
10	VMKY	20	1	170.9	1288.83	52256.2	75.31	0
11	VMKY	20	2	170.9	1549.86	41930.13	64.56	0
12	VMKY	20	3	170.9	1360.44	38330.78	81.8	0
13	VMKY	20	1	170.9	3141.7	32842.24	38.62	0
14	VMKY	20	2	170.9	2341.25	37823.54	77.88	0
15	VMKY	20	3	171.87	554.78	32267.71	337.07	0
16	VMKY	20	1	240	908.57	66190.07	117.7	0
17	VMKY	20	2	240	1246.76	65825.3	84.52	0
18	VMKY	20	3	240	2027.5	56815.85	69.82	0
19	VMKY	20	1	240	2697.73	52437.32	54.9	0
20	VMKY	20	2	240	4170.54	41521.59	41.6	1
21	VMKY	20	3	240	589.36	50572.72	179.99	0
22	VMKY	20	1	240	1098.49	65969.74	100.12	0
23	VMKY	20	2	240	1897.99	61185.19	68.8	0
24	VMKY	20	3	240	3273.17	44123.83	45.56	0
25	VMKY	20	1	240	1398.32	65555.22	89.87	0
26	VMKY	20	2	240	2042.06	58660.52	65.76	0
27	VMKY	20	3	240	2278.17	47803.57	72.72	0
28	VMKY	20	1	240	579.1	64115.31	138.35	0
29	VMKY	20	2	240	1274.26	64202.78	102.52	0
30	VMKY	20	3	240	1192.36	60042.46	117.18	0
31	VMKY	20	1	239.81	2275.98	45307.47	103.7	0
32	VMKY	20	2	240	1648.95	52391.47	100.55	0
33	VMKY	20	3	240	419.02	43527.53	203.7	0
34	SHR	Saline	1	240	2748.53	58890.28	-20.98	19
35	SHR	Saline	2	240	2287.28	63205.08	-21.32	6
36	SHR	Saline	3	240	1889.55	67440.71	-33.88	12
37	SHR	Saline	1	240	2975.54	59152.5	-20.26	10
38	SHR	Saline	2	240	2163.22	59305.8	-21.89	12
39	SHR	Saline	3	240	1763.58	63710.42	-34.18	9
40	SHR	Saline	1	240	3107.54	59643.5	-25.83	15
41	SHR	Saline	2	240	2654.42	63004.46	-17.73	22
42	SHR	Saline	3	240	1915.61	67538.4	-34.8	9
43	SHR	Saline	1	240	2726.94	57889.35	-15.86	10
44	SHR	Saline	2	240	2875.99	61040.88	-24.45	8
45	SHR	Saline	3	240	1276.49	60484.37	-41.89	1
46	SHR	Saline	1	240	2440.55	55292.28	-18.76	8
47	SHR	Saline	2	240	2024.43	63311.65	-36.26	29
48	SHR	Saline	3	240	1876.35	67207.42	-36.42	13
49	SHR	Saline	1	240	3265.75	53419.59	-17.84	13
50	SHR	Saline	2	240	1903.44	67274.4	-32.57	19
51	SHR	Saline	3	240	1788.32	63937.47	-50.43	17
52	SHR	Saline	1	240	2924.28	54611.78	-.11	14
53	SHR	Saline	2	240	2375.42	63233.85	-18.45	14
54	SHR	Saline	3	240	1469.39	61297.99	-41.87	14
55	SHR	Saline	1	240	3773.62	50900.8	-12.5	19
56	SHR	Saline	2	240	2783.53	65334.58	-23.88	21
57	SHR	Saline	3	240	2038.45	68363.34	-31.67	19
58	VMKY	Saline	1	240	276.95	38318.43	-98.9	0
59	VMKY	Saline	2	240	885.25	48782.21	-64.57	0
60	VMKY	Saline	3	240	1038.22	46723.23	-53.2	0
61	VMKY	Saline	1	240	1132.7	46986.52	-36.48	0
62	VMKY	Saline	2	240	1551.17	48182.65	-40.77	1
63	VMKY	Saline	3	240	233.68	45043.23	-123.9	0
64	VMKY	Saline	1	240.58	2100.43	47399.66	-37.95	5
65	VMKY	Saline	2	240	659.62	45679.17	-58.29	1
66	VMKY	Saline	3	240	463.16	32218.07	-100.04	0
67	VMKY	Saline	1	240	1932.4	52398.92	-27.71	1
68	VMKY	Saline	2	240	960.77	54622.07	-72.86	2
69	VMKY	Saline	3	240	264.6	56939.49	-132.45	0
70	VMKY	Saline	1	240	309.87	48952.11	-133.7	0
71	VMKY	Saline	2	240	281.53	46380.08	-129.14	0
72	VMKY	Saline	3	240	176.21	44910	-200.16	0
73	VMKY	Saline	1	240	1717.12	47929.58	-16.83	0
74	VMKY	Saline	2	240	1215.57	55168.92	-47.42	1
75	VMKY	Saline	3	240	370.99	54022.45	-119.6	1
76	VMKY	Saline	1	240	2473.73	54135.69	-25.48	2
77	VMKY	Saline	2	240	2236.46	60679.52	-22.83	2
78	VMKY	Saline	3	240	1546.75	62366.71	-50.23	4
79	VMKY	Saline	1	240	2175.65	42381.72	-16.44	4
80	VMKY	Saline	2	240	1816.01	54822.81	-31.26	5
81	VMKY	Saline	3	240	1016.7	51607.86	-56.38	2

	1 Strain	2 Ket	3 Bin	4 Time	5 Distance	6 TurnTotal	7 Meander	8 Rearing
1	SD	Saline	1	240	2992.23	56094.8	-25.46	8
2	SD	Saline	2	240	2386.71	64882.15	-23.07	11
3	SD	Saline	3	240	2179.61	72251.07	-55.58	12
4	SD	Saline	1	240	3983.83	54821.57	-8.51	11
5	SD	Saline	2	240	2699.1	68998.96	-22.55	16
6	SD	Saline	3	240	2601.58	67049.71	-82.13	10
7	SD	Saline	1	240	3439.07	50959.67	-11.05	3
8	SD	Saline	2	240	3871.2	61515.7	-25.02	5
9	SD	Saline	3	240	3142.25	62354.34	-20.51	8
10	SD	Saline	1	240	2775.39	58229.25	-11.75	6
11	SD	Saline	2	240	1968.32	65255.36	-24.77	2
12	SD	Saline	3	240	1629.99	67223.73	-116.76	6
13	SD	Saline	1	240	3049.36	53638.26	-15.91	7
14	SD	Saline	2	240	2591.1	62600.85	-19.66	6
15	SD	Saline	3	240	1643.96	74098	-33.88	12
16	SD	Saline	1	240	3998.51	44047.14	-16.66	3
17	SD	Saline	2	240	3025.8	67404.68	-16.61	6
18	SD	Saline	3	240	2313.76	67796.13	-19.01	13
19	SD	Saline	1	240	3327.76	47262.27	-10.54	3
20	SD	Saline	2	240	2575.46	60402.36	-22.19	12
21	SD	Saline	3	240	1792.5	67331.13	-36.6	9
22	SD	Saline	1	240	4237.1	51705.51	-14	6
23	SD	Saline	2	240	2792.25	56780.32	-21.45	3
24	SD	Saline	3	240	1997.76	64075.84	-26.43	8
25	SD	12	1	240	3107.84	61183.21	-30.96	3
26	SD	12	2	240	753.77	61703.35	-121.47	0
27	SD	12	3	240	541.09	48620.91	-98.09	1
28	SD	12	1	240	5629.66	52515.09	-9.86	0
29	SD	12	2	240	2745.18	54205.96	-47.03	0
30	SD	12	3	240	782.44	63350.75	-174.94	0
31	SD	12	1	240	4623.22	56023.69	-39.22	0
32	SD	12	2	240	970.02	60878.69	-132.87	0
33	SD	12	3	240	610.66	49676.7	-115.95	0
34	SD	12	1	240	3695.81	60194.13	-17.83	3
35	SD	12	2	240	1049.3	53800.06	-95.95	0
36	SD	12	3	240	602.25	67064.01	-117.71	2
37	SD	12	1	240	5788.98	41416.47	-8.19	3
38	SD	12	2	240	4024.8	53809.81	-29.49	0
39	SD	12	3	240	817.68	65687.1	-102.85	2
40	SD	12	1	240	5888.87	40512.25	-4.14	0
41	SD	12	2	240	2562.28	50685.1	-321.61	0
42	SD	12	3	240	839.8	66801.36	-130.76	0
43	SD	12	1	240	6544.05	40681.98	-1.67	2
44	SD	12	2	240	2599.7	63841.44	-41.68	0
45	SD	12	3	240	205.14	37590.42	-155.79	0
46	SD	20	1	240	5473.31	50012.27	-3.35	1
47	SD	20	2	240	6360.23	48842.25	-13.51	0
48	SD	20	3	240	3114.4	51486.37	-63.82	0
49	SD	20	1	240	3168.03	68482.02	-18.51	0
50	SD	20	2	240	4962.26	55060.16	-1.68	0
51	SD	20	3	240	2027.86	66590.55	-40.66	0
52	SD	20	1	240	6006.69	61612.22	-6.99	2
53	SD	20	2	240	7397.09	50611.2	-5.28	0
54	SD	20	3	240	5306.98	56330.64	-16.08	0
55	SD	20	1	240	4535.51	48082.55	-4.13	1
56	SD	20	2	240	5380.65	40712.09	-10.34	0
57	SD	20	3	240	2405.94	57484.49	-51.8	0
58	SD	20	1	240	3659.54	64766.99	-9.75	0
59	SD	20	2	240	5431.38	45989.64	-8.83	0
60	SD	20	3	240	4317.73	58423.76	-24.27	0
61	SD	20	1	240	3566.83	51126.97	-5.14	0
62	SD	20	2	240	1863.91	70834.4	-55.33	0
63	SD	20	3	240	772.56	67983.37	-93.89	0
64	SD	20	1	240	2743.86	81463.12	-12.33	0
65	SD	20	2	240	3217.34	67040.66	-13.54	0
66	SD	20	3	240	1278.78	57292.51	-91.72	0

Table B.23: Statistica output for non-parametric ANOVA, Fig. 3.17 on page 57, Distance travelled in 5 min bins

Multiple Comparisons p values (2-tailed); Distance (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =16.78500 p =.0002
Include condition: Ket="Saline" AND Bin=1

Depend.: Distance	SHR R:14.000	WKY R:4.6250	SD R:18.875
SHR		0.024030	0.503815
WKY	0.024030		0.000167
SD	0.503815	0.000167	

Saline, 0-5 min

Multiple Comparisons p values (2-tailed); Distance (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =14.48000 p =.0007
Include condition: Ket="Saline" AND Bin=2

Depend.: Distance	SHR R:14.500	WKY R:5.0000	SD R:18.000
SHR		0.021629	0.966596
WKY	0.021629		0.000708
SD	0.966596	0.000708	

Saline, 5- 10 min

Multiple Comparisons p values (2-tailed); Distance (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =15.69500 p =.0004
Include condition: Ket="Saline" AND Bin=3

Depend.: Distance	SHR R:14.375	WKY R:4.7500	SD R:18.375
SHR		0.019445	0.773697
WKY	0.019445		0.000349
SD	0.773697	0.000349	

Saline, 10-15 min

Multiple Comparisons p values (2-tailed); Distance (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =17.06788 p =.0002
Include condition: Ket=12 AND Bin=1

Depend.: Distance	SHR R:18.750	WKY R:6.1000	SD R:21.286
SHR		0.001563	1.000000
WKY	0.001563		0.000887
SD	1.000000	0.000887	

12 mg/kg, 0-5 min

Multiple Comparisons p values (2-tailed); Distance (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =9.601051 p =.0082
Include condition: Ket=12 AND Bin=2

Depend.: Distance	SHR R:20.333	WKY R:13.400	SD R:8.1429
SHR		0.171613	0.007829
WKY	0.171613		0.630761
SD	0.007829	0.630761	

12 mg/kg, 5- 10 min

Multiple Comparisons p values (2-tailed); Distance (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =16.68381 p =.0002
Include condition: Ket=12 AND Bin=3

Depend.: Distance	SHR R:22.667	WKY R:10.100	SD R:8.8571
SHR		0.001701	0.001948
WKY	0.001701		1.000000
SD	0.001948	1.000000	

12 mg/kg 10 - 15 min

Multiple Comparisons p values (2-tailed); Distance (Ketamine Open Field
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =15.58993 p =.0004
Include condition: Ket=20 AND Bin=1

Depend.: Distance	SHR R:19.615	WKY R:7.4545	SD R:22.714
SHR		0.003286	1.000000
WKY	0.003286		0.001554
SD	1.000000	0.001554	

20 mg/kg, 0 - 5 min

Multiple Comparisons p values (2-tailed); Distance (Ketamine Open Field by Bins)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =13.62645 p =.0011
Include condition: Ket=20 AND Bin=2

Depend.: Distance	SHR R:19.923	WKY R:7.9091	SD R:21.429
SHR		0.003774	1.000000
WKY	0.003774		0.006306
SD	1.000000	0.006306	

20 mg/kg, 5 - 10 min

Multiple Comparisons p values (2-tailed); Distance (Ketamine Open Field by Bins)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =11.70982 p =.0029
Include condition: Ket=20 AND Bin=3

Depend.: Distance	SHR R:21.923	WKY R:9.1818	SD R:15.714
SHR		0.001874	0.435660
WKY	0.001874		0.411831
SD	0.435660	0.411831	

20 mg/kg, 10 - 15 min

Table B.24: Statistica output for non-parametric ANOVA, Fig. 3.18 on page 58, Meandering in 5 min bins

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =11.18000 p =.0037
Include condition: Ket="Saline" AND Bin=1

Depend.: Meander	SHR R:13.250	WKY R:6.2500	SD R:18.000
SHR		0.143145	0.537328
WKY	0.143145		0.002668
SD	0.537328	0.002668	

Saline, 0 - 5 min

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =11.44500 p =.0033
Include condition: Ket="Saline" AND Bin=2

Depend.: Meander	SHR R:15.375	WKY R:5.6250	SD R:16.500
SHR		0.017462	1.000000
WKY	0.017462		0.006296
SD	1.000000	0.006296	

Saline, 5 - 10 min

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =10.97102 p =.0041
Include condition: Ket="Saline" AND Bin=3

Depend.: Meander	SHR R:16.188	WKY R:5.7500	SD R:15.563
SHR		0.009466	1.000000
WKY	0.009466		0.016540
SD	1.000000	0.016540	

Saline, 10 - 15 min

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =19.65080 p =.0001
Include condition: Ket=12 AND Bin=1

Depend.: Meander	SHR R:15.083	WKY R:22.600	SD R:4.0000
SHR		0.117695	0.018604
WKY	0.117695		0.000028
SD	0.018604	0.000028	

12 mg/kg, 0 - 5 min

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =16.62391 p =.0002
Include condition: Ket=12 AND Bin=2

Depend.: Meander	SHR R:16.667	WKY R:20.700	SD R:4.0000
SHR		0.805785	0.005281
WKY	0.805785		0.000207
SD	0.005281	0.000207	

12 mg/kg, 5 - 10 min

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =19.65080 p =.0001
Include condition: Ket=12 AND Bin=3

Depend.: Meander	SHR R:15.083	WKY R:22.600	SD R:4.0000
SHR		0.117695	0.018604
WKY	0.117695		0.000028
SD	0.018604	0.000028	

12 mg/kg, 10 - 15 min

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =20.72457 p =.0000
Include condition: Ket=20 AND Bin=1

Depend.: Meander	SHR R:15.692	WKY R:24.000	SD R:4.0000
SHR		0.077168	0.018259
WKY	0.077168		0.000016
SD	0.018259	0.000016	

20 mg/kg, 0 - 5 min

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =19.95308 p =.0000
Include condition: Ket=20 AND Bin=2

Depend.: Meander	SHR R:16.000	WKY R:23.636	SD R:4.0000
SHR		0.121052	0.014620
WKY	0.121052		0.000024
SD	0.014620	0.000024	

20 mg/kg, 5 - 10 min

Multiple Comparisons p values (2-tailed); Meander (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =18.75931 p =.0001
Include condition: Ket=20 AND Bin=3

Depend.: Meander	SHR R:16.538	WKY R:23.000	SD R:4.0000
SHR		0.248363	0.009795
WKY	0.248363		0.000046
SD	0.009795	0.000046	

20 mg/kg, 10 - 15 min

Table B.25: Statistica output for non-parametric ANOVA, Fig. 3.19 on page 59, Rearing in 5 min bins

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =17.71489 p =.0001
Include condition: Ket="Saline" AND Bin=1

Depend.: Rearing	SHR R:20.063	WKY R:5.2500	SD R:12.188
SHR		0.000084	0.077764
WKY	0.000084		0.149210
SD	0.077764	0.149210	

Saline, 0 - 5 min

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =16.77566 p =.0002
Include condition: Ket="Saline" AND Bin=2

Depend.: Rearing	SHR R:19.313	WKY R:4.9375	SD R:13.250
SHR		0.000144	0.259182
WKY	0.000144		0.056149
SD	0.259182	0.056149	

Saline, 5 - 10 min

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 24) =15.01868 p =.0005
Include condition: Ket="Saline" AND Bin=3

Depend.: Rearing	SHR R:17.750	WKY R:4.8125	SD R:14.938
SHR		0.000759	1.000000
WKY	0.000759		0.012558
SD	1.000000	0.012558	

Saline, 10 - 15 min

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =10.97591 p =.0041
Include condition: Ket=12 AND Bin=1

Depend.: Rearing	SHR R:20.000	WKY R:8.8500	SD R:15.214
SHR		0.006677	0.711866
WKY	0.006677		0.388013
SD	0.711866	0.388013	

12 mg/kg, 0 - 5 min

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =22.80131 p =.0000
Include condition: Ket=12 AND Bin=2

Depend.: Rearing	SHR R:23.292	WKY R:9.9500	SD R:8.0000
SHR		0.000758	0.000478
WKY	0.000758		1.000000
SD	0.000478	1.000000	

12 mg/kg, 5 - 10 min

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 29) =17.35739 p =.0002
Include condition: Ket=12 AND Bin=3

Depend.: Rearing	SHR R:22.333	WKY R:8.6000	SD R:11.571
SHR		0.000496	0.023613
WKY	0.000496		1.000000
SD	0.023613	1.000000	

12 mg/kg, 10 - 15 min

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =12.79858 p =.0017
Include condition: Ket=20 AND Bin=1

Depend.: Rearing	SHR R:21.615	WKY R:10.000	SD R:15.000
SHR		0.005455	0.361977
WKY	0.005455		0.766111
SD	0.361977	0.766111	

20 mg/kg, 0 - 5 min

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =17.03736 p =.0002
Include condition: Ket=20 AND Bin=2

Depend.: Rearing	SHR R:22.769	WKY R:11.500	SD R:10.500
SHR		0.007448	0.011989
WKY	0.007448		1.000000
SD	0.011989	1.000000	

20 mg/kg, 5 - 10 min

Multiple Comparisons p values (2-tailed); Rearing (Spreadsheet1)
Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 31) =14.05394 p =.0009
Include condition: Ket=20 AND Bin=3

Depend.: Rearing	SHR R:21.538	WKY R:12.000	SD R:12.000
SHR		0.031330	0.075705
WKY	0.031330		1.000000
SD	0.075705	1.000000	

20 mg/kg, 10 - 15 min

Table B.26: Correlation of Total distance travelled and rearing in the OF, Fig.3.20 and 3.21 on page 60

	Spearman Rank Order Correlations (Ketamine) MD pairwise deleted Marked correlations are significant at p <.05000	
Variable	Distance	Rearing
Distance	1.000000	0.229993
Rearing	0.229993	1.000000

All dosages

	Spearman Rank Order Correlations (Ketamine date as number) MD pairwise deleted Marked correlations are significant at p <.05000 Include cases: 47:70	
Variable	Distance	Rearing
Distance	1.000000	0.639983
Rearing	0.639983	1.000000

Saline

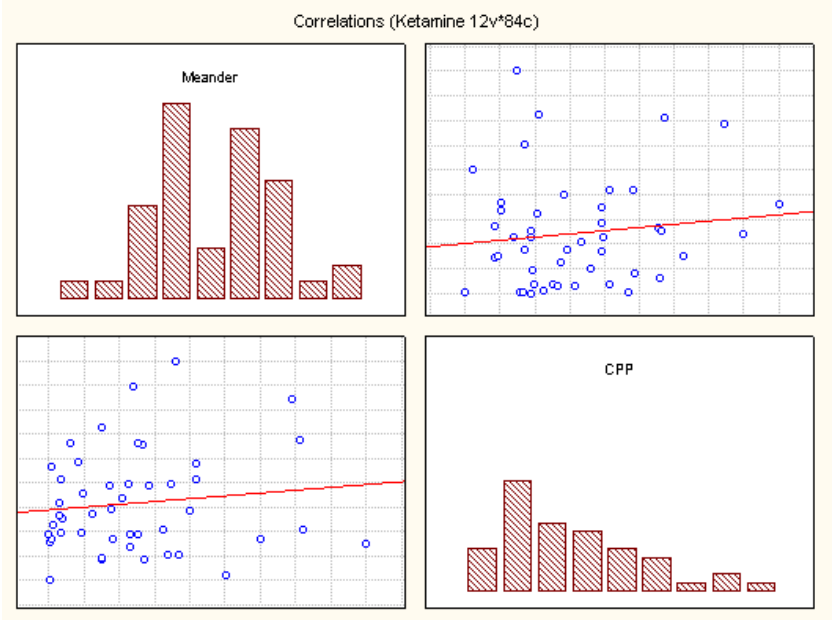
	Spearman Rank Order Correlations (Ketamine date as number) MD pairwise deleted Marked correlations are significant at p <.05000 Include cases: 1:77 Exclude cases: 23:70	
Variable	Distance	Rearing
Distance	1.000000	0.446528
Rearing	0.446528	1.000000

12 mg/kg

	Spearman Rank Order Correlations (Ketamine date as number) MD pairwise deleted Marked correlations are significant at p <.05000 Include cases: 23:84 Exclude cases: 47:77	
Variable	Distance	Rearing
Distance	1.000000	0.422649
Rearing	0.422649	1.000000

20 mg/kg

Table B.27: Correlation of Total distance travelled and meandering in the OF in 3.1.3 on page 48



Variable	Spearman Rank Order Correlations (Ketamine) MD pairwise deleted Marked correlations are significant at p <.05000 TurnsTotal	Meander
TurnsTotal	1.000000	-0.398279
Meander	-0.398279	1.000000

All dosages

Table B.28: Spreadsheet for average consumtion of SHR and WKY in Fig. 3.3 on page 63, OSA

Day	Total Liquid WKY			Total Liquid SHR		
	Average	SD	N	Average	SD	N
1	41.8667	14.4769	12	33.75	13.1083	12
2	35.8333	10.7145	12	27.8	5.29614	12
3	36.3417	9.16579	12	31.9	5.88295	12
4	37.8417	14.7866	12	31.2583	6.11934	12
5	32.3083	10.8972	12	28.475	8.25559	12
6	33.8167	4.82245	12	29.7833	6.20335	12
7	30.225	4.24759	12	28.525	5.01527	12
8	30.4917	5.44367	12	27.4083	5.73371	12
9	33.2833	3.92587	12	28.9583	4.37107	12
10	29.325	3.89198	12	29.8417	3.69802	12
11	31.225	4.95858	12	28.775	6.76665	12
12	32.4	5.78446	12	27.2667	4.29679	12
13	29.9083	6.34887	12	27.5833	4.17522	12
14	30.9	7.64056	12	29.325	2.09854	12
15	33.1333	8.90356	12	27.4333	4.74731	12
16	29.35	3.53952	12	26.4333	4.55259	12
17	30.9083	5.85405	12	20.95	10.3709	12
18	27.475	4.72539	12	26.25	4.38955	12
19	28.7167	4.36512	12	26.2583	5.68914	12
20	29.3833	4.03301	12	23.7917	7.63419	12
21	29.2083	5.47415	12	26.875	4.51807	12

Day	Water WKY			Water SHR		
	Average	SD	N	Average	SD	N
1	29.6583	9.17422	12	22.7667	10.8258	12
2	26.85	6.51006	12	22.9417	7.01079	12
3	28.7333	3.90792	12	25.4833	6.06392	12
4	29.2583	6.56137	12	22.1583	10.4399	12
5	27.35	8.01457	12	21.35	7.19277	12
6	30.1667	5.88022	12	20.9417	6.41153	12
7	28.35	5.37627	12	23.2333	6.03948	12
8	28.7917	6.72365	12	24.5167	5.10156	12
9	30.8667	4.53568	12	25.125	5.58735	12
10	25.0167	5.97912	12	25.475	4.2508	12
11	29.175	5.93748	12	26.3083	6.53629	12
12	30.5083	6.81204	12	23.75	4.01602	12
13	28.0833	6.90983	12	24.65	4.69325	12
14	28.2167	8.6485	12	26.0417	2.49141	12
15	31.5	8.01814	12	24.9167	4.84326	12
16	27.8667	4.06869	12	24.1333	4.55802	12
17	30.0417	8.01196	12	19.7083	10.0547	12
18	26.5833	4.32936	12	24.9917	4.1561	12
19	27.7583	5.46961	12	25.1583	4.96318	12
20	28.3417	4.81182	12	22.9417	7.43802	12
21	28	4.70289	12	25.5583	4.18792	12

Day	Ketamine WKY			Ketamine SHR		
	Average	SD	N	Average	SD	N
1	13.225	12.6771	12	11.4	10.9353	12
2	9.64167	13.5536	12	4.775	3.66552	12
3	6.98333	9.34693	12	7.35	5.34917	12
4	6.475	15.1005	12	8.5875	11.2565	12
5	7.025	5.70382	12	8.4125	5.8225	12
6	4.125	4.17513	12	7.525	7.26033	12
7	10.35	9.58212	12	2.525	3.96622	12
8	2.65	1.23865	12	12.5	5.86869	12
9	2.85	0.689477	12	8.3	3.0637	12
10	1.45	4.01969	12	2.15	3.3789	12
11	6.2	2.61781	12	2.85	1.4536	12
12	7.6	3.6863	12	2.15	1.8387	12
13	1.85	0.808478	12	1.6	1.74692	12
14	2.25	2.49998	12	5.2	2.35003	12
15	1.7	0.628792	12	3.05	1.20488	12
16	3.1	0.944682	12	5.05	1.88912	12
17	0.65	0.274552	12	0.4	0.894893	12
18	1.1	0.430908	12	0.95	0.917961	12
19	0.8	0.210878	12	1	0.657129	12
20	1.2	0.448144	12	1.05	0.480766	12
21	1.1	0.719638	12	0.7	0.294906	12