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**The Influence of Housing, Sex and Strain on Baseline and
Drug-Induced Behavioural and Neurochemical Parameters
in the Rat**

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September 2011

I hereby declare that this thesis is my own work and effort and that it has not been submitted elsewhere for any award.

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Abstract

A number of confounding factors are believed to affect both basal rodent behaviour and responsivity to psychotropic drugs. To date, few detailed comparative assessments of these confounds have been made. This thesis examined three such factors, namely, *housing conditions*, i.e. isolation (IC) and environmental enrichment (EE) compared to standard group conditions (SC); *sex* (males compared to females); and *strain* i.e. Wistar and Lewis compared to Sprague Dawley (SD) strain. Behavioural investigations involved the open field test (OFT), homecage activity (HCA), Morris water maze (MWM), elevated plus maze (EPM) and forced swim test (FST). The anxiolytic effects of diazepam (DZP), antidepressant effects of desipramine (DMI) and locomotor stimulant effects of amphetamines were assessed in the EPM, FST and HCA apparatus respectively. In addition, post-mortem brain monoamine levels were assessed. OFT, homecage, EPM and FST behaviours were unaffected by housing conditions or by strain; female rats were more active in all tests and had greater percent open arm entries in the EPM than males. Latencies in the MWM were not altered. EE had few effects on monoamine levels while IC housing reduced noradrenaline (NA), dopamine (DA) and serotonin (5HT) concentrations in the brain when compared to SC controls. Female rats had reduced levels of NA, 5HT and 5-hydroxyindoleacetic acid (5HIAA) compared to males; this was somewhat dependent on housing conditions however as sex differences were primarily observed in SC- and EE- reared rats. Lewis rats had significantly greater NA, 5HIAA and 5HT turnover in the hippocampus compared to SD and Wistar rat strains. The anxiolytic effects of diazepam in the EPM were blunted following EE rearing and in female rats but enhanced in the Lewis strain. Similarly, antidepressant effects of DMI were blunted for rats reared in social groups and in female rats. Finally the effects of stimulant drugs were enhanced by social housing conditions, in female rats and the Lewis strain; while stimulant effects were blunted in IC-housed and Wistar rats. In summary, baseline behaviour was most affected by sex differences, whilst psychotropic drug responses were affected by IC and EE housing, sex and strain. Monoamine levels differed according to housing conditions, sex and, to lesser extent, strain. These effects highlight the sensitivity of commonly-employed behavioural pharmacology tests to such variables. These findings stress the

importance of appropriate subject selection in the design of behavioural and neurochemical studies.

This thesis is dedicated to my parents George and Dervilla,
and in loving memory of Margaret.

“You don't always have to pretend to be strong, there is no need to prove all the time that everything is going well, you shouldn't be concerned about what other people are thinking...Cry if you need to, it's good to cry out all your tears, because only then you will be able to smile again...”

— Paulo Coelho, *Like the Flowing River*

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- Simpson J. & Kelly, J. P. (2010). The impact of housing conditions on behavioural responses to certain psychotropic drugs in the rat. *J Psychopharmacol*, **24** (supplement 3), A41. Presented at British Association for Psychopharmacology Summer Meeting, July 2010
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Glossary of Abbreviations

ACREC	Animal care and research ethics committee
ACTH	Adrenocorticotrophic hormone
ADHD	Attention deficit/hyperactivity disorder
AMP	Amphetamine
ANOVA	Analysis of variance
APO	Apomorphine
ASR	Acoustic startle reflex
BCA	Bicinchoninic acid
BDNF	Brain-derived neurotrophic factor
BN	Brown Norway
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CAST	Castration
CMS	Chronic mild stress
CNS	Central nervous system
COC	Cocaine
CONC	Concentration
CORT	Corticosterone
CPP	Conditioned place preference
CREB	cAMP response-binding element
CRF	Corticotrophin-releasing factor
CS	Conditioned stimulus
Cu	Copper
DA	Dopamine
DAT	Dopamine transporter
DCX	Doublecortin
DMI	Desipramine
DOPAC	3,4-dihydroxyphenylacetic acid
DZP	Diazepam
EE	Environmental enrichment
ELISA	Enzyme-linked immunosorbent assay

EP	Estradiol progesterone combination
EPM	Elevated plus maze
EPSP	Excitatory postsynaptic potential
FR	Fixed ratio
FRL	Flinders resistant line
FSL	Flinders sensitive line
FST	Forced swim test
GABA	Gamma-aminobutyric acid
GDNF	Glial-derived neurotrophic factor
GH	Genetically hypertensive
HCA	Homecage activity
HCM	Homecage monitoring
HCMA	Homecage monitoring apparatus
HPA	Hypothalamic pituitary adrenal
HPLC	High performance liquid chromatography
HRP	Horseradish-peroxidase
HVA	Homovanillic acid
IC	Isolated conditions
IL-1 β	Interleukin 1 beta
IS	Internal standard
ITI	Inter-trial interval
L-DOPA	L-3-4-dihydroxyphenylalanine
Lew	Lewis
LTD	Long term depression
LTP	Long term potentiation
MA	Methamphetamine
MAM	Methylazoxymethanol
mBDNF	Mature brain-derived neurotrophic factor
MS	Medial Septum
MWM	Morris water maze
NA	Noradrenaline
NaCl	Sodium Chloride
NGF	Nerve growth factor

N-methyl-5HT	N-methyl-5-hydroxytryptamine
NOR	Novel object recognition
NOS	Nitric oxide synthase
NT	Neurotransmitter
NT ₃	Neurotrophin 3
NT ₄	Neurotrophin 4
OFT	Open field test
OVX	Ovariectomised
PBS	Phosphate buffered saline
PFC	Prefrontal cortex
PH	Peak height
PND	Postnatal day
PR	Progressive ratio
PS	Prenatal stress
PSA-NCAM	Poly-sialated neural cell adhesion molecule
PSS	Predatory scent stress
RAM	Radial arm maze
RRF	Relative retention factor
SC	Standard group conditions
SD	Sprague Dawley
SEM	Standard error of the mean
SGZ	Subgranular zone
SHR	Spontaneously hypertensive rat
SNK	Student Newman Keuls
SPSS	Statistical package for the social sciences
SSRI	Selective serotonin reuptake inhibitor
SW	South west
TMB	3,3, 5-5 tetramethylbenzidine
Trk	Tyrosine kinase
UCS	Unconditioned stimulus
VTA	Ventral tegmental area
Wis	Wistar
WKY	Wistar Kyoto

5HIAA	5-hydroxyindoleacetic acid
5HT	Serotonin
5HTT	Serotonin transporter

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Chapter 1

General Introduction

Chapter 1

General Introduction

Animal models have been defined by van der Staay (2006) as

“A living organism used to study brain–behaviour relations under controlled conditions, with the final goal being to gain insight into, and to enable predictions about, these relations in humans and/or a species other than the one studied, or in the same species under conditions different from those under which the study was performed”

Animal models are employed in most areas of medical and scientific research and are accepted as the primary means of investigating disease states and potential treatments (Suckow et al., 2006). In accordance, all potential new drugs must undergo preclinical trials in rodent models to demonstrate features of safety and efficacy (Rang, 2005). The overall aim of using animals in research is based on the ability to model both normal and abnormal behaviours, thus allowing investigation of the brain-behaviour interactions and correlates of both states (van der Staay, 2006). Despite their extensive use, the predictive value of animal models in some disease research however remains controversial due to issues associated with poor internal and external validity; internal validity refers to the elimination of bias through experimental design and conduct, external validity refers to the extent to which the results of animal studies may be correctly generalised to human conditions (van der Worp et al., 2010).

Compared with other laboratory procedures, behavioural analysis can be difficult to standardise and interpret. Numerous factors can influence behaviour and its measurement; these have been classified as ‘trait’, ‘state’ and ‘technical factors’ (Wotjak, 2004, Sousa et al., 2006). Trait factors refer to genetic and developmental determinants such as sex, genetic makeup, maternal care, housing conditions and handling procedures. State factors include the time of testing, characteristics of the experimental setting, experimenter factors such as experience or bias, and animal characteristics such as general health or estrous cycle stage. Technical factors include the method of behaviour analysis, for example manual or automated scoring, the parameters of interest and data analysis (Sousa et al., 2006).

This thesis focuses on the analysis of rat behaviour and the effects of three ‘trait’ factors, namely housing conditions, sex and strain. Baseline activity and drug-induced responses are tested in a number of well-characterised tests which are commonly-employed in behavioural pharmacology research. Some of the behavioural tests frequently referred to in this thesis are summarised in table 1.1.

Table 1.1. Tests commonly employed in behavioural pharmacology

Test	Endpoints	Parameters		Reference
Open Field Test (OFT)	Exploration, activity and emotionality	Distance moved, grooming, rearing. Time spent in centre/centre crossings, defecation	Avoidance of the centre and high defecation scores indicate anxiety	(Gould et al., 2009)
Elevated Plus Maze (EPM)	Anxiety	Open arm time, open arm entries, distance moved, rearing, freezing, defecation	Fewer open arm time and entries, and increased faecal boli indicate anxiety	(Walf and Frye, 2007)
Forced Swim Test (FST)	Screen for antidepressant compounds, stress response	Time spent immobile, swimming and climbing	Increased time spent immobile/ decreased swimming and climbing indicate behavioural despair	(Porsolt et al., 1978)
Social Interaction Test	Anxiety	Time spent interacting with social partner	Reduced sniffing, following or grooming the partner indicates anxiety	(File and Seth, 2003)
Holeboard Test	Exploration of novelty, anxiety	Locomotor activity, exploring novel environment/objects	High levels of head-dipping indicates exploratory behaviour and reduced anxiety	(Casarrubea et al., 2009)
Morris Water Maze (MWM)	Spatial learning and memory	Latency to find the escape platform, time spent in the target quadrant, swim speed and path length.	Reduced latencies and greater time spent in the target quadrant indicate better spatial learning and memory	(Morris, 1984)
Novel Object Recognition (NOR)	Recognition memory	Time spent interacting with the novel object compared to the familiar object	Greater time spent with the novel object indicates better recognition memory	(Ennaceur and Delacour, 1988)
Radial Arm Maze (RAM)	Spatial working and reference memory	Number of correct and incorrect arm choices, time taken to finish trial	Entries into unbaited arms are reference memory errors; re-entries into baited arms are working memory errors.	(Olton, 1987)

1.1 Environmental Enrichment Effects

Environmental enrichment (EE) is a term for exposing laboratory animals to physical and/or social stimulation that is greater than they would receive under standard housing conditions (Rosenzweig and Bennett, 1996). EE can be classified into two types; physical and social enrichment. *Physical enrichment* strategies involve structural modifications, including increased floor space and the inclusion of features which allow exercise, play, exploration and permit animals some control over their environment (Stewart and Bayne, 2004); these include (in the case of rats) bedding enhanced with natural materials (paper parchment, fibre-based bedding), plastic tunnels, wooden objects to gnaw, ropes, swings, running wheels, balls, ramps, ladders and other appropriately-sized animal toys. *Social enrichment* on the other hand refers to housing social animals in groups wherever possible. Social enrichment is relatively easy to implement as cagemate(s) provide constant dynamic interaction and unpredictability, there are however situations where animals must be housed alone and in such cases physical enrichment is particularly useful. Ideally, a combination of both social and physical enrichment elements are thought to be preferable (Johansson and Ohlsson, 1996). Over the past number of decades there has been a growing obligation and legislation for the widespread implementation of EE policies in animal laboratories. The most recent EU directive for the protection of animals used for scientific purposes in 2010 recommends that laboratory animals be housed in social groups where appropriate and provided with enrichment to promote exercise, foraging, manipulative and cognitive ability (Directive 2010/63/EU of the European Parliament).

There has been growing interest in the area of EE research during the past number of years. In drafting a literature review, the search terms “Environmental enrichment, rats” were entered into the PubMed search engine and the results yielded 536 articles. After careful review, the article list was filtered to leave only 361 articles addressing the effects of environmental enrichment in rats. The number of articles and decade of publication between 1971 and 2010 are presented in figure 1.1. Immediately the intensity of the research field of EE is evident, particularly over the last decade. The steady growth of EE as a discipline since the 1970s is due to growing evidence that rodents reared in enriched conditions display a range of plastic responses in the brain including neurogenesis, increased dendritic branching,

increased cell size and improvements in learning and memory (Mohammed et al., 2002, Petrosini et al., 2009, Sale et al., 2009), which have implications for recovery in animal models of neurodegenerative disease, brain injury and psychiatric disorders (Will et al., 2004, van Praag et al., 2000, Nithianantharajah and Hannan, 2006, Laviola et al., 2008, Solinas et al., 2010). In such studies EE is introduced as an experimental variable and can contribute to assessing the external validity and robustness of various models as they are tested across a range of environmental conditions (Wurbel, 2002). The use of EE as an experimental variable however requires standardisation of procedures and outcome measures if conclusions are to be valid and not simply an artefact of a particular laboratory (Burke et al., 2007).

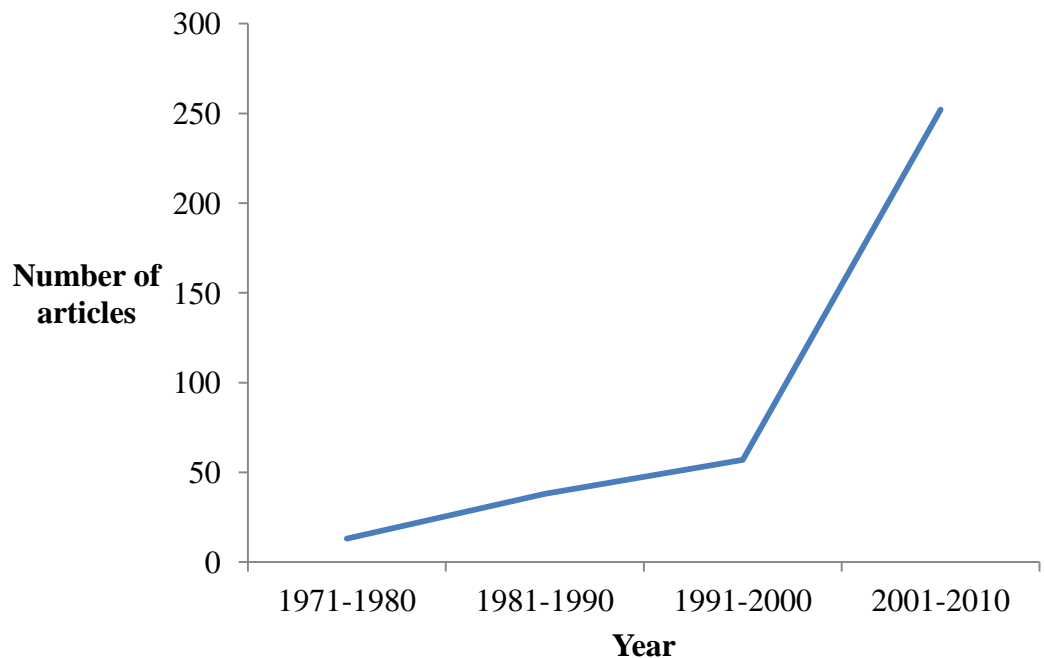


Figure 1.1: Number of articles returned when “Environmental Enrichment, Rats” was entered into the PubMed search engine. Between 1971 and the end of year 2010, 361 articles were published in this area

1.1.1 How does environmental enrichment vary?

A common theme in the area of EE is its inconsistent nature across scientific literature; furthermore, the definitions of EE can vary greatly according to different authors, as some are vague and overly-general (Newberry, 1995). When compiling a review of EE seven particular variables for consideration when assessing or implementing EEs protocol for rats were identified and outlined. Namely these were cage size and housing density; rat age; duration of EE, the EE protocol and enrichment items employed; rat sex and strain and the use of appropriate controls (Simpson and Kelly, 2011). Of the relevant EE articles searched in PubMed, a selection of 60 of these was selected to identify any consensus in the control groups employed in EE research and in the age of EE onset and its duration. The 60 were selected based on their relevance to behavioural and/or neurochemical consequences of EE housing for rats. They did not include studies of brain or spinal injury or of central nervous system (CNS) diseases; thus studies of EE as a rehabilitative therapy or the use of EE for the reversal of deficits (including prenatal stress and early life experience) were not included in the piecharts in the subsequent sections.

Enrichment cage size

A range of different cage sizes and density of animals have been employed in EE studies. Table 1.2 outlines the cage dimensions and number of inhabitants/cage from a sample of 13 studies; these are representative of the range of cage sizes and housing density found in the literature. The number of rats housed together ranges greatly, from a maximum of 15 to minimum of 2/cage. Such diversity makes it difficult to design and implement a standardised EE protocol, capable of replication across laboratories. Appropriate cage size and space are integral to animal husbandry and familiar cagemates are well tolerated but crowded conditions are aversive (to males) (Brown and Grunberg, 1995). Therefore, when designing experiments, animal density ought to be considered carefully.

Table 1.2. Cage dimensions and housing density in environmental enrichment studies

Cage Dimensions	Area	Number of rats	Animal Density	Reference
200 x 100 x 180 cm	20,000 cm ³	4	5000 cm ³ / subject	(Zimmermann et al., 2001)
35 x 55 x 19 cm	1925 cm ³	4	481 cm ³ / subject	(Zimmermann et al., 2001)
120 x 70 x 100 com	8400 cm ³	15	560 cm ³ / subject	(Brenes et al., 2008)
100 x 50 x 50 cm	5000 cm ³	10	500 cm ³ / subject	(Moncek et al., 2004)
70 x 70 x 45 cm	4900 cm ³	12	408.33 cm ³ / subject	(Carughi et al., 1989)
120 x 100 x 60 cm	12000 cm ³	8	1500 cm ³ / subject	(Segovia et al., 2006)
40 x 25 x 30 cm	1000 cm ³	2	500 cm ³ / subject	(Morley-Fletcher et al., 2003)
60 x 40 x 60 cm	2400 cm ³	6	400 cm ³ / subject	(Imanaka et al., 2006)
100 x 60 x 35 cm	6000 cm ³	8	750 cm ³ / subject	(Andin et al., 2007)
62 x 70 x 75 cm	4340 cm ³	3	1447 cm ³ / subject	(Schrijver et al., 2002)
70 x 50 x 22 cm	3500 cm ³	5	700 cm ³ / subject	(Leal-Galicia et al., 2008)
50 x 98 x 54 cm	4900 cm ³	5	980 cm ³ / subject	(Lores-Arnaiz et al., 2006)
43 x 27 x 19 cm	1161 cm ³	4	290 cm ³ / subject	(Brillaud et al., 2005)

The size of environmental enrichment cages used in the literature varies. The area of the cage / the number of conspecifics housed together allowed an estimation of a range of animal densities employed.

Types of physical objects used in environmental enrichment

The most commonly used physical enrichment objects are listed in table 1.3. Although nest-building is an acquired behaviour for rats (Gonder and Laber, 2007), the inclusion of nesting materials such as shredded paper, tissue paper and commercially-available pet bedding can also allow the rats an amount of control over their environment, as they establish territorial areas within the cage (Van Loo and Baumans, 2004). In most EE protocols the objects are moved around or changed a number of times each week to maintain the novelty of the environment. In addition, food treats or snacks such as cheese or apple pieces may be “hidden” in the cage to encourage foraging behaviour (Stewart and Bayne, 2004) and many studies alternate the position of the food hoppers and water bottles to increase exploratory behaviours in the EE cages. The addition of objects, bedding and structural change to EE cages can vary widely from modest enrichment to the formation of complex environments. Although general themes can be seen across studies (such as toys and supplementary bedding) there is no perceptible consistency in the objects employed.

Table 1.3. Types of physical objects used in environmental enrichment

Non-social enrichment	Examples	References
Structural Modifications	Increased surface area (larger cage) and/or inclusion of platforms to the cage or a 2-level cage	(Imanaka et al., 2006, Magalhaes et al., 2006, Brenes et al., 2009, Segovia et al., 2008, Tall, 2009)
	Use of shelters for hiding places	(Leggio et al., 2005, Tall, 2009, Zimmermann et al., 2001)
Exercise, play and exploration	Running wheels	(Zimmermann et al., 2001, Leggio et al., 2005, Pena et al., 2009a, Qian et al., 2008, Del Arco et al., 2007b)
	Plastic commercially available toys	(Lores-Arnaiz et al., 2006, Del Arco et al., 2007a, Segovia et al., 2008, Sparling et al., 2010, Pena et al., 2009a, Qian et al., 2008)
	Bells / chimes	(Varty et al., 2000, Sparling et al., 2010)
	Wooden chews and toys	(Andin et al., 2007, Brenes et al., 2009, Qian et al., 2008, Rahman and Bardo, 2008, Pena et al., 2009a)
	Swings, ropes, chains	(Magalhaes et al., 2006, Koh et al., 2007, Sparling et al., 2010, Pena et al., 2009a)
	Ladders, ramps	(Moncek et al., 2004, Lores-Arnaiz et al., 2006, Qian et al., 2008)
	Pipes/ tubes/ tunnels	(Magalhaes et al., 2006, Sparling et al., 2010, Harati et al., 2009, Pena et al., 2009a)
Foraging	Nesting material, rearrangement of food and water supply	(Zimmermann et al., 2001, Moncek et al., 2004, Brenes et al., 2008, Sparling et al., 2010)

Age at onset of environmental enrichment

The age at which differential housing is implemented varies considerably across studies, ranging from immediately after weaning to 2 years (Fig. 1.2). However the majority of authors of the surveyed articles commenced EE within the first month of life, most commonly introduced immediately post-weaning, at post-natal day (PND) 21-23. Five to eight week old animals were also favoured over rats aged 9 weeks and older and a minority of studies have looked at EE effects in rats of 1 year and older.

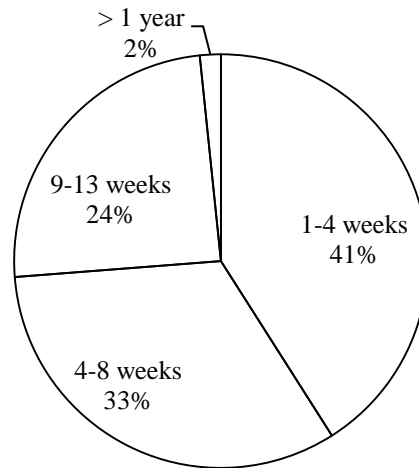


Figure 1.2: Pie chart depicting the age of onset of environmental enrichment for rats

Duration of environmental enrichment

The duration of EE chosen differs across various laboratories (Fig. 1.3). Almost one half of the sample examined employed a protocol of 1-2 months; 1-4 week EE schedules were also commonly used. While a minority have investigated the effects of longer periods of enrichment, most studies (82%) employ protocols of continuous enrichment for anything from 1 to 13 weeks. This however is a wide range, and the housing duration within the 12 weeks seems, in some cases, to be selected arbitrarily.

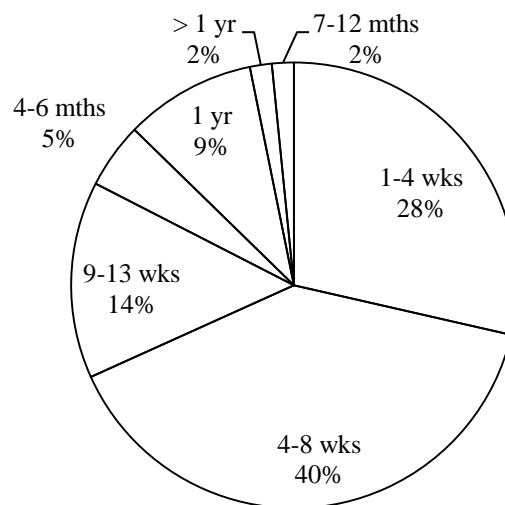


Figure 1.3: Pie chart depicts commonly employed environmental enrichment duration protocols

Rat sex

Male rats are over-represented in the literature in neuroscience research according to a survey carried out by Robert Hughes (Hughes, 2007). Similarly in EE research males are employed more often than females and fewer still include both male and female rats in experiments. The main reason for the omission of females from studies is that their estrous cycle may have effects on behaviour (Frye et al., 2007, Paris and Frye, 2008, Frye et al., 2000); in addition the inclusion of a female group alongside males increases the sample size twofold. As gender differences in overall activity (Fernandes et al., 1999, Pare and Redei, 1993) and in responses to cagemates (Brown and Grunberg, 1995) have been documented, it is important that the effects of housing conditions are assessed for both males and females.

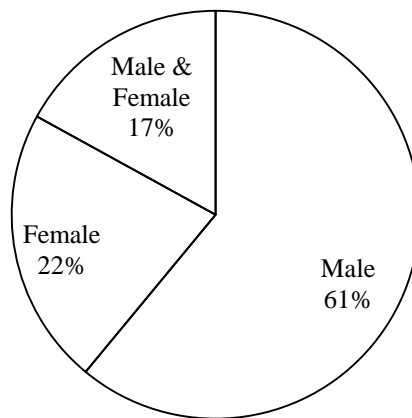


Figure 1.4: Pie chart depicts the gender representation in environmental enrichment research

Rat Strain

Strain and gender are two of the main sources of variations in laboratory animal research. A wide variety of rat strains are now available for behavioural pharmacological research, which may pose problems as inherent differences between the strains may affect baseline, as well as drug-induced, responses in behavioural tests (McDermott and Kelly, 2008) . Figure 1.5 depicts the rat strains typically employed in EE studies. It is apparent that most research has been carried out on the Sprague Dawley (SD) and Wistar rat strains. It may be that SD and Wistar rats are highly represented as these albino lines are widely used in behavioural pharmacology (McDermott and Kelly, 2008). However it is necessary to investigate housing effects in other strains, particularly the non-albino and hooded populations as pigmented and albino rats differ in visual acuity which may affect performance in complex behavioural tasks (Prusky et al., 2002).

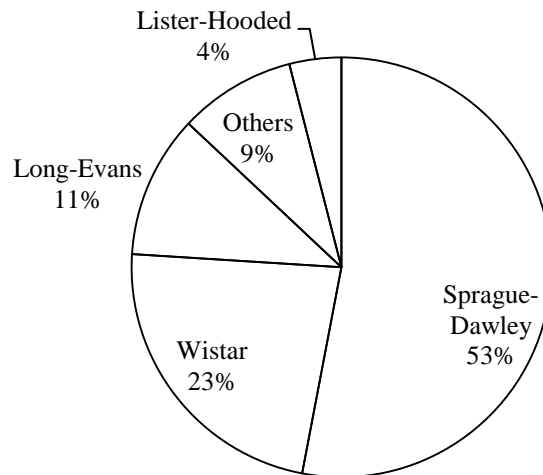


Figure 1.5: Pie chart depicts the commonly employed rat strains in environmental enrichment research

The use of appropriate controls

When evaluating the impact of EE, it is initially important to consider the use of appropriate controls against which comparisons can be made. In general, it is recommended that laboratory animals are housed in social groups; however in cases where this is not amenable to the conditions of particular experiments (e.g. homecage monitoring of individual behaviour, food/water consumption or cannulation) isolation housing is permissible. If one considers the spectrum of housing conditions, then isolation or impoverished conditions (IC) are at one end of the scale, EE conditions lay at the other end, and non-enriched standard group-housing conditions (SC) are at the fulcrum. Laboratories may typically employ group housing conditions but revert to singly-housing rats where necessary, thus it would seem that employing both conditions as controls in EE studies would be most suitable. The majority of EE studies solely employ IC to make control comparisons (Fig. 1.6); while the use of group-housed SC controls (ranging from 2-6 rats/cage) comprised 36% of the sample.

It has been shown that rats reared in isolation display a behavioural pattern called “social isolation syndrome” which is associated with hyperactivity in novel environments and poor adaptability (Brenes et al., 2008), as well as disrupted

prepulse inhibition (Varty et al., 2000) and higher impulsivity (Perry et al., 2008) compared to rats housed in EE. Furthermore, when compared to SC controls IC rats have been shown to display neurochemical and behavioural alterations (Hall, 1998). In addition, compared to EE, isolation rearing increased “depressive-like” behaviour in rats in the FST (Brenes and Fornaguera, 2008, Brenes et al., 2009), delayed sexual maturation (Swanson and van de Poll, 1983) and IC rats have been found to be more negatively affected by stressors than their EE counterparts. Some studies suggest that animals who are housed in SC (2-4 animals/cage) exhibit behavioural responses at a level somewhere between enriched and IC animals in behavioural tests (Zimmermann et al., 2001, Bardo et al., 2001). In a study of amphetamine (AMP) self-administration EE rats self-administered significantly less than the IC group and the SC group tended to administer less than those in IC (Bardo et al., 2001). In the FST, the EE animals showed the lowest despair behaviours, the IC rats showed the most despair, and the SC rats were intermediate (Brenes and Fornaguera, 2008, Brenes et al., 2009).

Overall therefore there are merits to the employment of both IC and SC as controls in EE experiments as both conditions are widely employed in behavioural laboratories. Notably Laviola (2008) proposed the use of EE as a control group to test the validity of animal models of neurodegeneration and psychiatric disorders (Laviola et al., 2008). Thus such models ought to be assessed using differential rearing conditions, including EE, in order to be proven as robust models or simply laboratory-specific phenomena. However before EE may be implemented as a control condition there is first a need to standardise EE procedures.

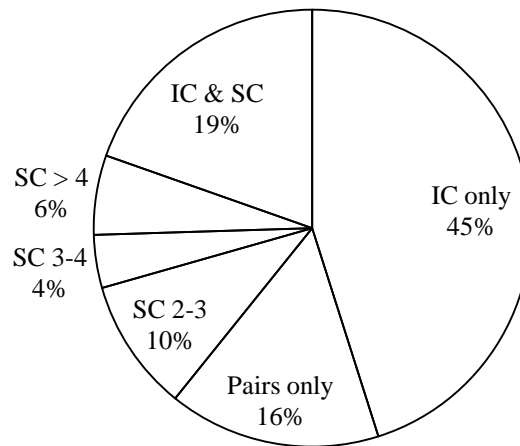


Figure 1.6: Pie chart depicts control housing conditions employed in environmental enrichment research. IC, isolated conditions; SC, standard social conditions (groups of 2-3, 3-4 or greater than 4 per cage).

1.1.2 Environmental enrichment “Categories”

Behavioural experiments must be carefully designed and controlled; the same is true of studies in the area of EE. Using the 60 articles selected previously to categorise the age of EE onset, EE duration and type of controls, the most commonly-employed EE procedures have been identified and summarised in table 1.4. As shown in the previous sections, the most common age of onset was 1-4 weeks, the preferred duration was 4-8 weeks and IC were the most common control group. Selecting those articles which employed the most common procedures, articles were coded for each of the variables; when a number of articles shared a protocol they were assigned to a category (A-E). When the emergent categories were expressed as a percent of the total sample selected they represent almost 80% of the total, this demonstrates the variability of EE in the studies and the range of possible EE protocols. The most prevalent procedure was category A; rats aged up to 4 weeks old were housed for 4-8 weeks in EE, this category represents EE during rat adolescence (approximately 6 weeks old); the three subgroups ($A_1, 2, 3$) represent the differences in control groups employed. The next category (B) includes studies in which EE was introduced at 4-8 weeks of age for a 4-8 week period and compared to IC controls. It is apparent from the categories A-E identified that EE protocols are widely diverse; which may make

the interpretation of experimental results more challenging than if a standardised approach existed. In the next section the consequences of EE on some common behavioural tests will be reviewed in light of the aforementioned variables.

Table 1.4: Commonly employed environmental enrichment protocols

Category	Age of EE onset	Duration of EE	Controls	Percent of total
A₁	≤ 4 weeks	4-8 weeks	IC & SC	15
A₂	≤ 4 weeks	4-8 weeks	IC	11
A₃	≤ 4 weeks	4-8 weeks	SC	11
B	4-8 weeks	4-8 weeks	IC	11
C	≤ 4 weeks	9-13 weeks	IC & SC	10
D	9-13 weeks	4-8 weeks	IC	10
E	≤ 4 weeks	9-13 weeks	SC	8

EE, environmental enrichment, SC, standard group conditions; IC, isolated conditions

1.1.3 Effects of Environmental Enrichment in Tests of Depression/Anxiety-like Behaviours

As summarised in table 1.5, previous studies suggest that rats housed in EE show better ability to adapt to a new environment and explore it more freely than controls housed in IC or SC. In the OFT and similar tests of locomotor habituation a number of authors have shown EE rats to display reduced locomotor activity and habituate more rapidly than controls. This appears to be a relatively consistent finding and was demonstrated when various EE protocols (including A, C, D and E as described in table 1.4 above) were employed. Category A rats exposed to a novel OFT environment showed lower activity (distance moved) than IC (Brenes et al., 2009, Varty et al., 2000) and SC controls (Brenes et al., 2008, Zimmermann et al., 2001), as well as decreased rearing and increased grooming behaviours compared to IC (Brenes et al., 2008). Similarly, rats from groups C and D displayed reduced activity compared to IC and SC controls (Schrijver et al., 2002, Hellemans et al., 2005, Larsson et al., 2002). On the other hand however when 3-month old rats were given non-continuous EE (exposure for 2 hours daily) for 15 days no difference was seen in overall activity in the OFT compared to SC controls (Leal-Galicia et al., 2007). It may be that for EE to have an effect on activity in this test it must be dynamic and continuous. Zimmermann and colleagues note that in the OFT the rats' desire to explore conflicts with their fear/emotionality and fear may reduce exploratory behaviour. The research suggests that EE housing reduces this fear and promotes faster habituation to novel environments, compared to IC or SC housing, and faster habituation implies improved information processing and better coping skills (Zimmermann et al., 2001). Activity in a novel arena therefore seems to be sensitive to the presence of continuous EE but the results are consistent even when the age at which EE is introduced and its duration are manipulated.

A number of studies have also found that EE reduces anxiety as assessed by the EPM; rats in category C displayed significantly greater percent open arm time and open arm entries than both IC and SC controls, while IC showed significantly lower percent open arm time and open arm entries than both EE and SC, displaying the effect of social background (in the absence of physical EE) in reducing anxiety in the EPM (Hellemans et al., 2005). EE for female rats in category D increased open arm time, decreased closed arm time and increased grooming duration (Sparling et al., 2010). Furthermore, post-weaning EE (categories A and E) increased the number

and percentage of open-arm entries on the EPM compared to SC controls (Pena et al., 2006, Pena et al., 2009a). All of these findings would suggest reduced anxiety in the EE rats. In contrast, however two studies (category A) found no significant difference in time spent on, or entries to open arms between EE, IC and SC rats (Hoffmann et al., 2009, Brenes et al., 2009). In one of these studies the EE rats made significantly more closed-arm entries than both control groups, which would suggest increased anxiety (Brenes et al., 2009). The authors suggest procedural factors accounting for this including bright lighting and anxiety due to tail-marking rats. The effects of EE on EPM endpoints therefore are somewhat inconsistent and maybe dependent on factors other than EE procedure as the EPM can be potentially affected by a number of experimenter and inter-animal variables (Hogg, 1996).

In other tests of anxiety, 2 weeks exposure to EE was shown to reduce defensive burying by adult Wistar rats compared to SC controls (Leal-Galicia et al., 2007). In the light/dark box, two studies using EE (category C) reported contradictory findings as one group demonstrated no effect of EE housing during adolescence on latency to enter the light side of the apparatus (Hellemans et al., 2004), while an earlier study showed that EE-housed rats took less time than IC and SC to enter the aversive white side (Schrijver et al., 2002). Though the two groups employed a similar EE protocol, Hellemans (2004) used a 5-minute test period while Schrijver (2002) observed rats' behaviour in the test box for 10 minutes; in addition different rat strains were employed, Long Evans and Lister Hooded rats respectively, which may impact on results in this test (van der Staay et al., 2009). In social interaction tests, category A EE had no effect on time spent in rough and tumble play or social rest compared to SC controls (Morley-Fletcher et al., 2003), while pre-weaning EE exposure decreased overall social behaviours observed in pre-pubertal rats compared to SC controls (Magalhaes et al., 2004). It may be that pre- and post-weaning EE have differential effects on social development, moreover, the age of rats (pre- vs. post-pubertal) at the time of testing likely affect patterns social interaction. A final factor which could further account for the disparate findings is that of the test arena used. In the study carried out by Magalhaes (2004) the social interaction test arena was furnished with bedding and EE objects, while Morley-Fletcher and colleagues (2003) used a novel, barren test arena. Tests of emotionality are thus sensitive to a number of factors in addition to EE, the tests employed and

endpoints measured vary across studies, as well as strain and experimental differences; all of which affect data and research conclusions.

Previous data would suggest that EE housing helped to improve rats' coping skills and display more a more active strategy (i.e. trying to escape) when faced with such stressors as the FST. In the FST, one group employing category A found EE rats engaged in significantly greater time swimming (Brenes et al., 2008) and climbing (Brenes et al., 2009) and significantly less time immobile than controls (Brenes et al., 2008, Brenes et al., 2009). However, when Cui and colleagues (2006) carried out the same test using a comparable EE protocol they found no effect of housing on immobility time (Cui et al., 2006). In one of the experiments carried out by the Brenes group, the FST was the penultimate test carried out and thus by this time rats had experienced up to 12 weeks differential housing. However this extended duration of EE cannot completely account for the EE effects on FST as in a second study carried out by the same group the test was carried out after just over 1 month of EE housing. Thus the reason Cui (2006) failed to find EE effects on FST behaviour may be due to other procedural variables such as rat strain or methodological differences as manual quantification of immobility behaviour.

Table 1.5: The effects of environmental enrichment in common behavioural tests

Attribute	Consequences of enriched housing			
	Compared to SC		Compared to IC	
Body Weight	↓ (males) - (females)	(Moncek et al., 2004, Hughes and Collins, 2009) (Harati et al., 2009)	↓ (adolescent males) ↓ (females)	(Zaias et al., 2008) (Harati et al., 2009)
General Activity	↑ habituation, ↓ activity	(Brenes et al., 2008, Brenes et al., 2009)	↑ habituation, ↓ activity	(Brenes et al., 2008, Brenes et al., 2009, Varty et al., 2000)
	↑ grooming, ↓ rearing	(Brenes et al., 2008, Brenes et al., 2009)	↑ grooming, ↓ rearing	(Brenes et al., 2009, Brenes et al., 2008, Pham et al., 1999a)
Anxiety	<i>Elevated Plus Maze:</i>			
	↑ open arm entries,	(Pena et al., 2006)	↑ open arm entries,	(Sparling et al., 2010)
	↑ open arm movement,	(Pena et al., 2009a)	↑ open arm duration,	(Sparling et al., 2010)
	↑ percent open arm entries and time	(Galani et al., 2007)	↑ percent open arm entries and time	(Hellemans et al., 2004)
	<i>Social Interaction:</i>			
	↑ social play, ↑ social rest and social exploration after prenatal stress	(Morley-Fletcher et al., 2003)		
	Pre-weaning ↓ social behaviour, exploration and play	(Magalhaes et al., 2006, Magalhaes et al., 2007)		
Depressive-like Behaviours	<i>Forced Swim Test:</i>			
	↑ swimming, climbing, diving, ↓ immobility	(Brenes et al., 2008, Brenes et al., 2009)	↑ swimming, climbing, diving ↓ immobility	(Brenes et al., 2008, Brenes et al., 2009, Konkle et al., 2010)

EE, environmental enrichment; SC, standard conditions; IC, isolated conditions; EE increases (↑), decreases (↓) or has no effect (-) on behaviours.

1.1.4 Environmental Enrichment Effects in Tests of Learning and Memory

Some effects of EE on performance in learning and memory tests are summarised in table 1.6. In tests of EE effects on learning and memory, the MWM is often employed. Rats housed in category C and D have displayed reduced latencies to the platform compared to IC (Larsson et al., 2002) and SC controls (Hellemans et al., 2004, Nilsson et al., 1999, Schrijver et al., 2002). Category A has also resulted in reduced latencies (Pham et al., 1999b); one such study recorded that IC controls took 6 days to reach the platform in the minimum time (7-10 seconds) whereas the EE group reached this criterion on day 3 of training (Schrijver et al., 2002). On the other hand, others have reported no differences in MWM latencies between EE and SC groups (Ueda et al., 2005). Rearing in EE for 1 year resulted in reduced latencies compared to IC controls (Pham et al., 1999a) and 9-13 weeks EE reduced latencies (Harris et al., 2009) and decreased thigmotaxis compared to IC (Sparling et al., 2010) and SC (Harati et al., 2009). Cui and colleagues (2006) found no effect of EE (4-8 weeks) on MWM performance in “normal” rats but EE did restore deficits in spatial learning due to early life stress (Cui et al., 2006). It is worth noting that the effects of EE in the MWM are primarily reported in studies of aging or cognitive decline as result of injury or disease. Overall EE seems to have positive effects on spatial learning and memory as assessed in the MWM though some exceptions have been found.

In the RAM, rats in categories A and E made significantly fewer working memory errors (i.e. fewer re-entries to arms) compared to SC controls (Hoffman et al., 2008, Leggio et al., 2005). Furthermore, mature and aging (7-22 month old) rats housed in EE for 70 days made more correct responses and fewer errors than IC and SC controls (Bell et al., 2009). On the other hand, Brillaud and colleagues (2005) found no effect of EE prior to RAM testing compared to non-enriched controls (Brillaud et al., 2005). However all animals in this study were IC-housed, thus the EE group lacked social enrichment, it is difficult therefore to compare these results with other EE results.

In the NOR and object exploration tests some inconsistencies have been found; category A showed decreased contacts with a novel object over time compared to SC (Van Waas and Soffie, 1996), as well as fewer object contacts, significantly less time in contact with the object and less time manipulating the

object than IC animals (Zimmermann et al., 2001), which the authors suggest is indicative of greater habituation by EE rats. Category E resulted in significantly greater discrimination ratios (i.e. time spent exploring novel object / total time spent exploring objects) for EE rats compared to SC, though no significant housing effect was seen when time spent exploring the object was analysed (Escorihuela et al., 1995). Following categories A and C EE rats showed a greater reaction to spatial displacement of an object (Van Waas and Soffie, 1996, Schrijver et al., 2002) than IC and SC controls. Spontaneously hypertensive rats (SHR) in category D showed improved novel object recognition (i.e. greater preference for the novel object), whereas there was no housing effect for Wistar rats in the same conditions (Pamplona et al., 2009). Many studies of EE effects on learning and memory are based on the reversal of cognitive deficits due to aging or disease; in one such study of EE effects on aging, eighteen months of non-continuous (3 hours daily) EE increased novel object recognition and exploration compared to IC controls (Leal-Galicia et al., 2008). The EE effects on object recognition and spatial memory are dependent on the endpoints measured and their interpretation as some authors assess adaptability of EE animals to a novel object (Zimmermann et al., 2001) while others quantify attention toward the novel object as a measure of memory (Escorihuela et al., 1995).

In other learning tasks young adult rats (9-13 weeks old) housed in EE for 9-13 weeks made significantly fewer errors in the Hebb-Williams maze than SC controls (Kobayashi et al., 2002). Category C EE had no effect on the development of conditioned taste aversion (Hellemans et al., 2004). EE (category A) rats showed significantly more freezing behaviour during a short pre-shock period, which implies better memory representation for aversive events, than SC controls (Woodcock and Richardson, 2000). Similarly, category A EE also resulted in increased corrected alterations in a water Y-maze (Van Waas and Soffie, 1996). Twenty-six months EE exposure improved working memory as assessed by correct choices in an 8-arm water maze compared to IC rats (Lores-Arnaiz et al., 2006). On the other hand, 12- (Del Arco et al., 2007a) or 21- (Segovia et al., 2008) months EE had no effect on performance in a water T-maze. Overall the effects of EE on cognitive tests vary depending on the age and strain of the animals employed as well as on the endpoint

measured as different tests and parameters of interest measure different types of learning and memory ability.

Table 1.6: The effects of environmental enrichment in common learning and memory tests

Attribute	Consequences of enriched housing			
	Compared to SC		Compared to IC	
MWM	↓ escape latencies	(Zhong et al., 2009, Leggio et al., 2005)	↓ escape latencies	(Schrijver et al., 2002, Pham et al., 1999a)
NOR	↑ novel object preference	(Bruehl-Jungerman et al., 2005, Pamplona et al., 2009)	↑ novel object preference	(Schrijver et al., 2002, Zimmermann et al., 2001)
RAM	↓ errors and time in arms	(Leggio et al., 2005, Hoffmann et al., 2009)	↓ errors in females - of modest EE	(Daniel et al., 1999) (Brillaud et al., 2005)

EE, environmental enrichment; EE increases (↑), decreases (↓) or has no effect (-) on behaviours compared to SC, standard conditions; IC, isolated conditions; MWM, Morris water maze; NOR, novel object recognition; RAM, radial arm maze.

Housing conditions may have differential effects on rats based on sex and strain. EE housing (category A) significantly increased locomotor activity of Long Evans rats, but had no effect on the Wistar rats compared to IC controls (Benloucif et al., 1995). Similarly the Wistar strain were not as responsive as the SHR strain to EE (category A₃); EE improved SHR rats' habituation to a second exposure of the OFT, decreased latency to the platform in the MWM acquisition and increased percent time spent in the target quadrant during the MWM probe phase, and increased NOR discrimination ratio compared to SC controls, while there were no differences between SC and EE rats of the Wistar strain (Pamplona et al., 2009). Regarding sex differences, no interaction of sex and housing was seen in the MWM (Harris et al., 2009, Zhong et al., 2009), nor was there any differential effect of housing in the problem-solving task the Hebb-Williams maze for male and female rats (Pena et al., 2009a). However in a social discrimination task EE (category E) improved social memory for males but impaired it for females, compared to SC controls (Pena et al.,

2009a). In the OFT, it has been shown that the separate elements of social and physical enrichment have different effects on open field activity in male and female SD rats. Male and female rats housed in IC or SC (3/cage), with or without EE (pet toys) were exposed to the OFT on three different occasions. The results suggested that over time the importance of social EE may be more salient than physical to both males and females and overall the effects of EE were greater for males than females as assessed by EE males' greater habituation to the OFT compared to female counterparts (Elliott and Grunberg, 2005).

1.1.5 Effects of Environmental Enrichment on Response to Stress

The hypothalamic pituitary adrenal (HPA) axis is activated by stress and its responsiveness can be measured by the serum concentrations of stress hormones corticosterone (CORT) and adrenocorticotrophic hormone (ACTH). There have been inconsistent results from studies addressing the effects of EE on HPA axis functioning (Table 1.7). Baseline CORT levels have been found to increase in male Wistar rats housed in EE from approximately 10 weeks old for 40 days (Moncek et al., 2004); decrease in IC rats housed with EE for an unspecified period (Belz et al., 2003); or remain unchanged using category C EE, compared to controls (Schrijver et al., 2002). As each of these studies employ different EE procedures, as well as different rat strains, it is difficult to compare their results. The provision of physical EE objects may therefore reduce stress for IC animals (Belz et al., 2003), while the provision of combined social and physical EE may actually induce elevations in CORT levels (Moncek et al., 2004). Notably Schrijver and colleagues (2002) saw no housing effect on CORT or ACTH, however the rats in their study were exposed to a number of behavioural tests in the weeks prior to basal blood sampling, moreover, basal samples were taken via the tail vein and rats were not habituated to this procedure previously, this may have had an effect their stress levels (Schrijver et al., 2002). ACTH concentration increases following a stressful event, however EE-rearing has consistently been shown to attenuate this increase following restraint stress (Moncek et al., 2004) and stress produced by administration of a saline injection (Belz et al., 2003) compared to controls. This would suggest then that EE animals have a lower HPA reactivity to stressful situations than controls.

Enriched animals are seemingly less emotionally reactive in novel situations, thus they can explore their environment more efficiently. This is thought to be due to

more efficient action of corticosteroids in these animals. Though no neuroendocrine parameters were measured, Larsson and colleagues (2002) found that increased stress primed rats for exploration as rats that were exposed to stress (footshock) showed increased exploratory behaviour in the OFT, and this was dependent on stress intensity (Larsson et al., 2002). Likewise, in the MWM, mild stress allowed for more efficient exploration by the EE group (category D), resulting in quicker escape latencies, whereas high intensity glucocorticoid release impaired memory and learning, in both IC and EE groups (however the IC group were more negatively affected). These results all imply that animals exposed to environmental manipulation were more reactive to stress but that mild stress (i.e. placed in an avoidance box but no shock received) facilitated their learning and coping responses (Larsson et al., 2002). EE animals are thought to be more affected by CORT due to increased glucocorticoid receptors in the hippocampus (Olsson et al., 1994) and therefore explore more in the tests such as the MWM and OFT, whereas impoverished animals have a reduced CORT response and habituate more slowly than EE animals. It may be that there is a more effective negative feedback loop in EE animals, whereby increased glucocorticoid receptor expression could inhibit corticotrophin releasing factor in both resting and stressed states; as a result EE animals would be more affected by changes in CORT levels (Olsson et al., 1994).

EE housing generally reduces both endocrine and behavioural reactivity to stress; further research on different rat strains and the action of other hormones are needed to fully understand the significance of alterations in glucocorticoid receptors and how these relate to behaviour, learning and memory. Sex differences must also be considered as males and female rats differ in HPA axis function (Chisari et al., 1995); it has been shown that female rats have greater plasma CORT and ACTH levels than males (Bakos et al., 2009, Pena et al., 2009a, Welberg et al., 2006) and this is thought to be dependent on ovarian hormones and the rat estrous cycle (Carey et al., 1995, Patchev et al., 1995). As EE and IC housing can also affect serum levels of stress hormones sex and housing effects can interact to influence stress reactivity. EE has sexually dimorphic effects on stress hormones as it increased ACTH levels in male, but not in female, Wistar rats (Bakos et al., 2009) and reduced ACTH and CORT in both male and female SD rats housed in isolation with modest enrichment (Belz et al., 2003). This difference in ACTH levels in males

suggest that EE males housed in groups are more stressed than those singly housed (Brown and Grunberg, 1995). EE can also reduce sensitivity to stress exposure (Pena et al., 2009a, Belz et al., 2003), particularly in females (Belz et al., 2003, Welberg et al., 2006). It is clear that the various EE housing methods employed in the above studies can have a profound effect on the endpoints measured and that these can also be altered between the sexes.

Table 1.7: Effects of environmental enrichment on circulating stress hormones in rats

Hormone	Effects of EE	Reference
CORT	↑ baseline level (vs. SC)	(Moncek et al., 2004)
	↑ baseline level (females only) (vs. SC)	(Pena et al., 2009a)
	↓ baseline level (vs. IC)	(Belz et al., 2003)
	- baseline (vs. IC & SC)	(Schrijver et al., 2002)
	- baseline (vs. SC)	(Welberg et al., 2006)
	↓ response to novelty (vs. SC)	(Pena et al., 2009a)
	↓ response to chronic stress (females only)	(Welberg et al., 2006)
	↓ response following saline injection (vs. SC)	(Belz et al., 2003)
	- following restraint stress (vs. IC & SC)	(Schrijver et al., 2002)
	- following handling stressor (vs. SC)	(Moncek et al., 2004)
ACTH	↓ baseline level (vs. IC)	(Belz et al., 2003)
	↑ baseline level (females only) (vs. SC)	(Pena et al., 2009a)
	↑ baseline level (males only) (vs. SC)	(Bakos et al., 2009)
	- baseline (vs. IC & SC)	(Schrijver et al., 2002)
	↓ following handling stressor (vs. SC)	(Moncek et al., 2004)
	↓ response following saline injection (vs. SC)	(Belz et al., 2003)
	- following restraint stress	(Schrijver et al., 2002)

EE, environmental enrichment, IC, isolated condition, SC, standard conditions, CORT, corticosterone, ACTH- adrenocorticotrophic hormone.

1.1.6 Effects of Environmental Enrichment on Monoamine Neurotransmitter Levels

The monoamine neurotransmitters serotonin (5HT), dopamine (DA) and noradrenaline (NA) have been investigated in studies of EE and IC housing due to their association with emotionality and they are often measured in addition to behavioural tests of anxiety/depression (Lanni et al., 2009, Brenes et al., 2009, Galani et al., 2007).

Following EE (category A) rats have enhanced expression of the gene for the 5HT_{1A} receptor in the hippocampus (Rasmuson et al., 1998) and in studies carried out by Brenes and colleagues 5HT levels were increased in the hippocampus and frontal cortex of EE (categories A and C) rats compared to SC and IC controls (Brenes et al., 2008, Brenes et al., 2009). Significantly greater concentration of hippocampal 5HT in EE compared to controls was correlated positively to time spent swimming and negatively to immobility in the FST. This would indicate that enhanced 5HT levels seen in EE rats could contribute to the coping behaviour seen the FST, a test for anti-depressant activity (Brenes et al., 2009). On the other hand, young adult female Long Evans rats housed in EE or SC (pairs) for 30 days showed no effect of housing on 5HT in the dorsal hippocampus or frontoparietal cortex and EE actually lowered 5HT and 5-Hydroxyindoleacetic acid (5HIAA) in the hippocampus (Galani et al., 2007). Del Arco and colleagues found no effect of housing on basal DA levels in the prefrontal cortex (PFC) of rats housed for 12 months in EE, IC or SC (Del Arco et al., 2007b), however the same group later showed that 12 weeks EE housing reduced D₁ receptor density in the PFC compared to IC controls (Del Arco et al., 2007a). In addition, decreased DA uptake and reduced expression of cell surface DA transporters (DAT) have also been reported in the PFC of rats housed in category A (Zhu et al., 2004, Zhu et al., 2005) . Furthermore, increased NA in the hippocampus (Brenes et al., 2009, Galani et al., 2007) and ventral striatum (Brenes et al., 2008) following categories A and D EE are further evidence of the ability of housing to promote changes in the brain's neurochemistry. Studies of long-term EE effects on acetylcholine (ACh) have shown no housing effects on baseline levels in the PFC (Del Arco et al., 2007b, Segovia et al., 2008), but EE does reduce stress-induced reduce ACh release following 40-minutes of handling and mild restraint; however EE had no effect on working memory impairments in the T-maze due to stress exposure (i.e. exposure to a

brightly lit OFT) (Del Arco et al., 2007b). In addition to being associated with emotionality, these neurotransmitters are related with learning, synaptic plasticity and neurogenesis in the brain (Brezun and Daszuta, 1999, Malberg et al., 2000).

The ability of EE to alter neurotransmitter levels in the brain can partially explain the capacity of EE to influence plasticity in the brain and enhance learning. The monoamines (such as 5HT and NA) are thought to play a role in neurogenesis, cholinergic parameters and the opioid system are also associated with synaptic plasticity and learning (van Praag et al., 2000). The interaction of EE with various neurotransmitter systems could prove to be an important factor to consider when investigating the neurochemical effects of pharmacological agents. For a more comprehensive review of EE effects on neurotransmission see (Solinas et al., 2010).

1.1.7 Effects of Environmental Enrichment on Neurotrophic Factor Expression

Neurotrophic factors are proteins known to promote cell survival and functions, particularly related to synaptic plasticity. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT3) are widely expressed in the hippocampus. Neurotrophic factors and their receptors p75 and the tyrosine kinase (Trk) receptors are involved in neuroplasticity for learning and memory (Ernfors et al., 1990). BDNF promotes long term potentiation (LTP) at hippocampal CA1 synapses (Korte et al., 1998) and in EE studies BDNF levels have been higher in the hippocampus, basal forebrain, hindbrain and cerebral cortex of rats housed in EE conditions for 1 year compared to IC controls (Table 1.8) (Ickes et al., 2000). Importantly both the hippocampus and cerebral cortex are involved in memory functions. BDNF can contribute to plastic changes in the brain and in support of this EE has been shown to affect neural morphology and synaptic growth (Nilsson et al., 1999, Lu et al., 2003). BDNF expression in various brain regions is also influenced by sex. Following 6 weeks rearing in SC (4/cage) or EE male and female rats' BDNF levels in the hippocampus and hypothalamus were assayed. BDNF levels were greater in females than males in both regions studied. EE increased hippocampal BDNF for both sexes compared to SC controls but this increase was greater in EE females than EE males. In addition, EE increased BDNF in the hypothalamus of female rats in EE compared to females in SC housing; however this effect was not seen between EE and SC males (Bakos et al., 2009). Overall BDNF levels were

greater in females than males and EE housing had greater effect on increasing BDNF levels in females than males.

Like BDNF, NGF is also thought to be important to LTP mechanisms and has been elevated in the basal forebrain, hippocampus and hindbrain of rats housed in EE compared to IC controls after 1 year (Ickes et al., 2000). Increased NGF in the hippocampus, entorhinal and visual cortex were associated with improved performance in the MWM by animals reared for 1 year in EE compared to IC controls (Pham et al., 1999a). No EE effect was found in the hypothalamus as NGF levels were low in this region regardless of housing condition (Pham et al., 1999a). Finally, category A EE rearing led to significantly higher NGF mRNA in the visual cortex and hippocampus compared to IC controls (Torasdotter et al., 1998).

Unlike BDNF and NGF, NT3 secretion is thought to be dependent on hormone and BDNF levels, thus NT3 might be indirectly affected by BDNF. As with BDNF, NT3 levels were increased in the basal forebrain and cerebral cortex of rats housed in EE for 1 year compared to IC (Ickes et al., 2000); in addition, EE (category A) rats had significantly increased NT3 mRNA in the visual cortex and hippocampal formation compared to IC controls (Torasdotter et al., 1998).

The changes in neurotrophic factor levels in the brain brought about by environmental manipulations would be expected to have an effect on the anatomical, morphological and behavioural functions of the brain. It is suggested that the decreased trophic factor levels in the IC rats indicate a long-term depression of neuronal activity in the limbic system. This gives rise to decreased BDNF and NGF production, followed by selective degeneration of basal forebrain cholinergic neurons. This would comprise a feedback system, wherein reduced trophic factor levels contribute to attenuated neuronal activity, leading in turn to reduced growth factor concentrations in certain brain regions (Ickes et al., 2000). The relevance of neurotrophic factors to EE research is primarily related to their role in synaptic growth and plasticity. Brain damage research could potentially benefit from any therapeutic effects of EE in the recovery of cognitive and behavioural deficits induced by traumatic brain injury (Will et al., 2004), stroke (Wurm et al., 2007) and spinal cord injury (Berrocal et al., 2007). Moreover, neurotrophic factors and their relationship with the cholinergic system have been associated with therapies for

neurodegenerative diseases (Pham et al., 1999a). Finally, decreases in neurotrophic factors have been associated with increased responsiveness to stress, anxiety and depression (Martinowich and Lu, 2008), possibly via interactions with monoamine neurotransmitters, which could also pose EE-related potential targets for the development of treatment for such disorders.

Table 1.8: The effects of environmental enrichment on central neurotrophic factor content

Neurotrophic factor	Effects of EE housing
BDNF	<p>↑ in hippocampus, basal forebrain, hindbrain and cerebral cortex (Ickes et al., 2000)</p> <p>↑ in hypothalamus of females (Bakos et al., 2009)</p>
NGF	<p>↑ in basal forebrain, hindbrain, hippocampus, entorhinal and visual cortices (Pham et al., 1999a, Ickes et al., 2000). ↑ mRNA in visual cortex and hippocampus (Torasdotter et al., 1998)</p>
NT3	<p>↑ in basal forebrain and cerebral cortex (Ickes et al., 2000), ↑ mRNA in visual cortex and hippocampus (Torasdotter et al., 1998)</p>

Abbreviations: EE, environmental enrichment, IC, isolated conditions; EE increases (↑) neurotrophic factor levels; BDNF, brain-derived neurotrophic factor, NGF, nerve growth factor and NT3, neurotrophin-3.

1.1.8 Effects of Environmental Enrichment on Neurogenesis and Plasticity

Housing animals in enriched conditions has been shown to aid memory and learning by improving brain plasticity (van Praag et al., 2000). Plasticity refers to the ability of the brain and nervous system to adapt and change according the challenges of the environment, thus allowing humans and animals to learn and store information. One mechanism by which this is achieved is neurogenesis, which refers to the birth of neurons. EE has been found to increase neurogenesis in the hippocampus of adult rats (van Praag et al., 2000). Other structural changes associated with EE and plasticity include increased in brain weight and size, (Bennett et al., 1969), increased dendritic growth (Leggio et al., 2005), increased neurogenesis (Lu et al., 2003) increased cortical thickness and synapse formation (Rosenzweig and Bennett, 1996).

Compared to IC controls, EE and SC rats have been shown to have greater LTP induction in the CA1 area of the hippocampus, which underlies synaptic plasticity. In a study of the lasting effects of EE, rats who were housed in category A EE and then transferred to SC for 3-5 weeks had greater hippocampal LTP than

controls who had not been exposed to EE (Artola et al., 2006). Similarly, rats housed in SC (5/cage) for 4 weeks had greater LTP in the hippocampus compared to those in IC (Lu et al., 2003). Category D enrichment protocol resulted in increased newborn cells in the sub-granule cell layer of dentate gyrus and evidence of superior memory skills in the MWM, as the dentate gyrus is important in memory and learning (Nilsson et al., 1999). This is further evidence that IC housing is not conducive to the normal neuronal development of animals. Interestingly, the administration of the anti-mitotic agent methylazoxymethanol acetate (MAM – a DNA methylating agent which has been shown to reduce adult generated hippocampal granule cells (Shors et al., 2002)) to adult rats housed in EE or in pairs (for 2 weeks prior to testing) prevented the increased hippocampal neurogenesis and improved water maze performance seen in saline-treated EE controls. This would suggest that the newborn cells in the dentate gyrus as a result of EE housing contribute to the behavioural effects of the housing environment on memory (Bruehl-Jungman et al., 2005). However a study showed only a partial reduction of adult neurons in the rat following MAM administration and warned that behavioural results obtained using this agent should be interpreted with caution as, when administered at doses sufficient to deplete neurons it may be detrimental to the animal's overall health (Dupret et al., 2005).

Dendritic branching and spine density are significantly increased in the somatosensory cortex of adult (3-months old) male rats following 3 weeks EE, compared to SC controls (Johansson and Belichenko, 2002). Increased spine density of parietal neurons due to EE housing (category E) were associated with superior problem-solving skills in the RAM and MWM latencies compared to pair-housed controls (Leggio et al., 2005). By inhibiting calmodulin activation, and thus inhibiting cAMP response, Le Zhong and colleagues (2009) showed that calmodulin activation is essential for mediating hippocampal neurogenesis following EE. Two weeks intermittent exposure of pups to EE promoted hippocampal neurogenesis and reduced latency to find the platform in the MWM, compared to controls. However administration of a calmodulin antagonist (trifluoperazine) completely inhibited the neurogenesis and memory skills seen in the non-treated EE controls (Zhong et al., 2009). Overall it seems that experiences within the complex environment (both social and physical EE) lead to greater plasticity in the brain via cAMP activation, as

well as neurotrophic factors and neurotransmitter levels; all of which contribute to improved performance in cognitive tests such as the MWM and RAM.

Sex differences have been found in neural plasticity markers in the rat brain. During a study of EE effects on the aging brain EE promoted a significant increase in the area of somas and increased dendritic processes of cortical and hippocampal neurons compared to SC controls in both sexes. Female rats in EE showed increased complex 1 activity in brain mitochondrial membranes (as assessed by NOS levels) compared to SC counterparts; however there was no such difference between EE and SC males (Lores-Arnaiz et al., 2006). Though males lived longer than females, it was females who were more responsive to EE effects on neural plasticity. Furthermore, female rats housed in EE for one month post-weaning had a larger dendritic tree than their male counterparts (Juraska et al., 1985). The male and female rat neocortex was shown to respond differently when exposed to the same enriching conditions; the EE-housed males showed significant increases in occipital cortex thickness compared to SC controls; however this effect was not as pronounced in females (Diamond, 2001). In contrast the female somatosensory cortex was significantly increased, whereas no such effect was seen in the male somatosensory cortex (Diamond, 2001). This may be due to females interacting more with the environment and manipulating objects more than the males (Pena et al., 2006). Finally an early study carried out by Juraska and colleagues (1988) reported that male and female rats housed in EE from PND 23-55 had a significantly larger corpus callosum than those housed in IC. In addition, EE rearing induced morphological changes in the corpus callosum of males and females differentially as EE females had a greater number of myelinated axons than EE males, while EE males had larger myelinated axons than EE females (Juraska and Kopicik, 1988). Thus EE has salient effects on promoting plasticity in the brain, which contribute to functional improvements in cognitive tests in studies of brain damage and these effects may also be influenced by sex hormones.

1.1.9 Effects of Environmental Enrichment on Responsiveness to Drugs of Abuse

EE during development can impact on responses to drugs of abuse and vulnerability to drug abuse, this is thought to be due to EE effects on brain's the reward system (Thiel et al., 2010). The majority of studies have been carried out on the psychostimulant drugs AMP and cocaine (COC).

Rats housed in EE (category A) displayed reduced COC self-administration at a low dose (0.1 mg kg^{-1}) but not at a higher dose (0.5 mg kg^{-1}), SC housing was also protective against self-administration in this study, suggesting the importance of social cohorts in drug use (Gipson et al., 2011). Female rats in EE (category A) were found to be more sensitive to the stimulant effects of high dose (1 mg kg^{-1}) COC on locomotor activity and to the development of COC-induced conditioned place preference (CPP) compared to IC controls. Furthermore, EE rats were more sensitive to COC toxicity as assessed by convulsive activity at 50 and 75 mg kg^{-1} doses, than IC (Smith et al., 2009). On the other hand, male rats (PND 23) were housed with or without social and physical enrichment for 20 days. The results showed that adolescent EE rats failed to develop CPP at 10 mg kg^{-1} COC compared to control IC rats (Zakharova et al., 2009), which suggests that the rewarding potential of the drug was reduced in the EE animals. Postnatal EE (introduced pre-weaning) has been shown to reduce the effects of prenatal COC exposure on social interactions (Neugebauer et al., 2004, Magalhaes et al., 2006). In addition, when pups were exposed to post-natal COC administration, EE throughout the first month of life reinstated social interactions and reduced the incidence of play fighting behaviours compared to COC-treated SC controls (Magalhaes et al., 2007).

A number of COC self-administration studies have shown that EE housing during abstinence can reduce COC-seeking behaviours compared to IC and SC controls during extinction (Thiel et al., 2010, Thiel et al., 2011), and during cue-elicited reinstatement (wherein rats were returned to self-administration chambers and lever-pressing was recorded) (Thiel et al., 2009) and stress-elicited reinstatement (wherein an anxiogenic drug administered and lever-pressing recorded) (Chauvet et al., 2009). However EE had no effect on COC-induced reinstatement regardless of COC dose (3 or 6 mg kg^{-1}) or EE duration (30 or 90 days) (Thiel et al., 2009, Chauvet et al., 2009). Overall therefore EE can reduce drug craving and the risk of

drug use in cue-elicited relapse but does not produce lasting protection against drug-elicited relapse (Thiel et al., 2009, Thiel et al., 2011).

Michael Bardo and colleagues have carried out a number of studies of the effects of EE (category A) on behavioural and neurochemical response to AMP. Similar to COC, EE can reduce self-administration of AMP compared to IC at low doses (0.006-0.03 mg kg⁻¹) but not at higher doses (0.1) mg kg⁻¹ (Green et al., 2002, Bardo et al., 2001). EE rats are more sensitive to the locomotor-stimulating effects of acute AMP, with increased distance moved and rearing compared to IC; however EE displayed reduced sensitivity to the effects of repeated doses, as measured by decreased distance moved and reduced rearing, compared to IC controls (Bardo et al., 1995). These effects are thought to be mediated via the glutamatergic neurotransmission in the nucleus accumbens, EE may play a protective role by blunting adaptations of the glutamatergic system as result of repeated drug use (Rahman and Bardo, 2008). EE rats showed greater extinction of AMP, as well as raising the reinstatement threshold of an AMP priming dose; IC reinstated AMP seeking at 0.25 mg kg⁻¹ while EE reinstated AMP seeking at the higher dose of 1 mg kg⁻¹ (Stairs et al., 2006). This is further evidence of the protective ability of EE against AMP addiction, but only at low drug doses. In contrast, EE (category A) and IC rats administered saline or monoamine-depleting doses of methamphetamine (MA) (4 x 10 mg kg⁻¹ doses) were then re-challenged with 0.3 and 0.1 mg kg⁻¹ MA and locomotor activity was assessed. The 0.1 mg kg⁻¹ dose induced significantly greater locomotor activity in EE rats previously administered high dose MA compared to EE saline controls rats, no significant difference between the MA and saline groups were seen in the IC rats. Thus rather than the expected protection against the behavioural consequences of monoamine-depleting doses of MA, EE actually enhanced the behavioural effects (Gehrke et al., 2006).

Housing conditions also impact on the effects of other drugs of abuse; EE rats (category A) are less sensitive to the psychostimulant effects of nicotine after both acute and repeated administration compared to IC, and are less sensitive to the hyperactivity effects of repeated nicotine injections compared to SC rats (Green et al., 2003). EE also reduced the reward value of ethanol as SHR in EE conditions consumed less ethanol in both a free- and forced-choice self-administration paradigm, in addition to reduced sucrose consumption and novelty-induced

hyperactivity compared to SC controls (de Carvalho et al., 2010). As the SHR strain are employed as a model of attention deficit/hyperactivity disorder (ADHD) such results may have implications for environmental influences on adulthood ADHD co-morbidity with drug abuse disorders (de Carvalho et al., 2010).

The effects of EE on drug use are likely mediated by the mesolimbic reward pathway and primarily via DA activity, in support of this EE (category A) rats show enhanced responsiveness (greater locomotor activity) to the DA agonist apomorphine (APO) than SC controls (Hoffmann et al., 2009). The increased stimulant effects seen in EE animals in response to DA-increasing drugs are partly due to decreased DAT and DA metabolism in the frontal cortex in EE rats (Zhu et al., 2004), as well as reduced CREB (Green et al., 2010) and elevated glutamate (Rahman and Bardo, 2008) in response to COC and AMP in the nucleus accumbens. In summary, the effects of EE on AMP and COC drug use suggest that EE-housed animals are more sensitive to the effects of acute drug doses, display reduced self-administration at low doses, improved extinction training and reduced cue-elicited reinstatement. However EE has no effect on COC-elicited reinstatement of drug taking, nor can it protect against the motivational properties of COC and AMP at high doses (Stairs and Bardo, 2009, Solinas et al., 2010).

Overall the existing literature would suggest that EE protocols for rats can vary greatly across laboratories, however a majority of studies included in this review employed category A, which is to say that most rats were housed in EE from post-weaning, for a period of 4-8 weeks. EE has profound effects on behavioural and neurochemical endpoints in addition to neuromorphology. However the variables such as controls employed, rat age, sex and strain can impact on results and contribute to variable results reported between laboratories. One of the objectives of this thesis is to assess the effects of IC and EE housing conditions on baseline behaviours in male and female rats using behavioural tests which are routinely carried out in this laboratory. In addition investigation of the effects of IC and EE implementation immediately post-weaning and in early adulthood on responses to psychotropic drugs were carried out.

1.2 Sex Effects

In the past, female animals have been largely excluded in scientific research, with the exception of sex-specific reproductive behavioural investigations. Increasing evidence however documents sex influences on the brain and behaviour, both clinically and preclinically. Though sex hormones can play a role in learning and memory (Dohanich, 2002) and affect responsiveness to stimulant drugs (Becker and Cha, 1989, Hu and Becker, 2003), there are other mediators of sex differences in the brain. In humans, the hippocampus is larger in women than in men, while men have greater CA1 volume as well as greater density of dentate gyrus neurons compared to women (Madeira and Lieberman, 1995). Experience (Juraska et al., 1985) and stress (McEwen, 2000) impact differentially on dendritic branching in the hippocampus of males and females, which may be important in the understanding and treatment of stress-related disorders such as depression and post-traumatic stress disorder (McEwen, 2000). Resting and experimentally-evoked responses in the amygdala are also known to be sexually dimorphic (Kilpatrick et al., 2006, Killgore and Yurgelun-Todd, 2001). Furthermore, neurochemical differences in the human brain include altered serotonin levels (Nishizawa et al., 1997) and sensitivity of brain regions to opioids (Zubieta et al., 1999).

In addition to neural differences, various diseases affect men and women differently; women tend to have earlier onset of Alzheimer's disease, elevated disease markers (neurofibrillary tangles and neurite plaques) and greater overall disease pathology. Women also have greater prevalence rates than men, though this is largely due to the fact that women live to an age at which Alzheimer's disease is more common more than men (Barnes et al., 2005). Sex differences exist in features of schizophrenia as men present with symptoms earlier than women (Hafner et al., 1994), while women experience more hallucinations or psychotic symptoms than men (Lindamer et al., 1999). Women are more sensitive to the reinforcing effects of stimulant drugs than men, which is likely to account for the more rapid development of dependence from initial drug use in women (Lynch et al., 2002). According to the World Health Organisation depressive disorders are among the most debilitating diseases in the Western world and incur huge economic costs yearly. Depression is twice as common in women than in men (Gorman, 2006), in addition depression and anxiety are the most common comorbid disorders and a sex difference exists in this

comorbidity rate (Linzer et al., 1996). Women are further at risk of depressive disorders during times of reproductive hormonal changes such as menstruation, pregnancy, post-partum and menopausal periods (Soares and Zitek, 2008).

Preclinical studies have been useful in modelling sex differences in drug abuse and the mechanisms underlying drug abuse vulnerability (Lynch et al., 2002). It has been found that female rats are more sensitive to the reinforcing effects of COC (Roberts et al., 1989) and display more intense behavioural responses to AMP (Becker and Cha, 1989). Such differences have been attributed to sexual dimorphisms in DA function in the brain and the effects of ovarian hormones on the same (Becker et al., 2001). In addition, male rats show greater antinociceptive responses to opiates as shown by tests such the hot plate and tail-flick tests (Kest et al., 2000). Male and female rats appear to have different innate mechanisms for dealing with stress (Ter Horst et al., 2009) and this may have clinical relevance in providing treatments for women with stress-related disorders.

In spite of clinical and preclinical evidence of sex differences female rats are often omitted from preclinical research studies, as illustrated in figure 1.7; this graph is adapted from Robert Hughes (2007). Data for this survey was gleaned from five journals regularly publish behavioural pharmacology studies using rats, namely, Behavioural Brain Research, Behavioural Pharmacology, Pharmacology, Biochemistry and Behaviour, Physiology and Behaviour and Psychopharmacology. *In vitro* studies of drug effects on brain neurochemistry in the absence of behavioural data were not included, nor were studies of sex-specific behavioural or sex-specific biological characteristics. Of 567 articles published using rats (during 2005-2006), the vast majority employ males only (Hughes, 2007). It must be said however that acknowledgement of sex differences and their investigation is increasing, between the late seventies and the late nineties there was a steady rise in the research of sex differences. Figure 1.8 was compiled based on a PubMed search using the search terms “sex differences, rat”.

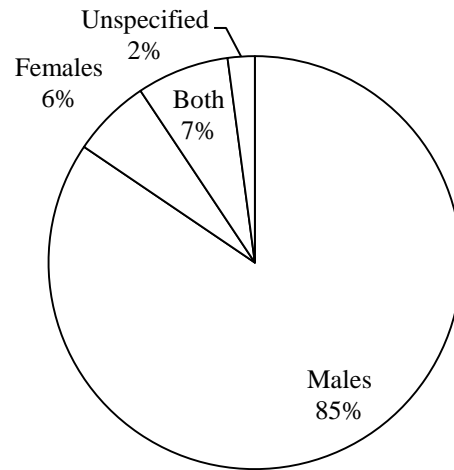


Figure 1.7: Survey of articles employing male and / or female rats, adapted from (Hughes, 2007).

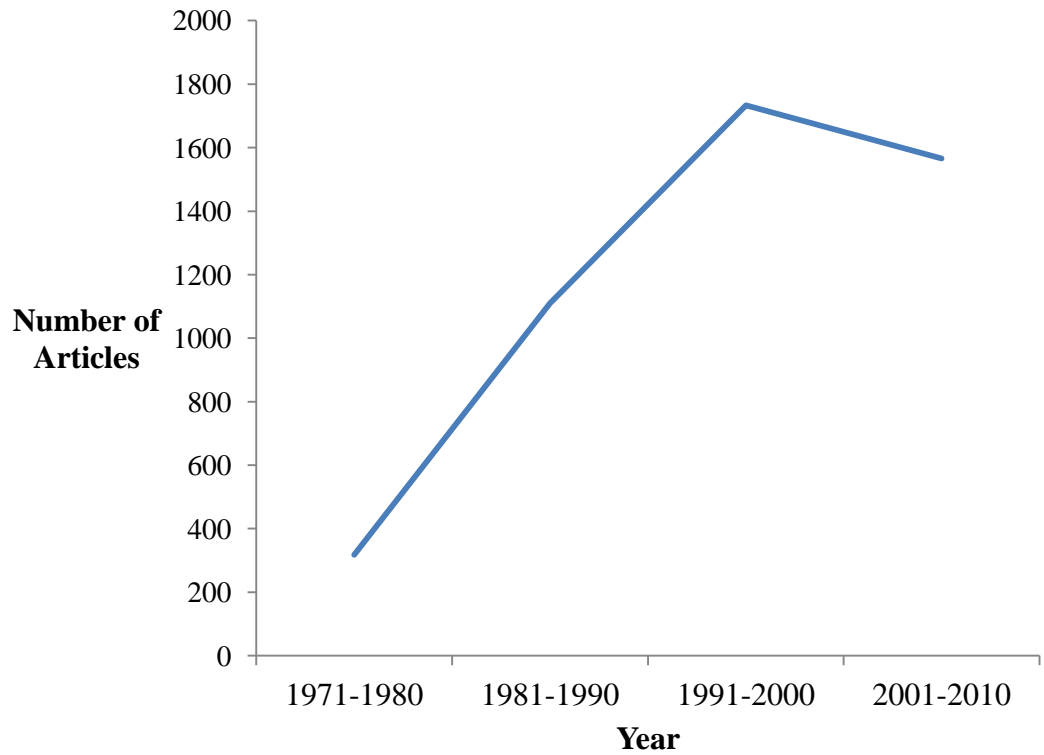


Figure 1.8: A PubMed search using the search terms “sex differences, rat” of articles published between 1971 and 2010. Overall the search retrieved 4862 articles.

The Estrous Cycle

The omission of females from some studies is due in part to the female estrous cycle. The rat cycle consists of 4 to 5 days (Fig. 1.9). Progesterone increases sharply, beginning early in the post ovulation phase (i.e. metestrous) on day 1 and drops sharply in diestrous on day 2. During proestrous, estrogen levels markedly surge, followed by increased progesterone secretion; this hormonal surge triggers ovulation. Hormones return to baseline levels when ovulation occurs (i.e., estrus) on day 4 followed by a brief temporary peak of estrogen on the evening of estrus (Emanuele et al., 2002). The estrous cycle is thought to interfere with experimental manipulations by introducing variability; however Camp and colleagues reported greater inter-subject variation in locomotor activity by male rats following amphetamine compared to females (Camp and Robinson, 1988). That being said, the female reproductive hormones and estrous cycle has been shown to influence behaviour in the OFT, EPM, FST, MWM, NOR test and other cognitive tasks. In the OFT some have shown there was no difference in distance moved or percent time spent in the centre of the arena for female rats in proestrous and diestrous phases (Hiroi and Neumaier, 2006), whereas others demonstrated that females in the proestrous phase experiencing peak estrogen levels made more central crossings than those in diestrous or male rats (Frye and Walf, 2002). Likewise treating ovariectomised (OVX) females with estradiol increased time spent in the centre of the arena compared to OVX oil-treated controls and females treated with estradiol and progesterone (EP) combined replacement. Thus estrogen may reduce anxiety in the novel open field arena, while progesterone effects can counteract this (Hiroi and Neumaier, 2006). Mirroring the effects in open field exploration female rats in the proestrous and estrous cycle phases displayed significantly greater percent open arm time and open arm entries in the EPM compared to male rats and to those in diestrous (who did not significantly differ from one another) (Mora et al., 1996, Marcondes et al., 2001). In addition, administration of estradiol to females in the diestrous phase increased time spent in the open arms to a level comparable to that of rats in the proestrous phase (Marcondes et al., 2001). In the FST, females generally show lower duration of immobility and greater time spent climbing and/or swimming than males and it has been proposed that this does not vary according to estrous cycle phase (Barros and Ferigolo, 1998). Varying doses of the tricyclic antidepressant imipramine (5, 10 and 15 mg kg⁻¹) had no effect on immobility

duration for female Wistar rats but did reduce immobility frequency, and this was evident in each phase of the estrous cycle (Barros and Ferigolo, 1998). On the other hand however Marvan and colleagues (1996) found clomipramine to reduce females' immobility time in the diestrous phase only (Marvan et al., 1996).

Some suggest that estrous hormones may contribute to female rats' performance in the MWM; intact females have higher latencies compared to OVX females (Daniel et al., 1999). Similarly, latencies to locate the platform were negatively affected by high estrogen levels as MWM latencies were reduced during the estrous phase of the female cycle compared to those in proestrous, during which estrogen levels are highest (Warren and Juraska, 1997, Pompili et al., 2010). On the other hand, as estrogen is associated with cognitive improvements Walf and colleagues (2006) examined estrous effects on NOR. During proestrous, time spent exploring the novel object was increased and correlated positively with serum estrogen and progesterone metabolites in the hippocampus and prefrontal cortex. OVX female rats treated with estradiol or progesterone improved novel object recognition compared to saline controls (Walf et al., 2006). These results highlight the importance of female steroid hormones in mediating the emotional responses in tests of anxiety and depression-like behaviours, and the sensitivity of these and memory/learning tasks to female hormonal states. Hormonal changes during the ovarian cycle can affect females' responsiveness to drugs (Becker and Cha, 1989, Hu and Becker, 2003). Cycle phase has thus been shown to affect anxiety behaviours, learning and even pain sensitivity (Frye et al., 1993). The alternatives proposed to avoid the estrous cycle effects are to test/administer drugs during diestrous only, employ males only, or use a counterbalanced design to average any variations (Hughes, 2007).

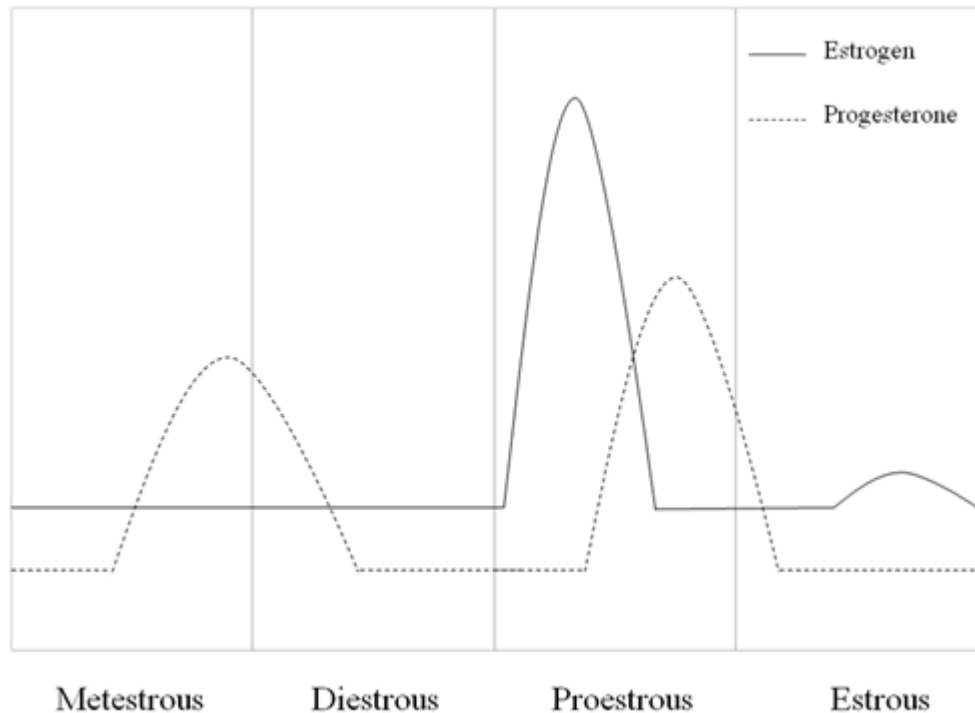


Figure 1.9: Rat estrous cycle. Progesterone and estrogen fluctuations across the 4-day rat estrous cycle, adapted from (Emanuele et al., 2002, Blaustein, 2008).

1.2.1 Sex Differences in Tests of Depression/Anxiety-like Behaviours

Sex differences have been identified in general activity and in a number of behavioural tests of anxiety and depression, as well as in cognitive and learning tasks. The differences in baseline activity between male and female rats contribute to their divergent profiles in some behavioural pharmacology tests. Though sex differences in various tasks cannot be fully explained by sex hormones, the rat estrous cycle does influence behavioural, cognitive and neurochemical endpoints (Cahill, 2006). Some of these sex differences in behavioural tests are summarised in table 1.9.

In the EPM, female rats displayed greater percent open arm time (Weintraub et al., 2010), increased percent open arm entries (Johnston and File, 1991a, Lucion et al., 1996) and increased overall activity as measured by greater total arm entries and distance moved (Pena et al., 2006) compared to male counterparts. On the other hand, Mazor and colleagues (2009) found that female SD rats displayed reduced open arm time on the EPM compared to males; and there was no difference in open arm entries or in overall distance moved between the two sexes (Mazor et al., 2009). Ovarian hormones can affect anxiety-like measures in the EPM as female rats

displayed significantly greater percent open arm time and open arm entries when in the proestrous and estrous cycle phases compared to those in diestrous and male rats (who did not significantly differ from one another) (Mora et al., 1996, Marcondes et al., 2001). Moreover, treating females in diestrous with estradiol produced plasma estrogen levels similar to those during proestrous and eliminated the difference in percent time spent in the open arms by proestrous and diestrous rats (Marcondes et al., 2001).

Overall EPM results suggest that male rats show a more anxious-like profile on the EPM than females. In an effort to address this, male and female hooded rats of equivalent weight (9–10 weeks old) were employed to assess sex differences across three different tests, all of which are thought to measure behavioural indices of rats' anxiety. In the EPM, females showed greater percent open arm entries than males, suggesting they were less anxious than males. On the other hand, in a social interaction test males spent more time engaged in social interaction and exploration than females, which might suggest greater anxiety by females. Finally, a Vogel conflict test was carried out; this test requires the rat to "choose" between their natural drive to consume water and the risk of being punished by a mild electric shock; an increase in water consumption is thought to reflect reduced anxiety. Male rats engaged in significantly more licking during the punished licking period compared to females, this would suggest female rats show more anxiety than males in the Vogel test. Though sex differences were revealed in all three tests, they were not consistent, implying that the three tests were not measuring the same variable and these were not reflective of the same traits in male and female rats. Moreover, the increased open arm activity in the EPM and the reduced level of social interaction shown by female rats compared to males may be due to innate sex differences in baseline activity and social interaction, and the authors propose differences in shock sensitivity contributed to the sex difference seen in the Vogel test (Johnston and File, 1991a). Overall, the results show that anxiety tests may not be measuring the same emotional-driven response in both male and female animals.

In the OFT female rats' baseline activity levels are generally found to be higher than males. Female rats display greater horizontal locomotor activity over 1 hour (Elliott and Grunberg, 2005) and greater ambulatory scores and wall-climbing in a three-minute OFT compared to male counterparts (Beck and Luine, 2002).

Females of the SD and Long Evans rat strains displayed greater distance moved and rearing behaviour compared to males (Padilla et al., 2009, Brotto et al., 2000); in a study of Wistar rats however no sex difference in locomotor activity was found but females displayed more rearing activity than males (Dalla et al., 2005). This illustrates the impact variables such as rat strain can have on behavioural endpoints and results comparisons between laboratories. In a study of estrous cycle effects on OFT behaviour there was no difference in distance moved or percent time spent in the centre of the OFT arena for female rats in proestrous and diestrous phases (Hiroi and Neumaier, 2006). However when OVX females were treated with estradiol they spent more time in the centre of the arena compared to OVX oil-treated controls and OVX females treated with EP. Thus estradiol may have reduced anxiety in the novel OFT environment, while progesterone effects counteract this (Hiroi and Neumaier, 2006).

Sex differences are also seen in studies of depression-like symptoms. In a “learned helplessness” stress model, male and female rats were exposed to a mild footshock, with or without the opportunity to escape, thus termed controllable or uncontrollable stress. One day later all animals were exposed to a more difficult escape task in a different context. Most males that were previously exposed to uncontrollable stress did not learn to escape, thus displaying “learned helplessness”. In contrast, most females learned to escape, regardless of previous stress exposure. OVX and/or castration (CAST) did not prevent sex differences in learned helplessness, prompting the authors to investigate the effects of exposure to testosterone during perinatal development on the expression of learned helplessness later in life. Even masculinisation of the developing female brain (achieved by administration of testosterone propionate within 24 h of birth) did not result in helplessness in adult females (Dalla et al., 2008b). Male and female rats thus respond differently to experimental models of depression. Accordingly, sex differences exist in activity in the FST, with females spending reduced time immobile and more time struggling / climbing compared to males (Barros and Ferigolo, 1998, Brotto et al., 2000). Kokras and colleagues (2009) found this to be true of Flinders Sensitive Line (FSL) rat strain; however no sex difference was seen for SD rats (Kokras et al., 2009); as the authors employed a single 5-minute swim test without the usual pretest,

this may not have been sufficient to promote immobility for the SD strain compared to the selectively bred “depressed” FSL strain.

Overall female rats appear to display greater activity levels in the OFT, greater open arm activity in the EPM and greater time spent struggling in the FST compared to male rats. However whether these results imply females are less anxious than males or merely reflect baseline differences in activity is not clear. The impact of the estrous cycle also modulates these behaviours, thus the results from studies employing these and similar types of tests with female rats must be interpreted with caution.

1.2.2 Sex differences in Tests of Learning and Memory

In addition to differences in common tests of anxiety and depressive-like behaviours, male and female rats differ in performance in memory and learning tasks. Sex differences in the MWM have been somewhat inconsistent. According to a meta-analysis of RAM and water maze experiments, male rats had a significant advantage over females in MWM performance, in that they demonstrated reduced latencies to locate the escape platform compared to females. Pretraining protocols however tended to reduce male advantages over females, compared with experiments without pretraining protocols (Jonasson, 2005). Mazar and colleagues (2009) found no significant sex difference in baseline latencies in the MWM, however males who had been previously exposed to stress (predatory scent stress [PSS]) showed reduced escape latencies compared to previously-stressed females (Mazar et al., 2009). Similarly Bucci and colleagues (1995) found no sex difference in latency to find the platform for male and female rats aged approximately 26 weeks and suggested the age of the animal contributes to sex differences (Bucci et al., 1995). In contrast to this theory however Harris and colleagues (2009) found adult (18-20 weeks old) male rats had reduced latencies to the platform during the first swim trials compared to females, however they failed to find a sex difference between younger rats (14-15 weeks old). The authors suggest that the younger rats of each sex were equally affected by the stress of the test as they displayed equivalent thigmotaxis; while the older rats were differentially affected by the acute stress of the test itself (males displayed less thigmotactic behaviour than females) (Harris et al., 2009). Estrogen appears to have a negative impact on MWM performance as gonadally intact female rats displayed significantly shorter times in the platform

quadrants and fewer target crossings during probe trials than OVX females and male rats (Daniel et al., 1999). Pompili and colleagues suggest that high levels of estrogen in the proestrous phase may have a negative effect on spatial reference memory as female rats in proestrous had longer escape latencies than those in the estrous phase (Pompili et al., 2010).

In a meta-analysis of the RAM male rats display significant advantages in performance over females and these advantages were greater for males in protocols which assessed working memory only, compared to working and reference memory combined protocols. Male advantages were also slightly smaller in working memory protocols which used 17-arm versus 8-arm mazes (Jonasson, 2005). Thus methodological variables can interact with sex differences to impact on performance in cognitive tasks. Similarly estrous cycle effects on RAM performance can depend on the nature of the task; working memory errors were higher when estrogen levels were low, compared to proestrous females, while reference memory errors were higher when females were in proestrous compared to estrous phases (Pompili et al., 2010). Thus estrogen can have differential effects on memory depending on the nature of the memory task.

The NOR test is also widely used to assess memory retrieval and spatial memory. In the NOR, females appear to have better memory for novel objects as female rats spent significantly more time exploring a novel object after 1, 2.5 and 4 hour retention periods compared to males (Sutcliffe et al., 2007, Beck and Luine, 2002). Female rats showed significant discrimination between novel and familiar objects for up to 3 hours inter trial interval (ITI), while males only showed significant discrimination for up to 1 hour (Ghi et al., 1999, Sutcliffe et al., 2007), demonstrating females' longer lasting memory retrieval compared to males. On the other hand however females' spatial recognition, i.e. recognition of a displaced object, is poorer than that of males. Female hooded rats exhibited novel location recognition for up to 1 hour ITI, while males showed preference for the moved object for up to 3 hours (Sutcliffe et al., 2007). Likewise, at 2.5 and 4 hours ITI female SD rats did not show any significant preference for a moved object versus an unmoved one, while males made significant spatial object discriminations at each time point (Beck and Luine, 2002).

Classical and operant conditioning paradigms are also used to assess learning and memory in laboratory rodents. Sex differences have been identified in these protocols, and these differences are often task-dependent. Females respond better to eyeblink conditioning tasks as they seem to anticipate the unconditioned stimulus and emit a more well-timed eyeblink response than males (Waddell et al., 2010, Wood and Shors, 1998, Dalla and Shors, 2009). The development of eyeblink conditioning is influenced by sex hormones as the sex difference is most evident when training is carried out during the proestrous cycle phase (Wood and Shors, 1998). However sex differences are evident throughout the estrous cycle, even in the diestrous phase when estrogen and progesterone levels are low (Dalla and Shors, 2009), thus other contributing factors play a role in the sex difference in eyeblink conditioning. Females also out-perform males in operant conditioning tasks as they learn to escape sooner than males do in avoidance tasks (Dalla et al., 2008b, Shors et al., 2007). Sex differences in these tasks are thought to be due in part to differences in baseline activity and sex-specific responses to stress as females typically engage more actively toward aversive stimuli, while males typically exhibit freezing behaviour (Padilla et al., 2009, Maren et al., 1994, Albonetti and Farabollini, 1995). Due to this difference in activity in response to stressful situations, males tend to outperform females in response to fear conditioning, with females displaying less conditioned freezing than males (Pryce et al., 1999). This also appears to be influenced by ovarian hormones as OVX females exhibited increased freezing behaviour compared to intact control females; moreover, estradiol administration to OVX rats reinstated active behaviour to the level of intact controls (Gupta et al., 2001).

Sex differences in tests of memory and learning appear to be dependent on the test employed and on the behavioural parameter of interest, in addition to the effects of the estrous cycle. Females perform better in NOR, but are poorer than males in tests requiring spatial memory. Sex differences in conditioning paradigms are somewhat governed by the requirements of the task, in procedures where active avoidance is rewarded females perform better than males, while in contrast, when passive responding is rewarded males outperform females.

Table 1.9: Sex differences in commonly-employed behavioural tests

Test	Parameter	Sex difference	Reference
OFT	Locomotor activity	Females > Males	(Padilla et al., 2009, Brotto et al., 2000)
		Females = Males	(Mazor et al., 2009)
	Rearing	Females > Males	(Dalla et al., 2005)
EPM	Locomotor activity	Females > Males	(Pena et al., 2006)
	Open arm time	Females > Males	(Marcondes et al., 2001)
		Females < Males	(Mazor et al., 2009)
	Open arm entries	Females > Males	(Lucion et al., 1996, Johnston and File, 1991a)
Vogel Conflict Test	Number of punished licking responses	Females < Males	(Johnston and File, 1991a)
Social Interaction	Time spent exploring unfamiliar cagemate	Females < Males	(Johnston and File, 1991a)
Learned Helplessness	Failure to escape based on prior inescapability	Females < Males	(Dalla et al., 2008b)
FST	Time spent swimming/climbing	Females > Males	(Barros and Ferigolo, 1998)
	Time spent immobile	Females < Males	(Brotto et al., 2000)
MWM	Latencies to escape platform	Females = Males	(Mazor et al., 2009)
		Females < Males (post-stress)	(Mazor et al., 2009)
		Females > Males	(Harris et al., 2009)
NOR	Time spent exploring novel object	Females > Males	(Sutcliffe et al., 2007, Ghi et al., 1999)
	Time spent exploring displaced object	Females < Males	(Sutcliffe et al., 2007, Beck and Luine, 2002)

Females >, greater than; < less than; or =, equal to males. OFT, open field test; EPM, Elevated plus maze; FST, Forced swim test; MWM, Morris water maze; NOR, novel object recognition test.

1.2.3 Sex Differences in Response to Stress

The stress hormones CORT and ACTH are measured to assess sex differences in stress responses. Some studies have demonstrated higher baseline plasma CORT levels in female rats compared to males (Yoshimura et al., 2003, Weiss et al., 2004, Weinstock et al., 1998), furthermore, female CORT levels are influenced by estrous cycle stage, with CORT levels reaching a peak during proestrous and declining during estrous (Atkinson and Waddell, 1997). In addition, male and female rats respond differently to stressful situations and a number of studies investigate the effects of stress exposure on subsequent behaviour. Exposure to PSS increased anxiety-like behaviour in the EPM for both male and female SD rats compared to non-stressed controls; however the magnitude of change was greater in males than females. On the other hand, post-stress females displayed a greater increase in acoustic startle response (ASR) than their male counterparts (Mazor et al., 2009). Thus the anxiety test employed may be sensitive to sex differences. Chronic mild stress (CMS) is a model of depressive symptoms in laboratory rodents. It involves exposing the animal to a number of mild stressors over a period of time; stressors include soiled cage, tilting of the cage, alterations of the light-dark cycle, periods of food or water deprivation, crowding or social isolation (Willner, 1997). CMS has differential effects on male and female rats; females' locomotor activity was reduced to a greater extent than males, as were 5HT levels in the hypothalamus and hippocampus, additionally, females' plasma CORT levels were increased to a greater level than males and the female estrous cycle was disrupted after CMS (Dalla et al., 2005). A slightly different CMS protocol employed by the same research group found that stress reduced sucrose consumption in both males and females but the effect was greater for males, however while stress exposure reduced DA levels in the PFC of females there was no such effect on males (Dalla et al., 2008a). Other studies have demonstrated CMS increased plasma CORT, increased adrenal weight and decreased thymus weight in both males and females equally, with males showing reduced growth after stress exposure while stress had little or no impact on female growth (Trentani et al., 2003, Westenbroek et al., 2003a, Westenbroek et al., 2004, Westenbroek et al., 2005, Lin et al., 2009, Lin et al., 2008). Thus the effects of anxiety tests such as the CMS can differ in male and female rats; effects can depend on the endpoint measured and the nature (how is mild defined?) and duration (how is chronic defined?) of the stressor.

Housing conditions can interact with sex to impact on stress responses. CMS enhanced locomotor activity in the OFT and this was most pronounced in females housed in IC compared to those in SC (Weinstock et al., 1998). On the other hand, in a foot shock stressor paradigm, housing condition had no effect on male rats' locomotor activity (Westenbroek et al., 2003a, Westenbroek et al., 2003b). Stress-induced increased adrenal weight was found only in IC females; while opposite effects of IC and SC housing were found for male rats as SC males displayed increased adrenal weight compared to those in IC (Westenbroek et al., 2003a). Similarly, Weintraub and colleagues (2010) demonstrated that female SD rats housed in IC during adolescence (PND 30-50) had greater CORT and ACTH increases in response to restraint stress than those reared in SC. Conversely, males housed in IC had lower stress responses to restraint stress than their SC counterparts (Weintraub et al., 2010). Thus, for male rats, SC housing appears to be more stressful than IC, while for female rats SC housing may be protective against the effects of stress exposure.

1.2.4 Sex Differences in Monoamine Neurotransmitter Levels

Sex differences in various behavioural tests are concomitant with differences in neurotransmitter levels. For the most part, data on sex differences in neurotransmitter levels focus on monoamines, DA in particular as this system is regulated by the ovarian cycle. Sex differences in DA levels have been detected in various brain regions, among the most robust are in the striatum. In both *in vivo* and *in vitro* analyses, fast cyclic voltammetry revealed female SD rats had greater DA uptake and greater DA release in the striatum compared to males (Walker et al., 2000). Similarly, though male and female SD rats did not differ in basal striatal tissue DA levels, females had a lower concentration of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) compared to males (Bisagno et al., 2003). Differences in the dopaminergic system of male and female rats have been demonstrated in studies of AMP-induced DA release. Female rats display enhanced behavioural responses to both acute and chronic AMP treatment (Bisagno et al., 2003, Becker et al., 1982) and this effect remains even when AMP levels in the brains of male and female rats are equivalent (Becker et al., 1982). The ovarian hormone system is implicated in mediating these sex differences in the striatal dopaminergic system; female SD rats have significantly higher basal extracellular

striatal DA during the proestrous and estrous cycle phases than in diestrous or after OVX surgery. In contrast, CAST has no effect on male rats' extracellular striatal DA (Xiao and Becker, 1994). Notably, sex differences in basal DA exist even in gonadectomised rats as CAST male rats have higher basal striatal DA than OVX females. Treating OVX females with estradiol produced significantly greater AMP-induced DA release in the striatum (relative to baseline DA levels) compared to oil-treated controls (Becker, 1990a, Castner et al., 1993), while no such effect was seen in CAST male rats (Castner et al., 1993).

Sex differences are also seen in DA levels in other brain regions. In the PFC female Long Evans rats had greater DA but lower DOPAC and DA turnover compared to males (Duchesne et al., 2009). On the other hand, Dalla and colleagues (2008) demonstrated lower DA levels and higher DA turnover in female Wistar rats compared to males (Dalla et al., 2008a). Finally Bisagno and colleagues (2003) failed to find any sex difference in PFC DA levels (Bisagno et al., 2003). Similar conflicting results were found in the hippocampus, with Duchesne (2009) showing lower DOPAC and DA turnover in the female dorsal hippocampus compared to males (Duchesne et al., 2009); while Dalla (2008a) reported lower hippocampal DA and homovanillic acid (HVA) levels, but higher DA turnover in females compared to males (Dalla et al., 2008a). The divergence in the results of these two studies may be related to the use of different rat strains and animal age. In addition, Duchesne (2009) pooled the neurochemical data for control and neonatally-handled rats to assess sex differences, which may have an impact on results. In other regions, female Long Evans rats had greater DA levels in the insular cortex, but lower DOPAC and DA turnover in the amygdala and insular cortex compared to males (Duchesne et al., 2009). In the hypothalamus, DA levels were lower, and DA turnover was higher, in female Wistar rats compared to males (Dalla et al., 2008a). No sex differences (Bisagno et al., 2003) or estrous cycle effects (Zhang et al., 2006) were found in DA levels in the nucleus accumbens or substantia nigra of SD rats (Bisagno et al., 2003).

Sexual dimorphisms have also been demonstrated in the 5HT system of the rat brain. Striatal 5HT levels were higher in female SD rats compared to males, while no 5HT differences were found between males and females in the nucleus accumbens, substantia nigra or ventral tegmental area (VTA) (Zhang et al., 2006,

Bisagno et al., 2003). Nor were differences observed in 5HT, 5HIAA or 5HT turnover in the hypothalamus, hippocampus or PFC (Bisagno et al., 2003, Pitychoutis et al., 2009). In a study of male and female SD rats, housed in IC or SC conditions and exposed to stress (21 days of 6 hour restraint stress) or unstressed controls, data were pooled for sex analysis of neurochemical data. Females had higher 5HIAA and HVA levels in the PFC; however there was no main effect of sex in the hippocampus or amygdala (Beck and Luine, 2002). On the other hand, female Long Evans adult rats had greater 5HT levels in the PFC, amygdala, insular cortex and dorsal hippocampus than males; while male rats had greater 5HT turnover in all of the above regions and greater 5HIAA in all but the dorsal hippocampus, compared to females (Duchesne et al., 2009).

Fewer studies have addressed sex differences in NA neurotransmitter levels, however females have been shown to have lower levels than males in the nucleus accumbens, without any differences seen in the striatum, PFC, substantia nigra or VTA (Bisagno et al., 2003). In young adult male and female rats (~ 60 days old) females had lower NA levels in the amygdala and hypothalamus than males, however a reversal took place around PND 120 and by 240 days old (8-9 months old) females had higher NA levels compared to males (Siddiqui and Shah, 1997). Thus rat sex and age may interact to effect neurochemical results. Finally Heal and colleagues (1989) found no difference in alpha or beta-adrenoceptors between male and female rats in a cortical binding study (rat strain was not specified) (Heal et al., 1989).

The sex differences observed in monoamine levels in the rat brain are somewhat inconsistent, as summarised in table 1.10. Sex differences are not homogenous due to factors such as rat age, strain, the effects of the estrous cycle and experimental challenges such as stress or environmental conditions, all of which can influence neurotransmission in a sex-specific manner.

Table 1.10: Sex differences in monoamine concentrations in various brain regions

Neurotransmitter	Brain region	Sex difference	Reference
DA	Striatum	Females = Males	(Bisagno et al., 2003)
	PFC	Females = Males	(Bisagno et al., 2003)
		Females > Males	(Duchesne et al., 2009)
		Females < Males	(Dalla et al., 2008a)
	Insular cortex	Females < Males	(Duchesne et al., 2009)
	Hypothalamus	Females > Males	(Dalla et al., 2008b)
	Nucleus	Females = Males	(Bisagno et al., 2003)
	Accumbens	Females = Males	(Bisagno et al., 2003)
	Substantia Nigra		
5HT	Striatum	Females > Males	(Zhang et al., 1999)
	PFC	Females = Males	(Bisagno et al., 2003)
		Females > Males	(Duchesne et al., 2009)
	Insular cortex	Females > Males	(Duchesne et al., 2009)
	Hypothalamus	Females = Males	(Pitychoutis et al., 2009)
	Nucleus	Females = Males	(Bisagno et al., 2003)
	Accumbens	Females = Males	(Bisagno et al., 2003)
	Substantia Nigra	Females = Males	(Zhang et al., 1999)
	VTA		
	Hippocampus	Females = Males	(Pitychoutis et al., 2009)
	(Dorsal)	Females > Males	(Duchesne et al., 2009)
	Amygdala	Females > Males	(Duchesne et al., 2009)
NA	Striatum	Females = Males	(Bisagno et al., 2003)
	PFC	Females = Males	
	Nucleus	Females < Males	
	Accumbens		
	Substantia Nigra	Females = Males	
	VTA	Females = Males	

Females >, greater than; <, less than; or = equal to males; DA, dopamine; 5HT, serotonin; NA, noradrenalin; PFC, prefrontal cortex; VTA, ventral tegmental area

1.2.5 Sex Differences in Neurotrophic Factor Expression

Neurotrophic factors, including BDNF, NGF and NT3 are growth factors, so called for their roles in cell survival and growth (Allen and Dawbarn, 2006). Ovarian hormones, estrogen in particular, can mediate neurotrophic factor levels in the brain. This is of clinical relevance as estrogen replacement treatment may be useful in reducing Alzheimer's symptoms in post-menopausal women (Yaffe et al., 1998). Loss of ovarian function leads to decreased basal forebrain cholinergic function (Gibbs, 1998a), while estrogen replacement increases forebrain cholinergic activity in rats (Gibbs, 2000). In studies of sex comparisons in neurotrophic factor expression in rats, BDNF is most commonly assayed and some results are summarised in table 1.11.

In terms of sex differences in growth factor levels, Lister-Hooded female rats had greater BDNF mRNA levels in the orbital, retrosplenial, frontal and parietal cortices than males (Snigdha et al., 2011). In contrast, no difference in BDNF mRNA in the retrosplenial cortex was found between male and female SD rats (Matsuki et al., 2001). Similarly Lin (2009) and Bimonte-Nelson (2008) did not find any sex difference in BDNF levels in frontal cortex tissue of male and female Wistar and Fischer rats (Lin et al., 2009, Bimonte-Nelson et al., 2008), thus BDNF mRNA levels do not necessarily directly translate to BDNF protein concentration. In the hippocampal CA3 region BDNF was significantly higher, while in the dentate gyrus BDNF was lower in females than males (Franklin and Perrot-Sinal, 2006, Lin et al., 2009); in addition there was no sex difference detected in tissue BDNF levels in the CA1 region of the hippocampus (Franklin and Perrot-Sinal, 2006). In a study of age and sex effects on neurotrophic factors, female rats had greater BDNF levels in the hippocampus at 20 months old and greater striatal BDNF at 7 months old compared to males. There was no sex difference in NGF levels in the hippocampus, frontal cortex or entorhinal cortex of male and female Fischer rats. At 4 months old, male rats had greater NGF levels in the striatum, however this was not significant at 7, 20 or 24 months old. Overall age had a different effect on NGF and BDNF concentration as NGF overall increased, while BDNF decreased, from 4 to 24 months old in both males and females (Bimonte-Nelson et al., 2008). In the cerebellum, no sex differences were found in BDNF, NGF or NT3 protein of young (PND 21) male and female SD rats (Sajdel-Sulkowska et al., 2009); nor were there

sex effects on BDNF mRNA in the ventromedial nucleus of adult male and female Wistar rats (Sugiyama et al., 2003).

A number of studies have investigated estrogen effects on growth factor expression, in a study of intact female SD rats, *trkA* (the NGF receptor) mRNA in the medial septum (MS) and BDNF mRNA in the hippocampus display estrous cycle-dependent fluctuations. *TrkA* and BDNF mRNA levels were at their highest during diestrous and at their lowest during proestrous, when estrogen levels are low and high respectively. NGF mRNA levels on the other hand were unaffected by cycle stage (Gibbs, 1998b). To further evaluate the effects of estrogen on neurotrophic factors, females undergo OVX surgery and are administered estradiol alone or estradiol followed 24-48 hours later by EP combined hormone replacement. Rats are then sacrificed at various timepoints following estradiol treatment and neurotrophic factor levels in various brain regions are compared to oil-treated controls. In such experiments estradiol treatment has been shown to decrease BDNF in the dentate gyrus compared to vehicle controls, but increase BDNF in the CA3 hippocampal region compared to EP treated animals (Franklin and Perrot-Sinal, 2006). A similar study also found hippocampal BDNF to be lower in estradiol-treated females than in controls (Gibbs, 1999). When BDNF mRNA levels were assayed in the hippocampus estradiol replacement increased levels in the dentate gyrus, CA1, 3 and 4 compared to controls (Gibbs, 1998b); in addition EP combined treatment also increased hippocampal BDNF mRNA compared to oil-treated controls (Gibbs, 1999). In the MS, estradiol resulted in increased *trkA* mRNA expression, and this increase was maintained over time in the EP combination condition (Gibbs, 1998b). Similarly, estradiol and EP enhanced BDNF mRNA in the pyriform cortex, while EP increased BDNF protein levels in the same region compared to oil-treated controls (Gibbs, 1999).

Overall, sex differences are seen in neurotrophic factor expression in the rat brain and may also be influenced by rat age. Incongruous results may be attributable to methodological approaches as the extent of mRNA expression does not necessarily translate to protein expression in the brain tissue. In addition neurotrophic factor expression is influenced by estrogen (and progesterone) in a region-specific manner. It is likely that estrogen receptor expression accounts for the

differences in estrogen effects in various regions (Jezierski and Sohrabji, 2000). Due to the estrogen/growth factor association BDNF protein levels in the hippocampus also vary in a sex-dependent manner. This may contribute to sex differences in stress responses as stress exposure can impact rat neurotrophin levels (Franklin and Perrot-Sinal, 2006, Lin et al., 2009). Furthermore, stress affects the levels of BDNF in various brain regions associated with the HPA axis; stress increases BDNF mRNA in the hypothalamus (Rage et al., 2002), and decreases BDNF mRNA in the hippocampus (Xu et al., 2002). Finally reduced neurotrophin levels are associated with depression and mood disorder in clinical populations (Sheline, 2003, Brunoni et al., 2008), which are more prevalent in women than men (Payne, 2003).

Table 1.11: Sex differences in BDNF expression in various brain regions

BDNF Measure	Brain region	Sex difference	Reference
mRNA	Orbital cortex	Females > Males	(Snigdha et al., 2011)
	Parietal cortex		
	Frontal cortex		
	Retrosplenial cortex	Females > Males	(Snigdha et al., 2011)
		Females = Males	(Matsuki et al., 2001)
BDNF protein	Frontal cortex	Females = Males	(Bimonte-Nelson et al., 2008, Lin et al., 2009)
	Hippocampus	Females > Males (CA3)	(Franklin and Perrot-Sinal, 2006)
		Females < Males (DG)	(Lin et al., 2009)
		Females = Males (CA1)	(Franklin and Perrot-Sinal, 2006)

Females >, greater than; <, less than; or = equal to males; BDNF, Brain-derived neurotrophic factor; DG, dentate gyrus.

1.2.6 Sex Differences in Neurogenesis and Plasticity

As ovarian hormones influence neurotrophic factors, it is not surprising that sex differences exist in hippocampal dendritic spine density and LTP, which are indicators of brain plasticity. Male SD rats displayed significantly greater hippocampal-dependent excitatory postsynaptic potential (EPSP) LTP, as measured by greater EPSP slope in the dentate gyrus, compared to females. This was accompanied by male rats displaying higher levels of Pavlovian fear-conditioned freezing behaviour following prior exposure to footshock than females (Maren et al., 1994). Sexual dimorphism in hippocampal plasticity, as measured by LTP, is likely to play a role in producing sexually dimorphic responses in hippocampal-related tasks such as Pavlovian fear conditioning (Maren et al., 1994). Though no sex difference in hippocampal cell birth or immature cell number was seen, Mandyam and colleagues (2008) found female SD rats had fewer mature neurons in the subgranular zone (SGZ) of the dentate gyrus than males. Moreover, female rats also had greater indices of cell death in the SGZ and reduced levels of IL-1 β , an anti-apoptotic cytokine, compared to males. However estimation of hippocampal and SGZ volume resulted in no sex differences, thus increased cell death in females did not translate to changes in the hippocampal structure (Mandyam et al., 2008).

Mandyam and others (2008) also investigated sex differences in adulthood hippocampal morphology following prenatal stress (PS); the dam was exposed to either restraint stress (PS restraint) or random stressors (PS random) such as saline injection or footshock. Cell proliferation was equally reduced in males and females exposed to PS. Overall stress had a greater effect on males than females in the SGZ; PS restraint increased markers of young neurons in both males and females, while PS random elevated young neuron markers, but only in male rats. Both stress conditions increased cell markers of apoptosis in males but not females, stress also reduced IL-1B expression in males compared to saline controls, but there was no such effect in females. Overall however, PS random reduced hippocampal and dentate gyrus volume in both male and female rats (Mandyam et al., 2008). Thus prenatal stress produced sex and stress specific effects on dentate gyrus neurons in addition to long-lasting effects on hippocampal structure in both male and female rats. Westenbroek and colleagues (2004) also found sex differences in baseline and stress – related responses in hippocampal cell proliferation and survival. Male and

female Wistar rats were housed in IC or SC conditions, then half of the animals from each rearing condition were exposed to a 3 week chronic stress protocol (footshock) and the other half remained undisturbed as unstressed controls. Cell proliferation and survival were assessed using bromodeoxyuridine (BrdU), this nucleoside is injected into the rat and becomes incorporated into the DNA of replicating cells, antibodies specific for BrdU can then be used to detect the incorporated chemical, indicating cells that are proliferating (Wojtowicz and Kee, 2006). Among unstressed animals housed in SC, females had significantly greater BrdU-positive cells than males; however there was no sex difference in baseline BrdU labelling in IC housed animals. Stress decreased proliferation in IC-housed male rats, but not SC-housed males. Females housed in IC however displayed stress-induced increased BrdU labelling, which was prevented by SC housing. As stress or housing had no effect on female proliferation, changes seen in the female dentate gyrus are likely due to cell survival (Westenbroek et al., 2004). Thus stress and social support or SC rearing conditions can affect neurogenesis in different ways in male and female rats. Others have also shown sex differences in response to stress; Shors and colleagues (2007) proposed that controllability of a stressor modulates neurogenesis in the adult male, but not female rat hippocampus. Male rats that learned to escape foot shock stress had significantly greater cell proliferation in the dentate gyrus compared to yoked counterparts. There was no difference in cell proliferation however between females who could or could not escape stress (Shors et al., 2007). Similarly, PSS decreased proliferation in the hippocampus of male rats but not females (Falconer and Galea, 2003).

Ovarian hormones affect dendritic spine density in the female hippocampus. During the estrous cycle, females in proestrous have significantly greater apical spine dendrites in hippocampal CA1 pyramidal neurons compared to males and females in diestrous (Shors et al., 2001) and estrous (Woolley et al., 1990). This also appears to be specific to CA1 apical spines as no sex effect was observed in basal dendrites or in CA3 pyramidal neurons or granule cells in the dentate gyrus (Woolley et al., 1990). The estrous cycle modulation of dendritic spines was mirrored in variations in LTP, females have significantly greater LTP responses to stimulation on the afternoon of proestrous than during diestrous, estrous or compared to male rats (Warren et al., 1995). These results suggest that estrogen levels

promoted dendritic growth and plasticity. To assess estrogen effects on CA1 apical spine density OVX females treated with estradiol or vehicle (oil) were compared to intact females. OVX oil-treated females had significantly lower spine density than intact females; while estradiol treatment increased spine density to the same level as seen in intact controls (Gould et al., 1990).

Some studies suggest that female rats have greater plastic potential in the brain and are less affected by stress than males, other evidence however does not agree. Individual markers of cells in the hippocampus differ between males and females but overall hippocampal volume, relative to body weight, does not seem to be affected by rat sex. The estrous cycle once more plays a role with an enhancing effect of estrogen on dendritic growth and plasticity.

1.2.7 Sex Differences in Responsiveness to Drugs of Abuse

Men and women differ in patterns of drug use and the development of drug abuse disorders (for review see (Becker and Hu, 2008, Brady and Randall, 1999). Animal models have extended our knowledge of the role of sex and gonadal hormones on behavioural and neurochemical responses to drugs of abuse. As previously alluded to (section 1.2.4) male and female rats respond differently to drugs which activate the mesolimbic reward system and much preclinical research has been done with psychomotor stimulants such as COC and AMP.

Female rats acquire COC self-administration more readily than males (Carroll et al., 2002, Lynch and Carroll, 1999b), and a greater percentage of females acquired self administration compared to males (Lynch, 2008). Upon acquiring self administration, female rats display higher response rates in the progressive ratio (PR) schedule (Carroll et al., 2002, Lynch and Carroll, 1999a). A PR schedule refers to a progressive increment in responses required in order to gain reinforcement or reward, higher PR responding in females implies greater incentive motivation than males. There was no sex difference in PR responding in sucrose self-administration implying this was a drug-specific effect, moreover, females' responding positively correlated with plasma estrogen levels (Lynch, 2008). Self-administration responding for COC is highest during estrous, when estrogen levels are high, and progesterone treatment attenuates estradiol effects on COC self-administration (Jackson et al., 2006). This hormone-dependent enhanced female responding for

COC reward is demonstrated using an OVX model; OVX females treated with estradiol show enhanced COC self-administration compared to males and saline-treated females (Jackson et al., 2006), while estradiol has no effect on responding by CAST males (Lynch et al., 2000).

Similar results have been found in studies using MA and AMP. Female rats achieve acquisition criteria for MA (0.02 mg kg^{-1}) self-administration more rapidly than males, females also responded for significantly more infusions than males in an MA-maintained PR schedule (Roth and Carroll, 2004). Female rats also show an enhanced behavioural response to AMP administration, they display a significantly greater number of AMP-induced rotations than males (Robinson et al., 1980), this is evident even when AMP concentration in the striatum is equivalent in males and females. Becker and colleagues (1982) administered a range of AMP doses and selected doses which resulted in equal striatal AMP levels in both males and females; 1.25 mg kg^{-1} and 1.56 mg kg^{-1} doses for female and male rats respectively. In spite of controlling for AMP concentration in the striatum, females made more net rotations than males (Becker et al., 1982). Furthermore, though there was no difference in striatal AMP levels across the estrous cycle females in estrous and proestrous made significantly more rotations than females in diestrous and males (Becker et al., 1982), suggesting high estrogen levels enhance AMP effects. The same authors later showed that OVX reduced female rotations following AMP, while estradiol treatment reinstated elevated AMP-induced rotations compared to oil-treated controls (Becker, 1990b).

Sexual dimorphisms also exist with other drugs as female rats tend to self-administer more opioid drugs such as morphine and heroin during a maintenance phase than males, which is reflective of greater reinforcing properties of the drug for females (Cicero et al., 2003). Similarly the motivation to self administer nicotine is greater in female rats than in males. They acquired self-administration more rapidly than males and while there were no sex differences in responding during stable fixed ratio (FR) self administration females displayed significantly higher responses in the PR schedule (Donny et al., 2000). An FR schedule is a reinforcement schedule wherein a response is reinforced after a specified number of responses. The higher PR responding in females implies greater incentive motivation than males. In free

choice paradigms female rats have significantly greater ethanol intake (compared to water intake) than males, moreover, females displayed an increase in ethanol consumption over 5 weeks whereas males do not significantly increase intake over time (Juarez and Barrios de Tomasi, 1999, Adams, 1995).

These sexual dimorphisms in response to stimulant drugs are likely due to differences in the DA system. It is understood that estrogen modulates DA concentration and its receptor expression in females. OVX females display reduced AMP-induced DA release in the striatum compared to CAST males (Becker, 1990a) and DA levels fluctuate significantly over the female estrous cycle (Xiao and Becker, 1994). Female rats exhibit significantly higher extracellular striatal DA when estrogen levels are elevated compared to OVX females, moreover, there is no effect of CAST on male DA concentration. Thus ovarian hormones, but not testicular hormones, modulate DA levels in the striatum of rats (Xiao and Becker, 1994). Estrogen can act directly on the striatum to promote changes in DA release and/or receptor activity (Becker and Hu, 2008); it can also act directly on the nucleus accumbens to enhance DA release (Thompson et al., 2001, Lammers et al., 1999). It was demonstrated by Becker and colleagues (2008) that estradiol treatment can attenuate gamma-aminobutyric acid (GABA) release in the striatum of OVX females compared to saline controls. This supports the theory that estradiol inhibits GABA and thus the enhanced DA release observed in response to estradiol may be partly mediated by a release from GABA inhibitory processes (Becker and Hu, 2008).

Female rats therefore tend to have a greater sensitivity to the rewarding effects of drugs of abuse compared to males. This is mediated by the effects of estrogen on neurotransmission (particularly DA) and the mesolimbic pathway. In addition however pharmacokinetic differences between male and female rats and the rate of drug metabolism also impact on sex differences in drug effects (Milesi-Halle et al., 2005). A further objective of this thesis was to analyse sex differences in baseline and drug-induced responses in commonly-employed behavioural pharmacology tests.

1.3 Strain Differences

Rats are widely employed as the primary animal models in research in many scientific disciplines including pharmacology, physiology, toxicology and psychology to name but a few. When the search term “rat” is entered to the PubMed search engine just over 230,000 articles have been published since the year 2000, while over 450, 000 studies employing rats were published in the past 20 years. Rat models are effectively employed in research of disorders and diseases of just about every type, including immunology, cancer, diabetes, schizophrenia and skeletal disorders. Numerous rat strains exist and the availability of various rat strains is advantageous for modelling specific disease states and symptoms. Some strains are inbred, derived from generations of brother-sister mating, resulting in genetic uniformity. Such inbreeding can be used to isolate and model specific genetic mutations or diseases. For example the SHR is used in cardiovascular research, the Lewis strain is employed in addiction research and the Wistar Kyoto (WKY) strain has been characterised as a model of depression. In addition, genetically-engineered animals (e.g. Goto-Kakizaki) and immunodeficient rat models (e.g. NIH nude) have been useful in modelling and identifying potential treatments for diseases such as diabetes and in tumour research (Rees and Alcolado, 2005, Hamada et al., 2005). Other strains are outbred; these are genetically undefined and represent a more heterogeneous population (Gill et al., 1992). Most of the currently-available rat strains are descendents of the outbred Wistar, and to a lesser extent, SD rat strain. The outbred Wistar and SD are very popular in scientific research as they are robust; that is to say they have a good life expectancy, show high disease resistance, they reach sexual maturity and fertility early, they have many and large litters and display rapid growth (Suckow et al., 2006). Though they are genetically undefined, an abundance of knowledge has been gathered about the Wistar and SD strains from toxicology studies (Suckow et al., 2006). Due to their heterogeneity however these strains are prone to genetic variations, thus animals purchased from different vendors, or even from the same vendors but from different locations, are open to considerable genetic variability which can affect experimental results.

To estimate the extent to which various commonly-employed rat strains were represented in the literature a PubMed search of behavioural tests (including the forced swim test, elevated plus maze, open field test, Morris water maze and

methamphetamine-induced activity) and rat strains (Sprague Dawley, Wistar, Fischer, Lewis, Lister Hooded and Long-Evans) was carried out and the results are depicted in Figure 1.10. These strains were selected based on their wide use in behavioural pharmacology literature and to provide an illustrative, not exhaustive, view of common rat strains. Of the general outbred strains, the SD and Wistar albino strains are widely employed in behavioural pharmacology research, while the hooded strains are less commonly used.

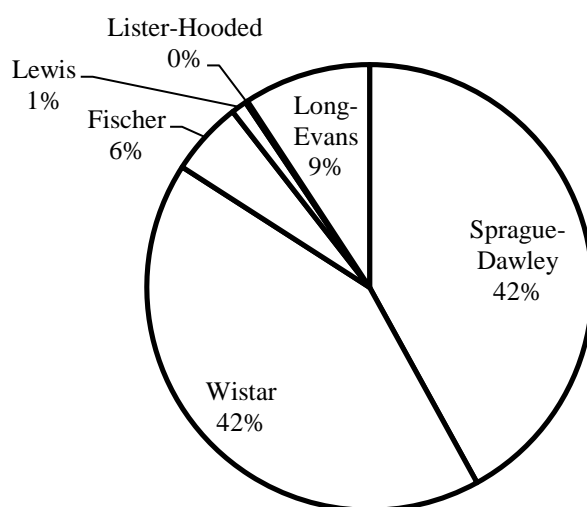


Figure 1.10: Commonly employed rat strains in behavioural tests

PubMed searches were carried out using rat strain and behavioural tests as search terms. The Sprague Dawley search yielded a total 1705 results, Wistar 1709 results, Fischer 218 results, Lewis 46 results, Lister-Hooded 7 and Long-Evans 375 results.

A large number of rat strains are currently available and employed in behavioural pharmacology research. Over 200 inbred strains alone have been characterised (Steen et al., 1999). Outbred strains often act as a control group when characterising inbred groups, however, differences can also exist between the outbred strains. The investigation of strain differences has increased in recent years as depicted in figure 1.11; the search terms “strain differences, rat” were entered to PubMed and the results over the past four decades show a steady increase in related articles.

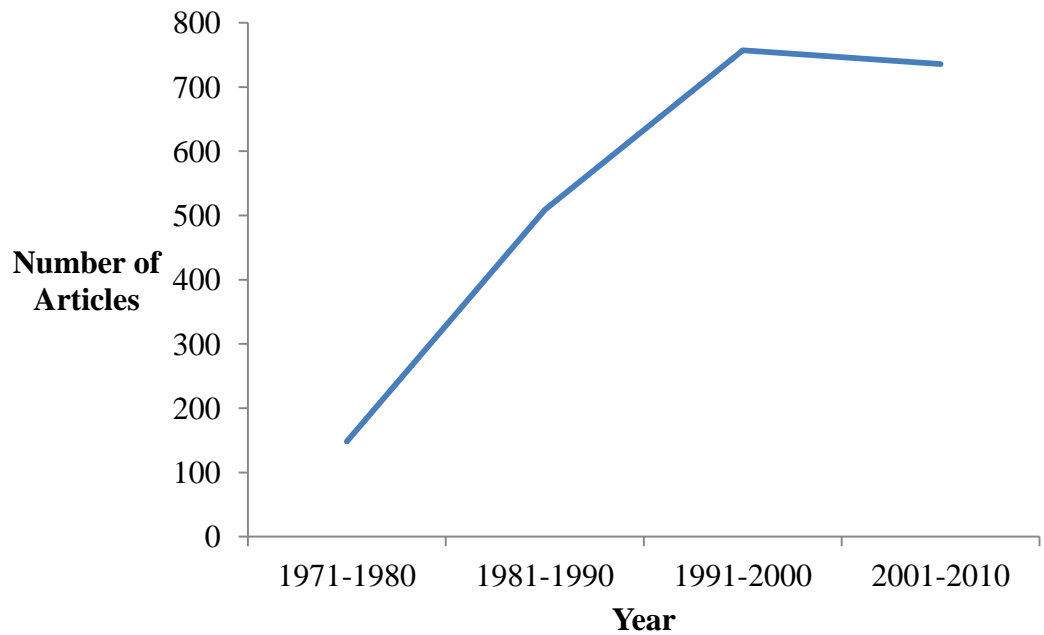


Figure 1.11: A PubMed search using the search terms “strain differences, rat” of articles published between 1971 and 2010. Overall the search retrieved 2212 articles.

1.3.1 Strain Differences in Tests of Depression/Anxiety-like Behaviours

Strain differences in tests of anxiety-like behaviours often include strains which are known to be sensitive to such tests, such as the WKY strain, compared to a control strain such as SD or Wistar. The WKY strain display depressive-like symptoms including reduced social interaction (Pardon et al., 2002), hypolocomotion (Tejani-Butt et al., 2003, Langen and Dost, 2011), symptoms of behavioural despair (O'Mahony et al., 2011, Jeannotte et al., 2009) and anxiety-like behaviours (Pardon et al., 2002, Ferguson and Cada, 2004, van der Staay et al., 2009). These are robust findings for the WKY strain but the results of other strain comparison studies can depend greatly on the “control” strain employed and on the nature of the task.

In terms of overall activity, Lewis rats engaged in significantly more jumping and had fewer rest periods during an observation of homecage activity during the dark phase compared to SD and Wistar rats (Hlavacova et al., 2006). SD and Wistar rats do not differ in homecage activity (Hlavacova et al., 2006, McDermott and Kelly, 2008) or distance moved in the OFT, however SD rats displayed greater habituation to the holeboard test compared to Wistar (Rex et al., 2004). The Lister-Hooded rats display greater homecage activity in the day-time, but reduced activity in the night-time, compared to the albino SD and Wistar strains (McDermott and

Kelly, 2008). Thus, the time of testing is an important consideration when designing experiments in which behavioural activity is measured.

In the EPM, SHR displayed significantly greater percent open arm entries and greater percent open arm time and than the Lewis strain (Kulikov et al., 1997, Ramos et al., 2002). Similarly in the OFT, SHR rats exhibit greater distance moved and greater activity in the centre of the arena than Lewis rats (Ramos et al., 2002); suggesting the Lewis rats are more fearful of the novel environment than SHR. When compared to SD and WKY strains however, Lewis rats have the greatest distance moved scores in the OFT (Pardon et al., 2002). Moreover, in a social interaction test Lewis rats engaged in significantly more time exploring a social partner compared to Fischer, Wistar and Brown Norway (BN) strains (Rex et al., 1996), implying reduced anxiety compared to the other strains when faced with an unfamiliar social partner.

In the OFT, Wistar rats displayed greater distance moved compared to BN, Lewis and Fischer strains (van der Staay and Blokland, 1996), while Wistar and BN spent more time exploring a familiar OFT than Lewis and Fischer strains (Rex et al., 1996), suggesting Wistar and BN were less fearful than Lewis and Fischer. However in the light/dark box the Wistar and Fischer strains spent equivalent time in the light section, significantly longer than the apparently more anxious BN strain (van der Staay and Blokland, 1996). In contrast, in a later study BN rats spent equivalent time in the light section as Fischer rats, significantly longer than the anxious WKY strain (van der Staay et al., 2009). In the EPM, the Fischer strain were least anxious, with increased percent open arm time and open arm entries compared to BN and Wistar, who did not differ from one another (van der Staay et al., 2009). In the EPM, SD and WKY rats made significantly fewer percent open arm entries and time, suggesting greater anxiety levels, compared to SHR and Wistar strains (Ferguson and Cada, 2004, Langen and Dost, 2011). There were no differences in EPM and social interaction anxiety tests between SD and Lewis strains (Pardon et al., 2002). Lister-Hooded rats displayed less anxiety-like behaviours in the EPM, as evidenced by greater percent open arm entries, percent open arm time and distance travelled in the open arms compared to both SD and Wistar albino strains, who did not significantly differ from one another (McDermott and Kelly, 2008). In a social interaction test SD rats engaged in more social interaction than the Wistar strain (Rex et al., 2004),

suggesting that the SD strain were less anxious than Wistar rats. Overall therefore, the perceived “anxiety” levels of the rat depend greatly on the test employed such as the OFT, EPM and social interaction tests, and on the strain of the comparator group.

Baseline and antidepressant effects on immobility time in the FST may be influenced by strain. There was no difference in baseline immobility time in the FST between Lister-hooded, SD and Wistar rats (McDermott and Kelly, 2008). As is their strain’s characteristic, WKY rats exhibit greater immobility and reduced time spent swimming than the SD and Wistar rats in the FST (Tejani-Butt et al., 2003, O'Mahony et al., 2011). The anti-depressants desipramine (DMI) (8 mg kg^{-1}) and nomifensine (10 mg kg^{-1}) significantly reduced immobility time and increased swimming in the FST, but only for the WKY strain; while paroxetine (10 mg kg^{-1}) had no effect on FST behaviour in WKY, SD or Wistar rats (Tejani-Butt et al., 2003). A similar study demonstrated DMI (10 mg kg^{-1}) decreased immobility for WKY rats compared to saline controls, but no such effect was found for Wistar strain (Jeannotte et al., 2009). The WKY strain thus provides a good model of behavioural despair in the FST which is attenuated by anti-depressant treatment, making them a valuable model for testing anti-depressant efficacy. In contrast the Fischer rat strain have failed to show adaptation to the pre-test and thus display low levels of immobility on the FST test day compared to SD rats, thus Fischer rats are unsuitable for use in the FST (Paul et al., 1990). Identification of such inherent differences between rat strains is important to results interpretation and experimental design. Some behavioural similarities and differences between the outbred SD and Wistar strains are depicted in table 1.12.

Table 1.12: Behavioural differences between the Sprague Dawley and Wistar rat strains

Test	Parameter	Strain difference	Reference
Homecage	Locomotor activity	SD = Wistar	(Hlavacova et al., 2006)
EPM	Open arm time / entries	SD = Wistar	(McDermott and Kelly, 2008)
Social Interaction	Time spent exploring an unfamiliar cagemate	SD > Wistar	(Rex et al., 2004)
FST	Time spent immobile	SD = Wistar	(McDermott and Kelly, 2008)
MWM	Distance swam	SD = Wistar	(Van der Borght et al., 2005)

SD, Sprague Dawley; EPM, elevated plus maze; FST, forced swim test; MWM, Morris water maze.

1.3.2 Strain Differences in Tests of Learning and Memory

Strain differences also exist in cognitive tests of learning and memory. Due to their phenotype it has been suggested that WKY rats exhibit more floating behaviour in the MWM and thus inefficient acquisition performance compared to the SD strain (D'Hooge and De Deyn, 2001). In the MWM, SD did not differ from SHR or WKY strains in swim path length; however the SHR strain had a shorter path length than WKY rats, implying that SHR had better spatial memory for the platform location and swam there directly. Overall, however, there were no strain differences in swim speed or latency to locate the platform between the SD, SHR and WKY strains (Ferguson and Cada, 2004). Similarly, Wistar and SD did not differ in distance swum to the hidden platform in the MWM; however in a cued trial, when the platform was visible, SD rats had greater swim distance scores than Wistar (Van der Borght et al., 2005), this suggests that SD rats had poorer spatial ability than Wistar. Compared to BN rats, Wistar and Fischer strains had significantly greater escape latencies in the MWM, suggesting BN had better spatial learning and memory skills than the albino strains (van der Staay and Blokland, 1996). On the other hand,

further cognitive tests in fact demonstrate a poor performance by BN rats (van der Staay and Blokland, 1996). In a delayed non-matching to position task, the rat is given a delayed food reward contingent on correct choice of lever press; a lever is presented during the “sample” phase, the correct lever press in the “choice” phase is the opposite to that presented previously in the “sample” phase. The BN strain had poor short-term memory ability compared to Fischer, while Wistar had intermediate scores (van der Staay and Blokland, 1996). In an active avoidance learning task a tone (i.e. a conditioned stimulus, [CS]) sounds prior to the presentation of footshock (i.e. an unconditioned stimulus, [UCS]). Twenty-four hours later when the rats are returned to the shock box they are expected to have learned to associate the CS with the UCS and escape the footshock when the tone is presented. In this task Wistar and Fischer showed a similar rate of learning, while BN failed to increase avoidance responding over the course of the acquisition sessions (van der Staay and Blokland, 1996). A later study carried out by the same authors supports the prior findings of poor memory ability in the BN strain. BN, Lewis and WKY rats were tested in passive avoidance task, when rats entered the dark compartment of a light/dark box they received a footshock, when re-exposed to the arena 24 hours later rats are expected to avoid the dark section. The BN strain showed the least aversion for the dark section compared to Lewis and WKY, who did not differ from one another (van der Staay et al., 2009).

In a complex 24-arm maze task, in which two arms were consistently baited with water reward, SHR rats earned more reinforcers and had higher efficiency ratios (i.e. number of reinforcers earned as a percent of number of arm entries), thus greater memory and spatial learning skills compared to WKY and SD strains (Ferguson and Cada, 2004). Long-Evans, Wistar and SD rat strains were tested in a variety of learning tasks; an autoshaping procedure consisted of the rat learning to release food pellets by pressing a lever within 30 seconds of its presentation, trials continued until the rat presses the lever on 5 consecutive occasions or a maximum of 300 trials are completed over 3 days. Long-Evans rats performed significantly better than Wistar and SD rats; SD rats were slightly better than Wistar but this did not reach significance; similarly, in the NOR test, only the Long-Evans strain showed significant preference for the novel object over the familiar one (Andrews et al., 1995). In a swim maze wherein rats were trained to swim to one of two seemingly

identical islands, SD rats were slower, but made more correct choices than Long-Evans, while there was no difference between SD and Wistar strains (Andrews et al., 1995). Overall therefore the requirements of the test and the comparator group employed can impact greatly on how well a particular strain group perform.

1.3.3 Strain Differences in Response to Stress

Strain differences in responses to tests such as the EPM and FST imply differences in response to stress and HPA reactivity, which is generally measured by plasma CORT and ACTH levels. According to Hlavacova and colleagues, SD, Wistar, Lewis and SHR rats do not differ in baseline plasma ACTH or CORT levels, as measured in the homecage during the dark phase of the day (Hlavacova et al., 2006). However differences exist between various inbred strains and “control” strains such as the SD. As would be expected, the WKY rat strain displayed greater elevations in ACTH and CORT in responses to stressors such as the FST compared to SD and Wistar rats (Rittenhouse et al., 2002b, Malkesman and Weller, 2009). ACTH release in response to restraint stress was enhanced by prior exposure to chronic cold (i.e. 7 days, 4 hours/day 4° C) and this increase was significantly greater in WKY rats compared to SD (Pardon et al., 2003). However there is no difference in baseline plasma CORT levels between WKY and SD controls (Rittenhouse et al., 2002b, O'Mahony et al., 2011). Notably, during the daylight hours there is no difference between plasma CORT and ACTH levels of Wistar and WKY rats, however during the night-time hours WKY had significantly higher levels of both stress hormones (Solberg et al., 2001). This reiterates the impact that time of testing may have on experimental measurements. Conversely, Armario and colleagues (1995) suggest that behaviour in the FST is not necessarily reflected in stress hormone response. In the FST, WKY and BN rats displayed long periods of immobility, Lewis and SHR strains were intermediate, and Fischer were most active (Lahmame et al., 1997, Armario et al., 1995). Analysis of corticotrophin releasing factor (CRF) binding demonstrated that BN rats had greater CRF binding in the hippocampus and PFC, and greater CRF content in the PFC compared to Lewis, SHR and WKY strains (Lahmame et al., 1997). Furthermore, though WKY spent more time immobile than Fischer and SHR strains, there was no difference in plasma CORT levels between strains after the FST (Marti and Armario, 1996). WKY rats do however have increased CRF and CRF receptors, CRF₁ and CRF₂, mRNA in various brain regions

compared to SD rats, this genetic difference is likely to contribute to their depressive profile when compared to the SD strain (Bravo et al., 2011).

In spite of low levels of immobility in the FST (Lahmame et al., 1997) and low levels of anxiety in the EPM (van der Staay et al., 2009) the Fischer strain is also more responsive to stress than Lewis and SD strains; they display enhanced elevations in plasma ACTH and CORT in response to restraint stress and take longer to recover from these increases compared to SD and Lewis rats (Dhabhar et al., 1995). In response to food deprivation, both Fischer and Lewis rats display similar increases of plasma CORT, however in response to restraint stress (20 minutes – 2 hours) Fischer rats displayed greater stress-induced increased CORT (Macho et al., 2008) and ACTH (Marissal-Arvy et al., 2007) compared to Lewis rats. This enhanced response to stress is likely mediated by increased CORT available and greater receptor binding as Fischer rats had greater levels of plasma corticosteroid-binding globulin and a greater magnitude of Type 2 adrenal steroid receptors activated following stress compared to Lewis and SD rats (Dhabhar et al., 1995). There were no differences however in basal ACTH or CORT levels between the three strains, Lewis, SD and Fischer (Dhabhar et al., 1995). Similarly others have reported no difference in baseline plasma CORT or ACTH levels between Fischer and Lewis rats (Marissal-Arvy et al., 2007).

Plasma hormone levels may not be always correlated with behaviours in tests of anxiety, thus the measurement of both may be beneficial to characterise “anxious” rat strains. In addition, it may provide information about the nature of stressors and the extent to which a particular stressor is aversive to a particular strain. Furthermore, binding assays have provided useful evidence of strain differences in HPA axis activity and its response to stressors.

1.3.4 Strain Differences in Monoamine Neurotransmitter Levels

Monoamine neurotransmitter levels are generally compared within genetic rat models of disease pathology. Fischer and Lewis rats are often compared in studies of drug abuse as the Lewis strain are more sensitive to drug effects than Fischer rats (Kosten and Ambrosio, 2002) and this is partly due to differences in monoaminergic neurotransmission. Lewis rats show a greater DA release in the nucleus accumbens in response to restraint stress, morphine, AMP, COC and nicotine (Lindley et al.,

1999, Cadoni and Di Chiara, 2007, Sziraki et al., 2001) compared to Fischer rats. Basal DA levels in the striatum are lower however in Lewis rats compared to Fischer (Lindley et al., 1999), while there was no difference between the two strains in basal DA in the nucleus accumbens, striatum or amygdala (Lindley et al., 1999, Cadoni and Di Chiara, 2007). Differences between the two strains are also seen at DA receptor and transporter level; Lewis rats have reduced D₂ receptor binding in the striatum, reduced D₃ binding in the nucleus accumbens shell and olfactory tubercle (Flores et al., 1998) and reduced DAT in the striatum and nucleus accumbens (Flores et al., 1998, Gulley et al., 2007) compared to Fischer rats. Basal 5HT levels were reduced in the Lewis strain nucleus accumbens and administration of 1 g/kg ethanol significantly increased 5HT in the region in Lewis rats but had no such effect on 5HT levels in the Fischer strain (Selim and Bradberry, 1996). Lewis rats also display reduced 5HT reuptake and 5HT transporter (5HTT) compared to the Fischer strain (Fernandez et al., 2003). Though there is no baseline difference in NA levels in the brain or periphery (Lindley et al., 1999, Bellinger et al., 2002), exposure to restraint stress induces a greater increase in NA turnover in the PFC, nucleus accumbens shell and striatum of Fischer rats compared to Lewis (Lindley et al., 1999). Such differences in DA, 5HT and NA activity mediate the differences in drug sensitivity between the two strains (see section 1.3.7).

The “depressed” WKY strain display deficits in monoamine activity. Burke (2010) and others demonstrated reduced NA in the hypothalamus, hippocampus, amygdala, thalamus and nucleus accumbens shell of WKY compared to SD rats (Burke et al., 2010, Scholl et al., 2010) and reduced NA in the striatum and amygdala core compared to Wistar rats (Scholl et al., 2010). WKY also display an attenuated NA response to stress and significantly lower hippocampal β_2 adrenoceptor density (Pardon et al., 2002), in addition to increased expression of proteins involved in NA metabolism compared to SD controls (Pearson et al., 2006). Notably mid-aged (7-9 months old) WKY and SD rats do not differ in plasma levels of NA, plasma levels therefore may not be an accurate indication of central NA concentration (Jablonskis and Howe, 1994). WKY rats also display reduced DA in the VTA, reduced DOPAC in the PFC, and significantly higher DA turnover in the nucleus accumbens compared to Wistar and SD controls, with no differences seen in the amygdala or striatum (Scholl et al., 2010, Ferguson and Cada, 2003).

DAT binding was also decreased in the hippocampus, amygdala, hypothalamus and substantia nigra of WKY rats compared to Wistar and SD controls (Jiao et al., 2006a). On a receptor level the WKY strain display reduced D₁ receptor binding in the nucleus accumbens and caudate putamen, and increased D₁ binding in the substantia nigra compared to Wistar rats (Novick et al., 2008), in addition to modulated D₂ and D₃ receptor binding in various striatal subregions and hypothalamus compared to Wistar rats (Yaroslavsky et al., 2006). Studies have demonstrated reduced 5HT in the cortex, amygdala, hypothalamus, striatum and nucleus accumbens, but no difference in the hippocampus, of WKY rats compared to Lewis, SD and Wistar rats (van der Staay et al., 2009, Scholl et al., 2010). Stress differentially affected 5HT_{1A} receptor and 5HTT binding in the hippocampus, cortex and hypothalamus of WKY and SD rats (Pare and Tejani-Butt, 1996). Furthermore, O'Mahoney and colleagues (2011) showed stress decreased hippocampal 5HT and 5HIAA in SD rats, but this response was blunted in WKY (O'Mahony et al., 2011). Altered monoamine levels in the rat brain, particularly in regions such as the amygdala, hippocampus and striatal regions contribute to the depressive phenotype which characterises the WKY strain (Pare and Redei, 1993b).

Fewer studies have investigated differences in monoamine levels between the control strains. Some neurochemical similarities and differences between the outbred SD and Wistar rat strains are presented in table 1.13. Rex and colleagues (2004) showed SD rats had reduced 5HT in the hippocampus and PFC compared to Wistar (Rex et al., 2004), while Scholl and others (2010) found no difference between SD and Wistar rats in 5HT or 5HIAA levels in the amygdala, hypothalamus, striatum or PFC (Scholl et al., 2010). Scholl (2010) also found Wistar had increased NA only in the nucleus accumbens core, and increased DA in the VTA compared to SD. The Wistar strain were also found to have increased DA concentrations in addition to increased DAT and D₁ and D₂ receptor binding in the caudate putamen and increased D₁ receptor and DAT binding in the nucleus accumbens compared to SD rats (Zamudio et al., 2005).

Overall therefore strain differences are prevalent in monoaminergic neurotransmission and these may govern the specific characteristics of various strains, for example the Lewis and WKY strains outlined above. In addition differences can exist between control strains; this is an important consideration when

designing experiments, particularly when carrying out neurotransmitter analysis. Scholl and colleagues go so far as to suggest the employment of multiple strain comparison groups in such studies and for the true validation of animal models of various states (Scholl et al., 2010).

Table 1.13: Neurochemical differences between the Sprague Dawley and Wistar rat strains

Neurochemical measure	Parameter	Strain difference	Reference
ACTH / CORT	Baseline plasma levels	SD = Wistar	(Hlavacova et al., 2006)
DA	DAT binding	SD = Wistar	(Jiao et al., 2003)
	DA in VTA	SD > Wistar	(Scholl et al., 2010)
	DA in Caudate Putamen	SD < Wistar	(Scholl et al., 2010)
5HT	5HT in hippocampus	SD < Wistar	(Rex et al., 2004)
	5HT in PFC	SD < Wistar	(Rex et al., 2004)
		SD = Wistar	(Scholl et al., 2010)
	5HT in amygdala	SD = Wistar	(Scholl et al., 2010)
	5HT in hypothalamus	SD = Wistar	(Scholl et al., 2010)
NA	NA in Nucleus Accumbens	SD < Wistar	(Scholl et al., 2010)

SD, Sprague Dawley; ACTH, adrenocorticotrophic hormone; CORT, corticosterone; DA, dopamine; 5HT, serotonin; NA, noradrenaline; VTA, ventral tegmental area; PFC, prefrontal cortex.

1.3.5 Strain Differences in Neurotrophic Factor Expression

Few studies directly compare the levels of neurotrophic factors in naive rats of various strains; rather inbred strains are compared to a “control” strain. For example the genetically hypertensive (GH) strain of Wistar rat has been shown to be deficient in NGF. Compared to outbred Wistar, the GH strain displayed impaired induction and maintenance of LTP; however this was reinstated by intraventricular injection of NGF (Kelly et al., 1998). Levels of NGF and Trk receptors in the dentate gyrus were also significantly lower in GH compared to Wistar controls (Kelly et al., 1998). A later study extended these findings; GH rats displayed impaired LTP as expected and the EPSP slope was greater and maintained over a longer period of time in Wistar rats than in the GH strain. In the dentate gyrus, GH rats had decreased expression of NGF and BDNF, but there was no difference in NT3 or NT4 levels. Furthermore, GH rats showed decreased expression of TrkA and B, but not C, receptors in the dentate gyrus compared to normotensive Wistar controls (Hennigan et al., 2009). This was reflected in functional ability as the GH rats failed to show preference for an unfamiliar object in the NOR test (Hennigan et al., 2009).

As reduced BDNF is associated with chronic stress exposure (Murakami et al., 2005, Smith et al., 1995) and antidepressant treatments elevate neurotrophic factors over time (Nibuya et al., 1995, Zhang et al., 2010), studies have compared characterised neurotrophic levels in “depressed” rat strains compared to controls. The WKY strain have similar serum BDNF levels as SD rats but exposure to chronic stress (2 hours restraint stress for 10 days) reduces BDNF in WKY rats but in the control SD strain (O'Mahony et al., 2011). In another genetic model of depression serum and blood BDNF levels of FSL rats were significantly increased compared to Flinders resistant line (FRL) rats. However opposite results were seen in brain tissue BDNF levels; in the hippocampus the BDNF levels were significantly decreased in FSL compared to FRL rats while no differences were found in the frontal cortex (Elfving et al., 2010). Caution must therefore be exercised when interpreting serum neurotrophic levels alone as they may be prone to misinterpretation.

There were no strain differences in baseline hippocampal BDNF mRNA of Lewis, SD and Fischer rats. However PS (random mild stressors on gestational days 14-21) significantly increased BDNF mRNA compared to non-stressed controls in Lewis and SD strains, but not in Fischer rats. Exposure to acute (restraint) stress

immediately prior to sacrifice increased BDNF mRNA equally in each strain compared to baseline levels (Neeley et al., 2011). There were no significant strain differences in basal pro-BDNF and mature BDNF (mBDNF) proteins in the hippocampus, as assessed by western blot analysis. PS significantly reduced both pro-BDNF and mBDNF levels in Lewis and SD rats compared to non-stressed controls, again however stress had no effect on protein expression in the Fischer strain (Neeley et al., 2011). Fischer rats are often used in animal models of pain due to high pain tolerance (Herradon et al., 2007). Compared to Lewis and SD rats they were the only strain to show full functional recovery from pain, as measured by mechanical allodynia, 28 days after chronic constriction injury of the sciatic nerve (Herradon et al., 2007). Moreover, BDNF gene expression was upregulated in the dorsal root ganglia of Fischer rats compared to Lewis and SD, and this correlated with resistance to neuropathic pain (Herradon et al., 2007). Thus these observed alterations in BDNF may contribute to the Fischer strain phenotype.

In addition to stressors, environmental factors can affect neurotrophic levels in a strain-dependent manner. Following long-term (1 year) EE rearing conditions SD rats displayed significantly increased BDNF levels in the basal forebrain, cerebral cortex, hindbrain and hippocampus compared to IC controls (Ickes et al., 2000). On the other hand, Wistar rats reared in similar conditions for the same period showed no difference in BDNF levels in the frontal cortex, amygdala or hippocampus (Segovia et al., 2008). The increased neurotrophic factor levels are thought to be due to interaction with a more complex environment and exercise can have the same effect (O'Callaghan et al., 2009).

A number of environmental factors and stressors can thus impact on growth factor and receptor expression in the rat brain. Baseline differences also exist and these genetic differences contribute to performance in behavioural tests and to brain plasticity.

1.3.6 Strain Differences in Neurogenesis and Plasticity

Differences between strains in stress responses and levels of neurotrophic factors can contribute to altered neurogenesis and neuron proliferation in the hippocampus. Research has illustrated strain differences in neural responses to various environmental challenges such as the MWM, stressors and housing conditions. The effects of MWM learning on hippocampal cell growth and survival were assessed in adult male SD and Wistar rats. Rats were administered BrdU and assigned to one of three groups. Two groups were exposed to the MWM; “place learners” were required to learn the location of the hidden platform while “cue learners” were tested in a cued version of the MWM, i.e. the platform was visible at all times. The third “homecage” group were not exposed to the MWM. There was no effect of exposure to the MWM on cell survival (as measured by BrdU positive cells) in the dentate gyrus, nor was there any difference in cell proliferation (as measured by Ki67 labelling of proliferating cells at the time of sacrifice) in the SGZ. Immature neurons express poly-sialated neural cell adhesion molecule (PSA-NCAM) and thus this is accepted as a marker of plasticity. Place learning increased PSA-NCAM positive cells in the SGZ compared to homecage controls for both SD and Wistar strains. As there was no effect of PS-NCAM on performance or BrdU labelled cells, the learning-induced increase in PS-NCAM may be due to indirect mechanisms such as neurite growth or dendritic branching (Van der Borght et al., 2005). Regarding strain differences between homecage controls, Wistar rats had 42 % more BrdU-and 40 % more PSA-NCAM - positive cells in the dentate gyrus than SD rats. As there were no strain differences in cell proliferation, the reduced cell number in SD may reflect a greater level of neuron death compared to Wistar (Van der Borght et al., 2005). Compared to Lister-hooded rats however the SD strain had up to 60% more Ki67-labelled cells in the dentate gyrus. As plasma CORT levels were also lower in the SD compared to Lister strains differences in corticoids could account for the differences in cell proliferation (Alahmed and Herbert, 2008). Lister-Hooded rats have also shown impaired long term depression (LTD) in the hippocampus compared to Wistar and SD rat strains (Manahan-Vaughan, 2000) and impaired plasticity in the dentate gyrus as demonstrated by reduced LTP intensity and duration compared to Wistar controls (Manahan-Vaughan and Schwegler, 2011).

A number of studies have investigated the effects of stress on neurogenesis and proliferation in the hippocampus. CMS has been demonstrated to reduce BrdU-labelled cells and neurogenesis ratio in the in the ventral hippocampus, dentate gyrus and granule cell layer of the hippocampus of Wistar and SD male rats (Jayatissa et al., 2006, Toth et al., 2008, Lee et al., 2006). Due to its effects on the brain and behaviour CMS is widely used as a model of depressive symptoms (Willner, 1997), however it may not be an appropriate model for use with all rat strains. IC housing and CMS exposure increased BrdU and doublecortin (DCX)-positive immature cells in Lewis rats, moreover, IC housing increased the neurogenesis ratio for Lewis rats compared to SC controls. Lewis rats also displayed reduced CORT in IC housing, thus it may be that this strain prefer IC housing and that the CMS protocol was not detrimental to rats of the Lewis strain compared to SD and Fischer strains due to their HPA hypo-activity (Wu and Wang, 2010).

Baseline strain differences in cell survival and immature neuron numbers were seen between Lewis, SD and Fischer rats; SD rats had significantly lower BrdU cell counts in the dentate gyrus than Fischer, but significantly greater counts than Lewis. Dentate DCX-positive cell counts were different in all three strains; Fischer rats had the highest count, SD the lowest, and Lewis had an intermediate DCX-positive cell count (Wu and Wang, 2010). Male and female SHR rats had significantly greater numbers of BrdU labelled cells in the dentate gyrus compared to SD rats; this was evident on day 1 and day 30 following a week of daily BrdU injections (Perfilieva et al., 2001). This may reflect improved cognitive function as SHR rats have been shown to outperform SD and other rat strains in tests of learning and memory (Ferguson and Cada, 2004, Langen and Dost, 2011). On the other hand however older SHR rats (aged 6 months and older) have been employed in studies of neuroprotection and dementia due to their hippocampal abnormalities (Pietranera et al., 2011). Compared to the WKY strain, 6-month-old SHR rats have displayed decreased hippocampal volume, increased number of astrocytes (Sabbatini et al., 2002) and increased glial fibrillary acidic protein expression (Tomassoni et al., 2004) in various brain regions. Increases in astrocyte size and number are associated with neurodegenerative processes in the brain (Maragakis and Rothstein, 2006), thus the hypertensive nature of this rat strain is a risk factor for dementia and makes them

useful during mid-adulthood in studies of Alzheimer's disease and similar neurodegenerative disorders.

1.3.7 Strain Differences in Responsiveness to Drugs of Abuse

Strain-dependent alterations in reward pathway, particularly in the dopaminergic system, of the rat brain are likely to account for differences in self-administration and response to drugs of abuse. Fischer and Lewis rats are often compared for their divergent levels of vulnerability to drug abuse, mediated in part by differences in HPA responsiveness and neurotransmitter systems (Kosten and Ambrosio, 2002). Decreased DA and 5HT activity in Lewis rats may mediate behavioural differences such as increased impulsivity and higher operant responding in food reinforcement tasks seen in the Lewis rat compared to Fischer rats (Anderson and Diller, 2010, Martin et al., 2003). In addition, Lewis rats display greater self-administration of drugs of abuse such as morphine at a range of doses ($0.25\text{--}2\text{ mg kg}^{-1}$) (Martin et al., 2003, Sánchez-Cardoso et al., 2007, Garcia-Lecumberri et al., 2011) compared to Fischer counterparts. Furthermore, while Fischer rats showed a preference for the 0.5 mg kg^{-1} dose of morphine, Lewis displayed equal responding for all doses, suggesting there was no difference in the reinforcement value of increasing doses (Garcia-Lecumberri et al., 2011). Strain differences in DA receptor binding are likely to mediate these different responses as baseline DA D_1 and D_2 receptor binding is reduced in various brain regions in Lewis rats compared to Fischer and morphine self-administration decreased receptor binding in the Lewis rats but increased binding in the Fischer strain (Sánchez-Cardoso et al., 2007). These results would support the use of Lewis rats as a model of drug addiction; however other studies suggest that the reinforcement value of psychomotor stimulant drugs such as COC and AMP may be greater in Fischer rats than Lewis (Christensen et al., 2009, Freeman et al., 2009, Kosten et al., 2007, Stohr et al., 1998). Notably Lewis rats are more sensitive to the aversive effects of COC, while they are less sensitive to the aversive effects of morphine compared to Fischer rats, which may account for differential responses (Lancellotti et al., 2001, Glowa et al., 1994).

The locomotor-stimulating effects of increasing doses of AMP (0.5 and 1.0 mg kg^{-1}) were greater in WKY rats than in SHR compared to saline controls, while there was no significant drug effect seen in Wistar rats (Langen and Dost, 2011). A single dose of AMP (2 mg kg^{-1}) significantly increased homecage activity compared

to saline controls in both Lister-Hooded and Wistar, but not in SD rats (McDermott and Kelly, 2008). In a passive-avoidance learning test AMP produced amnesic-like effects equally in SD and Lewis rats (Kaminsky et al., 2001), similarly there was no strain difference in AMP-induced DA increases in the striatum of SD, SHR and WKY rats (Ferguson et al., 2003). COC (10 mg kg⁻¹)-induced rearing activity in the OFT was greater in Long-Evans compared to SD rats, but there was no difference in overall distance moved however (Gulley, 2007). While on the other hand the Lewis strain displayed greater AMP (0.5 mg kg⁻¹)-induced horizontal activity, while Fischer rats displayed greater AMP-induced rearing and stereotypical behaviours in the OFT (Gulley et al., 2007). Furthermore, the Lewis strain displayed moderate increases in stereotypical behaviours in response to 0.25 mg kg⁻¹ AMP and enhanced increases in COC-induced activity compared to Fischer rats (Cadoni and Di Chiara, 2007).

Regarding other drugs of abuse, WKY rats consume more ethanol than Wistar (Jiao et al., 2006b) and ethanol (3.7 %) increased DA D₂ receptor binding in striatal subregions in WKY rats compared to water-treated controls, whereas ethanol decreased receptor binding in Wistar rats (Morganstern and Tejani-Butt, 2010). In a free-choice paradigm WKY rats consume significantly more ethanol than SD; in addition, ethanol (7 %) had significant anxiolytic effects in the EPM for WKY but not SD rats (Pare et al., 1999). In response to nicotine (0.4 and 0.8 mg kg⁻¹), Lewis rats displayed attenuated conditioned taste aversion compared to Fischer rats, suggesting a greater reward value of nicotine for Lewis rats (Pescatore et al., 2005). Similarly Lewis rats were more sensitive to nicotine discrimination at 0.4 mg kg⁻¹, while 0.9 mg kg⁻¹ was required to observe the same effect in Fischer rats; in addition, Lewis rats developed CPP at lower nicotine doses than Fischer (Philibin et al., 2005). Compared to SD rats Lewis also have greater sensitivity to nicotine and are more likely to continue self-administration even as the dose is decreased (Brower et al., 2002). Thus Lewis rats are greatly sensitive to the reinforcement value of nicotine and provide a good model for long-term dependency.

Strain differences in sensitivity and responses to drugs of abuse such as psychomotor stimulants including COC and AMP, nicotine, ethanol and opioids are primarily driven by differences in monoaminergic neurotransmitter systems. The WKY rat strain is sensitive to the effects of stimulant drugs and the anxiolytic effects

of ethanol, while the Lewis strain have been employed in many studies to model vulnerability to drug self-administration and addiction. Strain sensitivity to the rewarding effects of drugs can depend on the comparator strain employed and this is reflected the Lewis - Fischer model of drug use, however recent experiments are questioning the usefulness of this model. Strain considerations are therefore integral to proving or disproving the validity of an animal model.

An additional objective of this research thesis was to identify any differences between three albino rat strains, namely the inbred albino Lewis strain and the outbred Wistar, compared to the outbred control SD strain. Baseline and drug-induced behaviours as well as post-mortem brain monoamine levels were analysed.

Chapter 2

Materials and Methods

Chapter 2

Materials and Methods

2.1 Materials

2.1.1 Animals and Animal Husbandry

Item	Source
Sprague Dawley (males $n=235$, females $n=81$)	Charles River (UK)
Wistar (males, $n = 24$)	
Lewis (males, $n = 24$)	
Sawdust bedding	Wonham Mills Ltd, RH6 0UW, UK
Rat chow pellets	Harlan, Bicester, U.K.
Environmental enrichment objects:	Petworld, Headford road, Galway
Bamboo Tunnel (Criticter's Choice, U.K.)	
Baguette Hamster House Hideaway	
Super Pet Igloo small animal shelter	
Living World Dome small animal shelter	
Wooden Shelters (Happy Pet Products Ltd, U.K.)	
Super Pet Hide and See T.V. pet toy	
Just 4 Pets Roll 'N' Chew	
Plastic Tunnels	
Cat toys with bell and with rattle	
Super Pet Grassy Tunnels	
Ka-Bob wooden chew sticks and balls	
Burgess gnaw sticks	
Nestledown bedding	

2.1.2 Behavioural Testing

Item	Source
EthoVision XT (v 3.1 and 7)	Noldus, Tracksys Ltd., Nottingham
Video cameras	Ganz FCH 30C, Sanyo Digital Colour CCD
Personal computer monitor	Dell
DVD recorder	Panasonic (DMR – EX77)
Open Field Test	
Elevated plus maze	Pharmacology Department, National University of Ireland, Galway.
Homecage monitoring and tracking apparatus	
Novel object recognition arena	
Pyrex (10L) cylinders	Alkem, Cork, Ireland
Morris water maze	Engineering and Design Plastics Ltd., Cambridge, U.K.

2.1.3 Drugs and Injection Materials

Item	Source
Diazepam	“Diazemuls” Actavis, Iceland
Desipramine hydrochloride (D3900)	Sigma Chemical Co.
Methamphetamine hydrochloride (M8750)	Sigma Chemical Co.
D-amphetamine sulphate (A-5880)	Sigma Chemical Co.
Apomorphine hydrochloride (A-4393)	Sigma Chemical Co.
Needles and syringes	BD Microlance, Drogheda, Ireland

2.1.4 Biochemical and Associated Materials

Item	Source
Micro punchers	Harvard Apparatus, Kent, England
Citric acid monohydrate (C1909)	Sigma Chemical Co.
Sodium dihydrogen phosphate (S9638)	Sigma Chemical Co
1-Octane sulfonic acid (O8380)	Sigma Chemical Co
Ethylenediaminetetra-acetic acid (E9884)	Sigma Chemical Co
Noradrenaline (A9512)	Sigma Chemical Co
Dopamine (H8502)	Sigma Chemical Co
5-hydroxyindoleacetic acid (H7752)	Sigma Chemical Co
L-3-4-Dihydroxyphenylalanine (D9628)	Sigma Chemical Co
3,4-Dihydroxyphenyl-acetic acid (D9128)	Sigma Chemical Co
Homovanilic acid (H1252)	Sigma Chemical Co
5-hydroxytryptamine (H9772)	Sigma Chemical Co
N-Methyl-5- hydroxytryptamine (M1514)	Sigma Chemical Co
Sonicator	Mason 1621
Centrifuge	Mason Mikro 22R
PBS tablets (P4417)	Sigma Chemical Co.
Tween 20 (T2700)	Sigma Chemical Co.
Reagent Diluent (DY995)	R & D Systems
Sodium chloride (71382)	Sigma Chemical Co.
Trizma HCL (T5941)	Sigma Chemical Co.
Glycerol (G6279)	Sigma Chemical Co.
Igepal (I7771)	Sigma Chemical Co.
3-3, 5-5-Tetramethylbenzidine (T8665)	Sigma Chemical Co.
Sulphuric acid (320501)	Sigma Chemical Co.
BCA Protein Assay Reagent A (23223)	Thermoscientific
Copper sulphate (C7631)	Sigma Chemical Co.
96-well plate	Starstedt microtest, flat bottom

Incubator	Merck, Cultura
Plate reader	ELX 800 Universal microplate Reader, Bio-tek instruments INC
GraphPad prism (version 5)	Graphpad Software, CA, USA
BDNF DuoSet ELISA kit (DY248):	R & D Systems
Capture antibody; part 840180 (1 vial)	
Detection antibody; part 840181 (1 vial)	
BDNF Standards; part 840182 (3 vials)	
Streptavidin-HRP; part 890803 (1 vial)	
Nunc maxisorb 96-well plates (NC9229197)	Fischer Chemical Co.
Plate-washer	Autowash Biotech ELX50

2.2 Animals

A total of 336 rats of both sexes were employed in behavioural and post - mortem tests, while a further 21 dams and 7 male SD rats were employed for breeding purposes only (see Fig. 2.1). All rats were purchased from Charles River (UK). Procedures were approved by the Animal Care Research Ethics Committee (ACREC certificate 005/09) and carried out under the guidelines of the Animal Welfare Committee, National University of Ireland, Galway and in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC).

2.2.1 Experimental Design

The first three studies investigated the effects of IC, SC and EE conditions for SD rats, while the two final studies addressed sex and strain as influencing variables on pharmacological responses in behavioural tests:

Study 1: Investigation of the effects of differential housing on baseline behaviours and pharmacological responses of young adult male SD rats in various behavioural tests.

Study 2: Investigation of housing condition effects on baseline activity and monoamine levels in various brain regions for both male and female young adult SD rats.

Study 3: Investigation of the differential housing conditions when introduced at the age of from weaning (PND 21) and assessed its effects on pharmacological responses of male SD rats and on brain monoamine and BDNF levels.

Study 4: Examination of any effects of gender on baseline and drug-induced behaviours.

Study 5: Examination of any strain differences in male SD, Wistar and Lewis rats in baseline and drug-induced behaviours, and on brain monoamine concentrations.

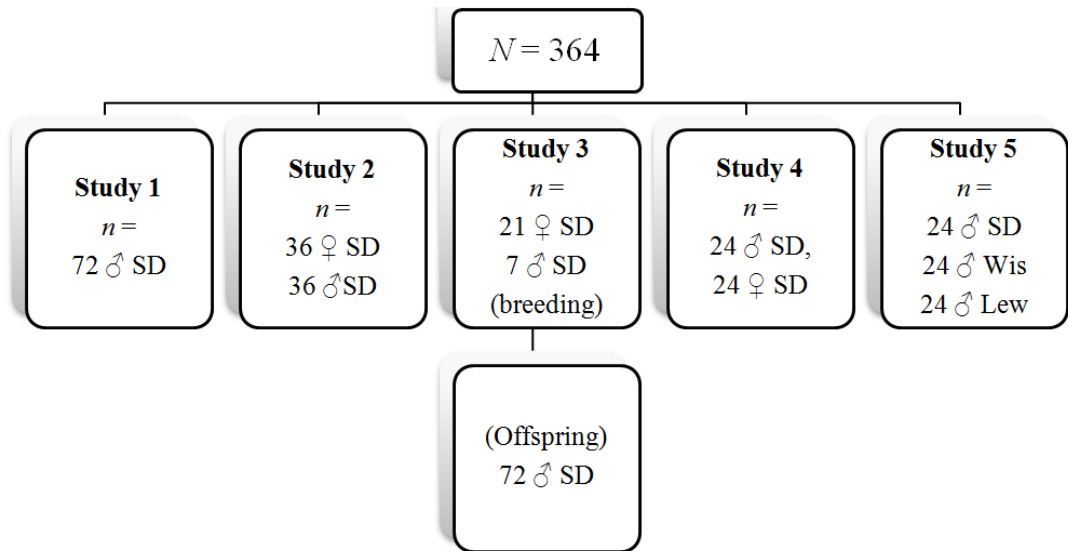


Figure 2.1: Animal numbers employed in studies 1–5. SD, Sprague Dawley; Wis, Wistar; Lew, Lewis.

2.2.2 Animal Husbandry (Figure 2.2)

Rats were housed in plastic-bottomed cages with sawdust bedding. Rats housed in social conditions and with environmental enrichment were housed in same sex groups of 4, others were singly housed. Those housed in social and isolation conditions were maintained in the standard sized cages (42 x 25.5 x 20 cm), while additional larger cages (54 x 38 x 20 cm) were employed in enrichment studies (chapters 3, 4 and 5). All rats were provided continuous access to rat chow pellets and tap water. They were maintained on a 12-hour light:dark cycle (lights on 08:00) in a temperature controlled room (20 ± 2 °C), with relative humidity of 45–70%. All rats were handled and weighed weekly; bedding was changed once weekly for singly-housed and 3 times weekly for group-housed animals. Food and water were replenished as necessary.

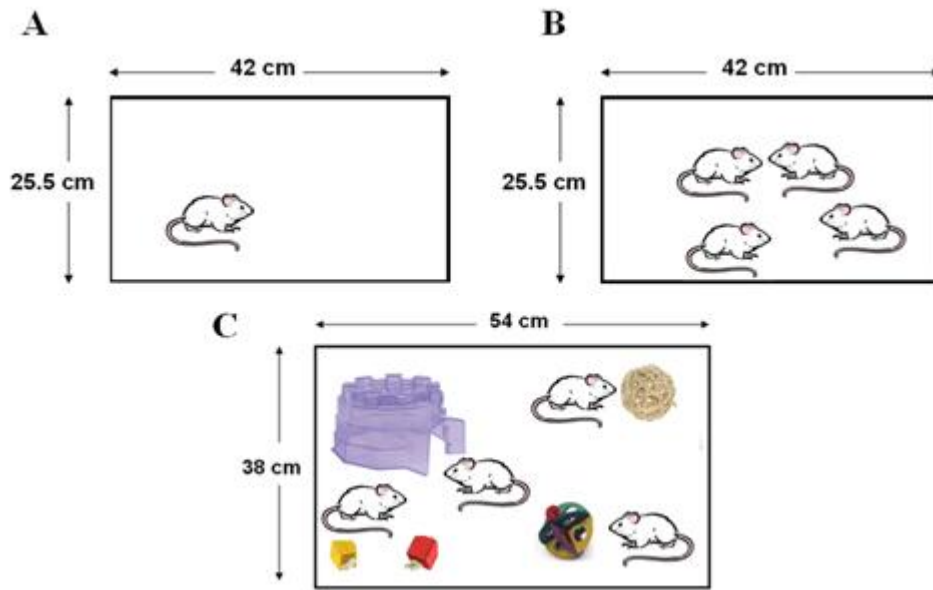


Figure 2.2: Rat housing conditions. Rats were singly (A) or group housed (B) in standard rat laboratory rat cages, larger cages were provided for environmental enrichment conditions (C).

Enrichment Housing

In addition to the normal rat chow, rats housed in EE were also provided with supplementary food treats such as coco pops (Kelloggs®), pop corn (Tayto®) and dried fruit and nuts. Environmental enrichment objects were provided and changed at least twice weekly. The provision of nutritional enrichment and maintaining novelty has been recommended by others (Baumans, 2005). Objects and toys were thus rotated between the EE cages and a record was kept of the objects received by each group. In this way all groups were exposed to all objects and the combinations of objects were varied to enhance their novelty value.

2.3 Behavioural Tests

2.3.1 Behavioural recording and Ethovision XT

EthoVision® was used for automated and semi-automated behavioural tracking. Video cameras were used to record behaviours and the image output was displayed on a computer monitor. Behavioural testing was recorded using a DVD recorder.

2.3.2 Open Field Test (Figure 2.3)

The OFT (Hall, 1934) consisted of a large, well-lit (floor surface lux approximately 200) circular arena (90 cm diameter) with aluminium high walls (60 cm). The rat was placed in the centre of the open field and allowed to explore freely for 5 min. Between each rat exposure the arena was cleaned with hot soapy water. A video camera approximately 94 cm above the arena floor recorded activity in the OFT to DVD and behaviour was tracked live using EthoVision® software. The arena was divided into inner and outer arenas and parameters of interest derived by EthoVision® included total distance moved, velocity of movement, time spent and movement in the inner and outer arenas.

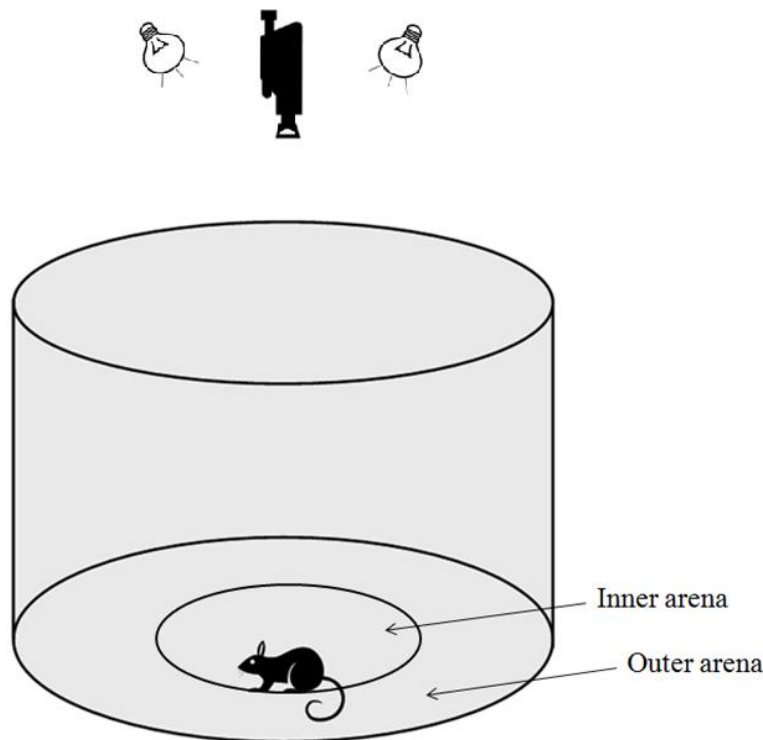


Figure 2.3: The open field test

2.3.3 Elevated Plus Maze (Figure 2.4)

The EPM is a validated animal model of anxiety (Pellow et al., 1985), it consisted of a 4-arm maze (each arm is 40 cm length x 12 cm width) elevated approximately 50 cm from the floor. The 4 arms intersect (centre section 12 cm x 12 cm) and form the shape of a plus (+); two of the arms are open and two are enclosed. The closed arms (lux 36) are surrounded by walls approximately 30 cm high, the open arms centre sections of the arena (lux 90) are lit by two bulbs located above the maze. Thirty min after receiving saline or the anxiolytic drug diazepam (DZP) each individual rat was placed into the centre of the maze facing an open arm and allowed to freely explore for 5 min. After each rat exposure the maze was cleaned down and the rat returned to its homecage. A camera located approximately 1.3 m above the maze recorded behaviour to DVD and behaviour was tracked live using EthoVision®. The parameters of interest were entries to and time spent in the open/closed arms of the maze.

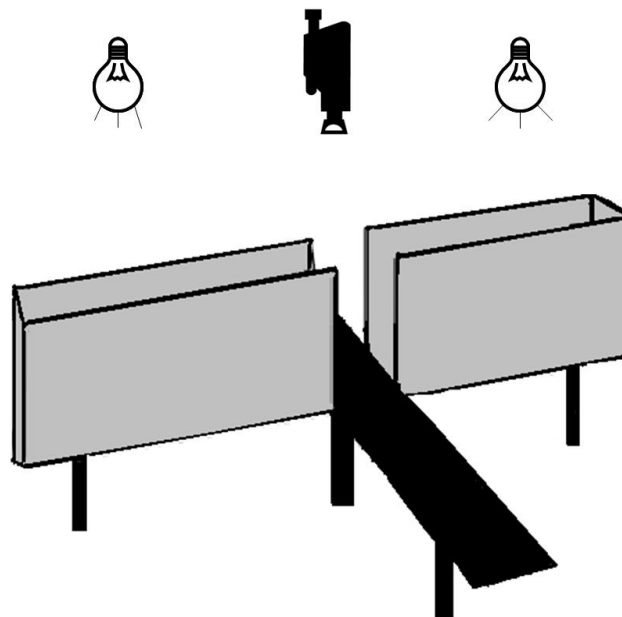


Figure 2.4: The elevated plus maze

2.3.4 Forced Swim Test (Figure 2.5)

The FST is a test of behavioural despair used to evaluate the antidepressant activity of compounds (Porsolt et al., 1978). The FST procedure consisted of a 15 min pretest followed 24 hours later by a 5 min test. Saline vehicle or the antidepressant drug DMI was administered at 24 hours, 5 hours and 1 hour prior to the test. During the test, individual rats were placed in glass cylinders, filled with water (23–25 °C) to a depth of 25 cm. The test was recorded onto DVD and later scored manually. The parameters of interest were time spent swimming, climbing and immobile (i.e. making minimum movements except those necessary to keep the rat's head above water).

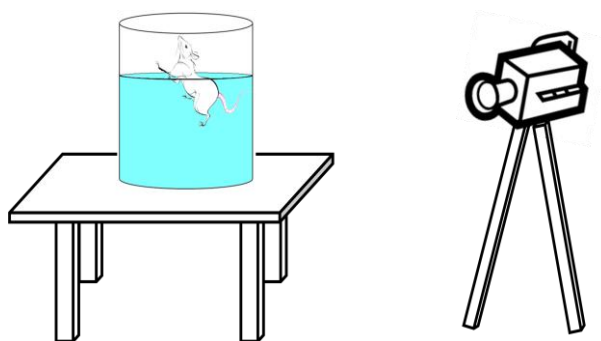


Figure 2.5: The forced swim test

2.3.5 Homecage Monitoring

Homecage Tracking (Figure 2.6)

Home cage activity (HCA) monitors were used to continuously monitor rats' locomotor activity, using a method described in detail elsewhere (O'Halloran et al., 1993). The apparatus consisted of a wooden and steel rack (2.07 m long, 1.7 m high and 0.5 m wide) in which the home cages were placed. Each monitor located above the cages contained a passive infrared sensor, split into zones that record the individual animal's movement across the zones in the cage as a count. The number of activity counts was recorded for 23 hours of each day for one week; values were then

summed to represent total activity counts over the 23-hour monitoring period and mean activity counts during the light and dark periods of the day were calculated.

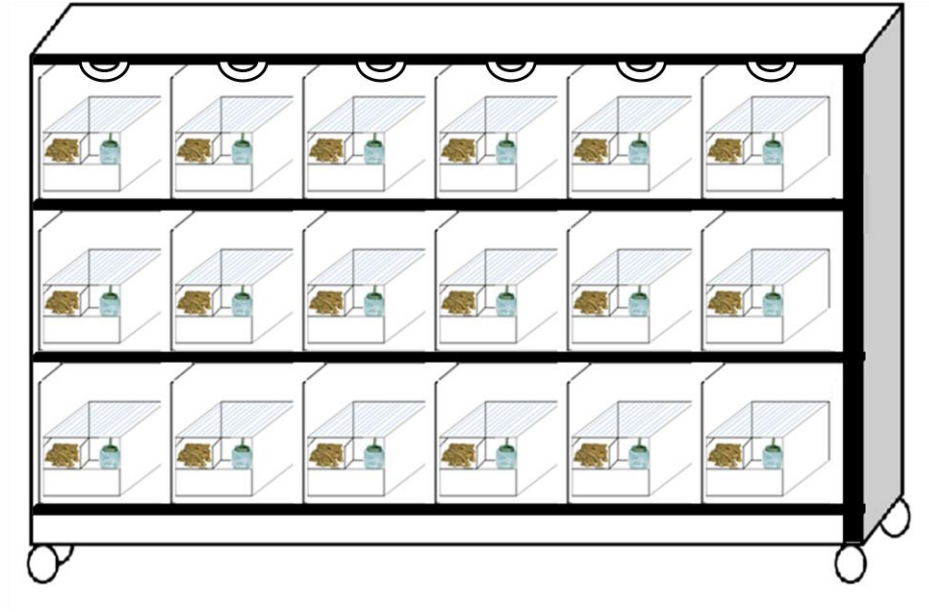


Figure 2.6: Homeage tracking cage rack

Homeage Monitoring (Figure 2.7)

The homeage monitoring apparatus (HCMA) consists of a mobile, steel structure measuring 163 cm in height, 127 cm in length and 52 cm in width which contains four adjacent wooden chambers (44 x 29 x 46 cm) each of which can accommodate a rat cage. An adjustable surveillance camera was positioned (approximately 62 cm) above the base of each chamber, and this was connected to a DVD recorder and monitor which displayed the output. Rats were habituated in individual homecages with dark bedding and dark plates which covered the cage base (thus minimising interference for EthoVision® tracking) overnight prior to drug treatment. Following administration of saline or a stimulant drug the homecages were placed in the HCMA and their activity was recorded for 1-2 hours. EthoVision® was used to record locomotor activity throughout, the parameters of interest were distance moved (cm) and velocity (cm/sec).

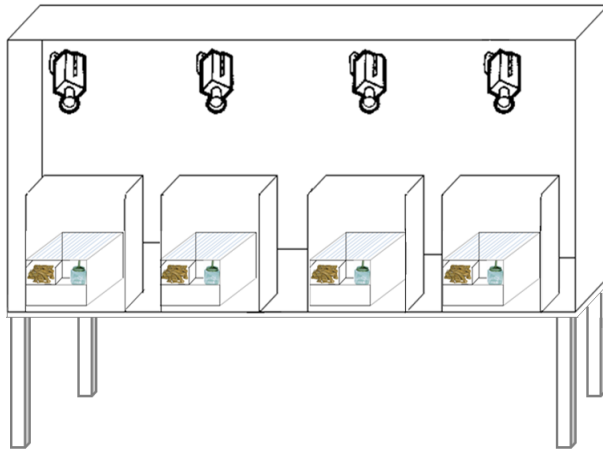


Figure 2.7: The Homecage monitoring apparatus

2.3.6 Morris water maze (Figure 2.8)

The MWM (Morris, 1984) is a test of learning and memory, it consists of a large circular tank (200 cm x 61 cm) filled with water to a depth of 32 cm. Large geometric shapes (oval, rectangle, vertical lines) are placed at three locations (North, South and East) on the shower curtains surrounding the maze to provide extramaze visual cues (see Fig. 2.8 B), these cues remained constant throughout training and testing. The water temperature was maintained at 26 °C (\pm 2). Four spot lights and diffusers (lux approximately 120) were situated approximately 174 cm from the water surface and a video camera above the tank recorded behaviour to DVD. The MWM procedure consisted of a four-day acquisition phase followed by a probe trial on day 5. During the acquisition phase a transparent escape platform (30 cm height x 10 cm diameter) was submerged in the southwest (SW) quadrant of the tank. The rat was gently placed in the pool, facing the wall, at one of four pre-determined release points (South-East, North-West, North or East). Each rat was given 2 min to find the platform and if it failed to reach the platform in this time the rat was gently guided to the platform and remained there for 10 seconds before being removed, dried and placed in a recovery cage. Each rat received four trials per day across the four days of the acquisition phase. The time taken to reach the escape platform was recorded. During the acquisition phase the primary parameter of interest was latency (i.e. time required) to reach the escape platform, the four daily trials were averaged to get a mean latency value for each animal on each day. The rats' swim speed and path length were also recorded using EthoVision®. On day 5, the probe trial was carried

out during which the platform was removed from the maze and the rat was placed in the pool at a novel starting point (North-East). The trial consisted of 2 min free swimming before the rat was removed from the pool. The parameter of interest was time spent in the target (SW) quadrant. In both phases of the water maze task rats' performance in the task was recorded to DVD and later tracked using EthoVision ®.

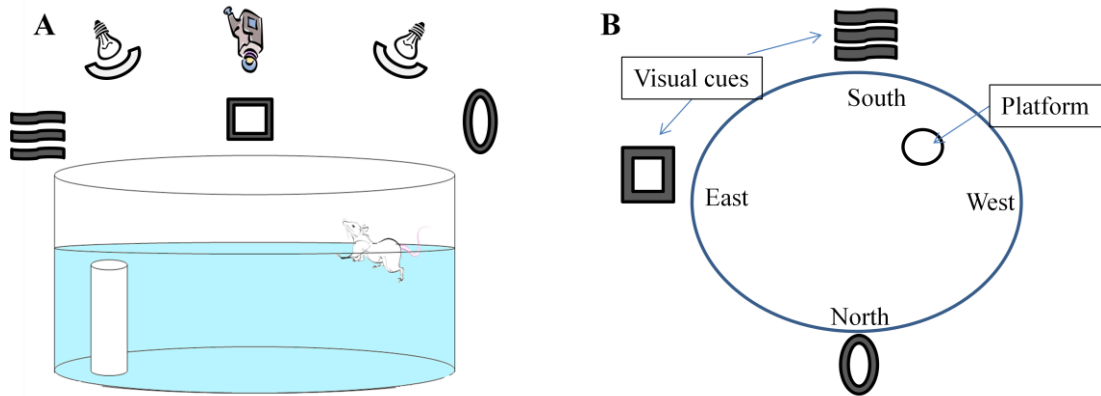


Figure 2.8: The Morris water maze; diagram of the Morris water maze (A) and schematic view of the maze quadrants (B).

2.3.7 Novel Object Recognition Test (Figure 2.9)

The NOR test was carried out to investigate any housing effects on memory in the rat. This test is based on the tendency of rats to interact more with a novel object than with a familiar one (Ennaceur and Delacour, 1988). The apparatus consisted of a circular dark open field arena (75 cm diameter, lux 25) with high walls (approximately 60 cm). On the first day each rat was placed in the empty arena for 30-min habituation. On the second day each rat was re-habituated to the arena for 3 minutes, then removed to their homecage for 7 minutes. This was followed by two 3-min exposures to objects in the apparatus. In the first phase the rat was exposed to two identical (familiar) objects (plastic Coca-Cola® bottles, 24 cm high, 20 cm circumference). The objects were placed at opposite sides of the arena (60 cm apart and each object was approximately 12 and 38 cm from the arena walls). After the first exposure, each rat was removed to their homecage for 2 minutes ITI. In the second phase the rat was exposed to one of the previously experience objects and a novel object (a tower-shaped Stickle Brick® construction, 15 cm high x 5 cm wide). It was expected that the rats would spend more time exploring the novel object than

the familiar one in exposure 2. Behaviour was recorded using a video camera above the NOR apparatus and behaviour was manually scored. Time spent exploring the novel and familiar objects during exposure 2 were recorded. In addition, a

discrimination ratio was calculated as:
$$\frac{\text{Time spent exploring novel object}}{\text{Time spent exploring novel + familiar objects}}$$

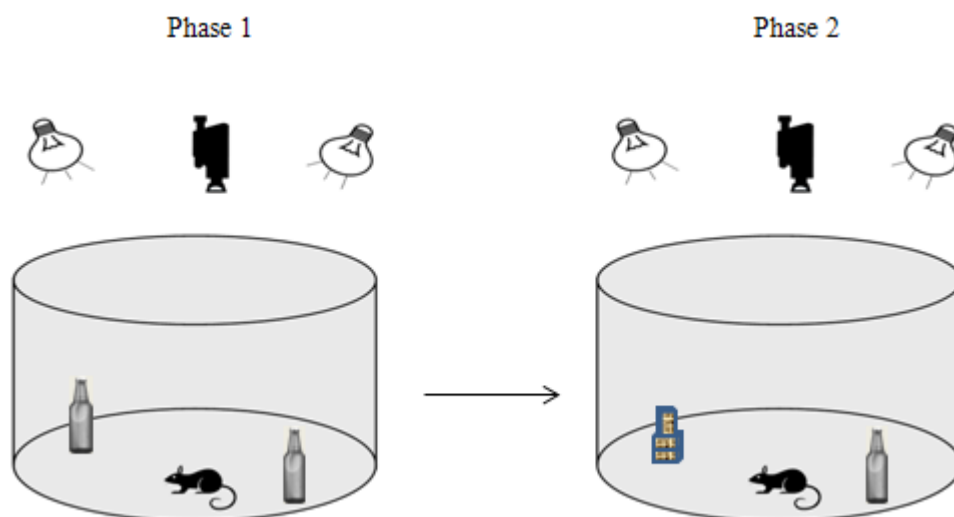


Figure 2.9: The novel object recognition test

2.4 Drugs

All drugs were made up in 0.89% w/v NaCl and were administered in a dose volume 1 ml/kg, with the exception DMI, which was administered 2 ml/kg to avoid irritation at the injection site. The drugs employed are summarised in table 2.1.

Table 2.1: Drug doses and routes of administration

Drug	Dose	Route
DZP	0.625, 1.25, 2.5 and 5 mg kg ⁻¹	i.p.
DMI	2.5, 5 and 10 mg kg ⁻¹	i.p.
MA	0.25, 0.5, 1, 2 and 4 mg kg ⁻¹	s.c.
AMP	0.75, 0.2, 0.5, 1, 1.5, 2, 3, 3.75 and 5 mg kg ⁻¹	s.c.
APO	0.75, 1.5 and 3 mg kg ⁻¹	s.c.

i.p., intraperitoneal; s.c., subcutaneous injections; DZP, diazepam; DMI, desipramine; MA, methamphetamine; AMP, amphetamine; APO, apomorphine.

2.5 Animal sacrifice and brain dissection

Upon completion of each experiment rats were sacrificed by decapitation, the brain removed from the skull and frozen on dry ice before being stored at -80 °C for later analysis. Some brains were gross dissected while micropunch samples were obtained from others.

2.5.1 Gross Brain Dissection

All procedures were carried out on ice. Brains were gross dissected (adapted from the Neuropharmacology laboratory manual, department of pharmacology, National University of Ireland, Galway, 2011) and the hypothalamus, frontal cortex, striatum, hippocampus and cerebellum removed and placed in labelled and weighed eppendorfs. The brain was turned ventral side uppermost and the olfactory tubercles were first removed using a forceps. The frontal cortex was removed by making a cross-sectional cut anterior to the hypothalamus, which was then removed using a forceps. Next a horizontal cut to the boundary of the hypothalamus was made and the tissue section was flattened out to identify the outer cortex and inner striatal tissue. The inner striatal tissue was separated from the surrounding cortex using a forceps. The brainstem was then separated from the tissue containing the hippocampus by making a horizontal cut through the tissue where the brainstem was connected to the rest of the brain. To dissect the hippocampus a horizontal cut was made through the centre of the brain, through the connection point of the hypothalamus. This middle section of the brain was “folded” and was flattened out gently to reveal the hippocampus, which was removed using a forceps. The cerebellum was removed by making a horizontal cut along the side of the brain stem.

2.5.2 Cryostat and Punch Procedure

Whole brain samples were placed in the cryostat box which was set to -18 °C. Weighed, labelled eppendorfs were kept on dry-ice, as were the tips of the micro-dissection punches. Once the tissue reached a suitable temperature, the hindbrain and olfactory bulbs were removed by making a horizontal cut along the brain, creating a smooth edge to glue the brain in position using Tissue-Tek®. The brain was fixed with the frontal cortex facing outwards and the ventral surface facing upwards. When the tissue was firmly set some thin (20 µm) slices were taken to ensure the surface was even. Then the blade was adjusted to 300 µm sections to take sections of brain regions of interest. Frozen coronal brain sections (300 µm) were collected from

the start of the PFC through to the end of the hippocampus. The following brain regions were identified using a brain atlas (Paxinos and Watson, 1998) (see Fig. 2.10); the frontal cortex (start: Bregma 3.70 mm), nucleus accumbens (start: Bregma 2.70 mm), striatum (start: Bregma 1.20 mm), amygdala (start: Bregma -1.80 mm) and the hippocampus (start: Bregma -2.12 mm). Once cut, the sections were placed onto labelled gelatine-subbed slides and samples were punched from sections using cylindrical micron biopsy punches (internal diameter 2.0 mm for all regions). Punch samples were placed in pre-weighed and labelled eppendorfs for later analysis.

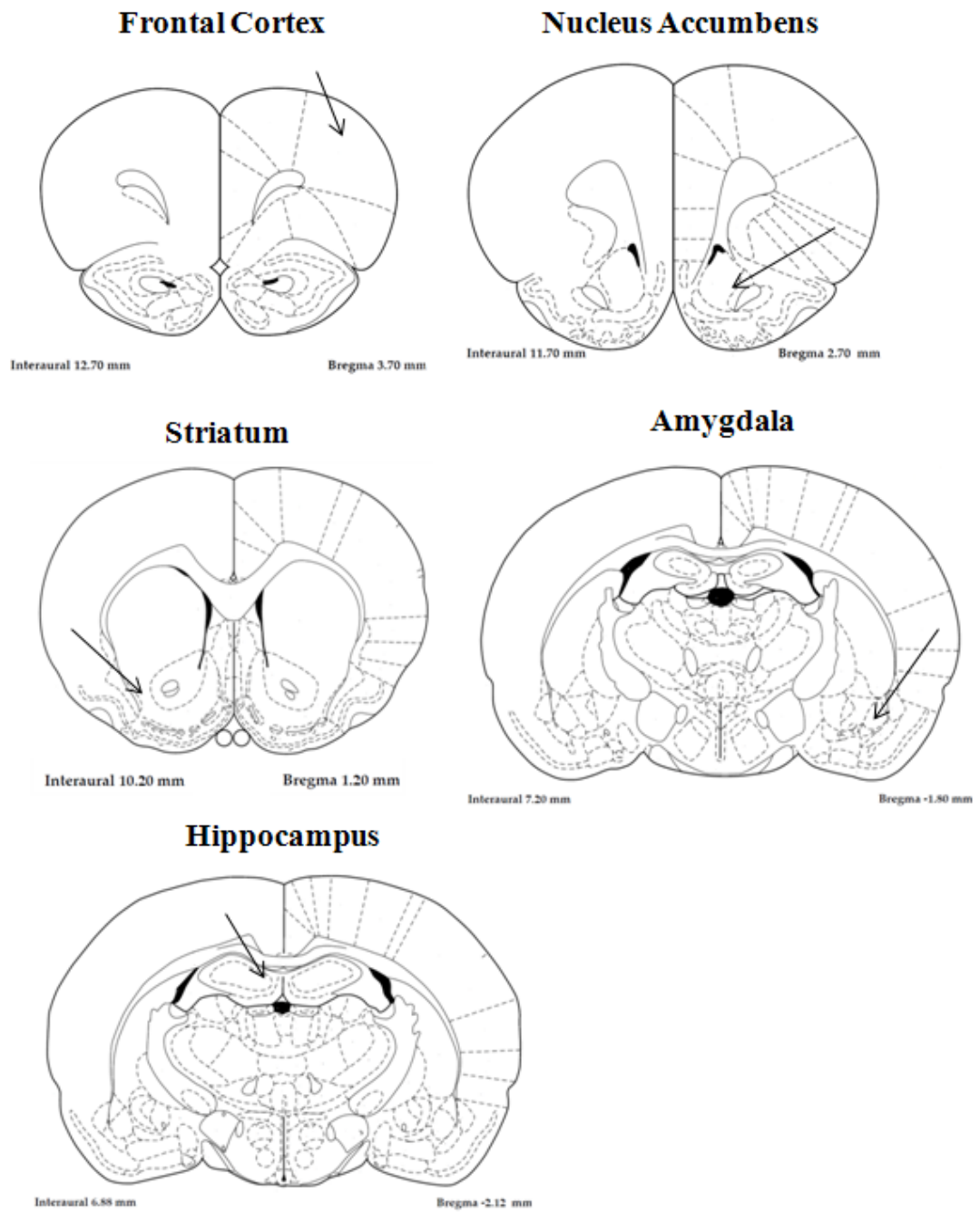


Figure 2.10: Rat brain regions of interest (from Paxinos and Watson, 1998). Punched regions are indicated with an arrow.

2.6 Determination of brain monoamine levels using high - performance liquid chromatography (HPLC)

Concentrations of NA, 3-4-Dihydroxyphenylalanine (L-Dopa), DOPAC, DA, 5HIAA, HVA and 5HT were measured by high performance liquid chromatography (HPLC) with electrochemical detection. The HPLC method used was based on that of (Seyfried et al., 1986).

2.6.1 Preparation of Mobile Phase

Reagents (Table 2.2) were fully dissolved in nano-pure water and made up to a final volume of 4 L, following which methanol (400 ml) was added and the final pH of the solution was corrected to 3.5 using 4 M NaOH.

Table 2.2: Reagents used to prepare HPLC mobile phase.

Compound	Weight (g)/4 L
0.1M Citric acid monohydrate	84.056
0.1M Sodium dihydrogen phosphate monohydrate	55.186
1.4mM 1-Octane sulfonic acid	1.211
0.1mM Ethylenediaminetetra-acetic acid (EDTA)	0.1488

2.6.2 Preparation of HPLC Standards

- Allowing for the attached salts, the standard amines (Table 2.3) were individually dissolved in 10 ml of HPLC mobile phase solution to give solutions of 10 mg/10 ml of each amine.
- 100 µl of each (10 mg/10 ml) solution was then added to 10 ml of mobile phase to yield amine concentrations of 200 ng/20 µl.
- A standard mix solution was prepared by adding 100 µl of each (200 ng/20 µl) standard to 10 ml mobile phase buffer, yielding a single solution containing each amine at a concentration of 2 ng/ 20 µl.

2.6.3 Preparation of Homogenising Buffer

To prepare the homogenising buffer, 1 ml of the N-Methyl-5-HT (internal standard) (200 ng/20 μ l) solution was diluted in 100 ml mobile phase buffer to provide a working homogenization buffer containing 2 ng/20 μ l of internal standard. This provided an identifiable reference peak which was present in every sample analysed.

Table 2.3: Internal and external standard mixtures used in the HPLC analysis of rat biogenic amine concentrations.

Compound	Weight (mg) [*] /10 ml
NA	19.8
L-DOPA	10
DOPAC	10
DA	12.3
5-HIAA	10
HVA	10
5-HT	22.2
N-Methyl-5-HT(IS)	14.7

^{*}Denotes the weight of the compound required to produce an initial concentration of 10 mg/10 ml taking into account the existence of salts or hydrated forms of the compound. NA, noradrenaline; L-DOPA, L-3-4-Dihydroxyphenylalanine; DOPAC, 3,4-Dihydroxyphenyl-acetic acid; DA, dopamine; 5HIAA, 5-hydroxyindoleacetic acid; HVA, Homovanilic acid; 5HT, 5-hydroxytryptamine; IS, internal standard.

2.6.4 Sample preparation for HPLC

All procedures were carried out on ice with samples derived from gross dissected tissue sections (see section 2.5.1) or tissue punches (section 2.5.2). The tissue was sonicated in 1 ml HPLC homogenising buffer, ensuring that no intact tissue remained in the vial. Sonicated samples were then centrifuged at 14,000 g for 15 min at 4 °C. The samples were then frozen for use on the following day. On the day of the analysis the samples were thawed and centrifuged again to ensure full separation of pellet and supernatant at 14,000 g for 5 min. Aliquots (300 μ l) of the supernatant of each sample were dispensed into vials and placed in the HPLC auto-injector. One standard mix was run between every 10 samples to recalibrate the system and minimize any drift in amine retention times as sampling proceeded.

2.6.5 HPLC analysis:

Biogenic amine concentrations were determined using an automated HPLC system connected to an electrochemical detector and coupled with an integrator which produced a chromatogram for each sample and standard mixture.

The retention times of biogenic amines varied between 4 and 60 min (see Fig 2.11 representative chromatograms), depending on which amine, on a Merck Licrosorb RP-C18 column. The flow rate of mobile phase through the column was 1 ml/min at a pressure of 200 bars. The column oven was maintained at 30 °C and the electrochemical detector set at 0.8 v. The sample injection volume was set at 20 µl and the sample run time maintained at 65 min for the duration of the analysis, to make sure all amines had eluted. Peak height data from chromatograms together with data obtained from brain tissue weights and internal and external standards mixtures was used to calculate neurotransmitter concentration in each brain sample in terms of ng/g of fresh tissue.

After HPLC analysis the peaks in the standards were identified from chromatograms (Fig. 2.11) and using the retention time found using the standards, the biogenic amines in samples were also identified. First of all, the relative retention factor (RRF) was calculated. The RRF describes the ability of a HPLC system to discriminate between two compounds, for example: a reference compound and an unknown compound. It is dependent on the temperature, properties of the mobile phase and stationary phases and is heavily influenced by the presence of impurities in the mobile phase. The RRF calculation is described in equation 1.

$$RRF_{mixture} = \frac{CONC_{IS\ mix} \times PH_{NT\ mix}}{CONC_{NT\ mix} \times PH_{IS\ mix}}$$

Where:

$CONC_{IS\ mix}$: Weight of Internal Standard (2ng) in the volume (20 μ l) of mix injected

$CONC_{NT\ mix}$: Weight of Amine Neurotransmitter (2ng) in the volume (20 μ l) of mix injected

$PH_{NT\ mix}$: Peak height of the Amine Neurotransmitter in the mix

$PH_{IS\ mix}$: Peak height of the Internal Standard in the mix

Equation 1. Calculation for Relative retention Factor (RRF).

As previously described, N-Methyl-5-HT (2 ng/20 μ l) was the internal standard within each sample. To determine the concentration of a particular amine neurotransmitter (NT) in the brain tissue sample in terms of ng NT per 20 μ l, equation 2 was used.

$$NT\ Concentration(ng/20\mu l) = \frac{PH_{NT\ sample} \times [IS]_{sample}}{PH_{IS\ sample} \times RRF\ of\ the\ NT_{mix}}$$

Where:

$PH_{NT\ mix}$: Peak height of the Amine Neurotransmitter in the mix

$PH_{IS\ mix}$: Peak height of the Internal Standard in the mix

$[IS_{sample}]$: Concentration of the Internal Standard in sample (2ng/20 μ l)

RRF of the NT: Relative Retention Factor (calculated previously)

Equation 2. Calculation for NT concentration (ng/20 μ l).

To convert the NT concentration from units of ng/20 μ l to units of ng/gram of fresh tissue equation 3 was used.

NT concentration (ng/g tissue) =	$\frac{[\text{NT}] \text{ in sample} \times 50}{\text{Weight of sample (mg)}} \times 1000$
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Equation 3. Calculation of neurotransmitter concentration in ng/gram of fresh tissue.

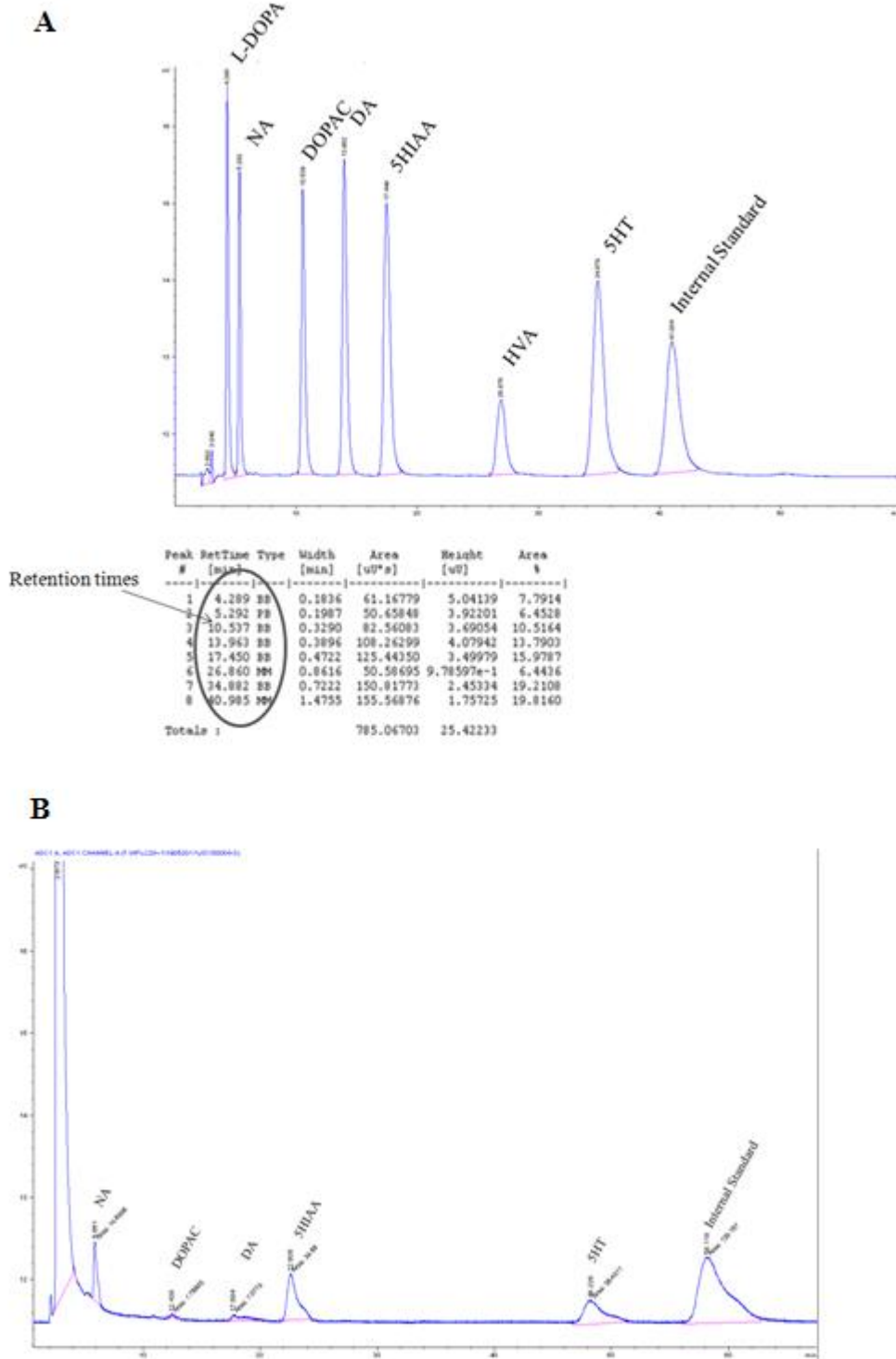


Figure 2.11: Sample HPLC chromatograms. Chromatogram from a mix analysis (A) and a hippocampus tissue sample (B). L-Dopa, L-3-4-Dihydroxyphenylalanine; NA, noradrenaline; DOPAC, 3,4-Dihydroxyphenyl-acetic acid; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, Homovanilic acid; 5HT, 5-hydroxytryptamine.

2.7 Enzyme Linked ImmunoSorbent Assay (ELISA)

In this study, the Sandwich ELISA was used to detect sample BDNF concentrations. The Sandwich ELISA measures the amount of antigen between two layers of antibodies (i.e. capture and detection antibody).

2.7.1 Solutions required:

- a) Phosphate-Buffered Saline (PBS): PBS tablets were used, 1 tablet dissolved in 200 ml Distilled H₂O. 2 L PBS was made up at a time (10 tablets in 2 L DH₂O).
- b) Wash Buffer: 500 µl Tween 20 (0.05% v/v) was added to 1 L of PBS to make up 1 L of wash buffer.
- c) Reagent Diluent: 1% bovine serum albumin (BSA) (w/v) in PBS
- d) Lysis Buffer: 0.80062 g NaCl
0.315 g Trizma HCL
10 ml Glycerol (10% v/v)
1 ml Igepal (1% v/v)

Made up to a final volume of 100 ml; 1 µl protease inhibitor is added to each ml of lysis buffer immediately before use.

- e) Substrate solution: TMB (3-3, 5-5-tetramethylbenzidine).
- f) Stop solution: 2N sulphuric acid (H₂SO₄)

2.7.2 Sample Preparation

All procedures were carried out on ice. Brains were gross dissected and the hypothalamus, frontal cortex, hippocampus and cerebellum were removed as described previously in section 2.4.1. The samples were placed in labelled eppendorfs then sonicated in 750 µl lysis buffer, ensuring that no intact tissue remained in the vial. Sonicated samples were then centrifuged at 14,000 g for 15 min at 4 °C. The supernatant was removed for ELISA and protein assays.

2.7.3 Bicinchoninic Acid Protein Assay Protocol

BDNF concentration in tissue samples was expressed per unit of tissue protein; tissue protein was determined using bicinchoninic acid (BCA) protein assay (Smith et al., 1985). The BCA assay was carried out due to the potential interactive effects of igepal and glycerol (in the lysis buffer) on the Bradford protein assay which is more routinely carried out in this laboratory. The peptide bonds in protein reduce Cu^{+2} ions from the cupric sulfate to Cu^{+} . The amount of Cu^{+2} reduced is proportional to the amount of protein present in the sample. Next, two molecules of bicinchoninic acid chelate with each Cu^{+} ion, forming a purple-coloured product that exhibits a strong absorbance at a wavelength of 570 nm that is nearly linear with increasing protein concentrations over a broad working range

- All solutions, samples and standards are kept on ice.
- Protein standards as per table 2.4 were made from a stock solution of 0.2 % BSA; 0.02 g BSA was added to 10 ml lysis buffer and allowed to dissolve with gentle agitation.
- The BCA solution was made up by combining reagent A and B; BCA reagent A (materials section 2.1), reagent B was copper sulphate (4% w/v), i.e. 4g copper sulphate dissolved in 100 ml H_2O .
- BCA Reagent A was added to reagent B in a ratio 50:1, thus 29.4 ml reagent A was added to 0.6 ml reagent B to make 30 ml BCA solution. Samples and standards (10 μl) were pipetted in duplicate to a 96-well plate and 200 μl BCA solution was added.
- The plate was gently shaken on a plate rocker for 30 seconds before heating at 37 ° C in a mini-incubator oven for 30 min.
- After the heating period the plate was read plate was read in a plate reader at 570 nm.
- A standard curve was plotted using GraphPad Prism[®] as in figure 2.12. Sample concentration was calculated by multiplying the absorbances obtained from each sample by 1/slope obtained from the curve.

Table 2.4: Preparation of protein standards

BSA Stock solution (μl)	Lysis Buffer (μl)	Final concentration BSA (μg/ml)
0	200	0
5	195	50
10	190	100
25	175	250
50	150	500
75	125	750
100	100	1000

BSA, Bovine serum albumin.

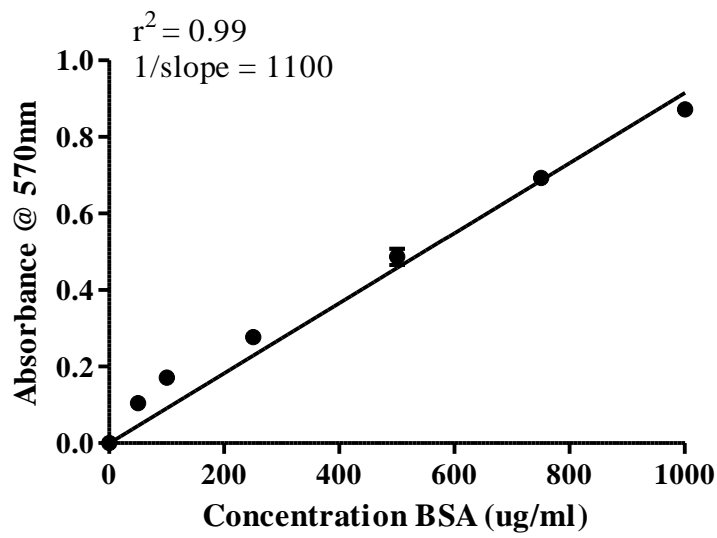


Figure 2.12: BSA protein standard curve. Concentration BSA (μg/ml), data expressed as mean \pm standard deviation ($n = 2$ replicates), created in GraphPad Prism®.

2.7.4 BDNF ELISA Kit

The human BDNF ELISA kit used was the DuoSet ELISA Development kit from R & D Systems (Johnson and Mitchell, 2003). This contained the basic components required for the development of a sandwich ELISA to measure natural and recombinant BDNF in cell culture supernates and serum. Previous work in this laboratory has also validated this assay for use with rat brain lysates.

Materials provided in the kit:

- a) *Capture antibody*: Supplied 360ug/ml of mouse anti-human antibody when reconstituted with 1 ml PBS (i.e. 1 ml PBS added to the vial). The solution was dissolved fully with some gentle agitation. To make this to a working concentration of 2 µg/ml a 1 in 180 dilution was required. The 1 ml stock solution was added to 179 ml PBS. The solution (2 µg/ml) was gently stirred then stored in 15 ml tubes (5 ml per tube) at -20°C. All tubes were labelled with a name and date.
- b) *Detection Antibody*: Supplied 4.5 µg/ml of biotinylated mouse anti-human BDNF when reconstituted with 1 ml reagent diluent (i.e. 1 ml reagent diluent added to the vial). The solution was dissolved fully with some gentle agitation. To make this to a working concentration of 25 ng/ml a 1 in 180 dilution was required. The 1 ml stock solution was added to 179 ml reagent diluent. The solution (25 ng/ml) was gently stirred then stored in 15 ml tubes (5 ml per tube) at -20 °C. All tubes were labelled with a name and date.
- c) *Standards*: Supplied 110 ng/ml of recombinant human BDNF when reconstituted with 0.5 ml reagent diluent (i.e. 500 µl added to each vial). The solution was dissolved fully with some gentle agitation. To make this up to a high standard of 2000 pg/ml (as recommended) a 1 in 55 dilution was required. Thus 27 ml of reagent diluent was added to each 0.5 ml of stock solution. The solution (2000 pg/ml) was gently stirred then stored as 1 ml in eppendorfs at -20 °C. All tubes were labelled with a name and date.
- d) *Streptavidin-HRP (horseradish-peroxidase)*: Supplied 1 ml of streptavidin conjugated to horseradish peroxidase. This was stored at 2–8 °C. Immediately before use the streptavidin was diluted 1 in 200 with reagent diluent.

2.7.5 ELISA Protocol

Plate Preparation:

1. Capture antibody (2 µg/ml) was thawed and 100 µl per well was added to 96-well plates (Nunc maxisorb). Each plate was sealed with an adhesive strip and incubated overnight at room temperature.
2. The next day the plates were washed and aspirated with 400 µl wash buffer three times using an automated plate-washer. The plates were then inverted and blotted dry on clean paper towel.
3. Next the wells were blocked by adding 300 µl reagent diluent and incubated at room temperature for 2 hours.
4. Aspiration/washing was repeated as in step 2

Assay procedure:

5. BDNF standards (100 µl) in reagent diluent (as shown in table 2.5, vortex samples) and undiluted supernatant samples were added to the plates in duplicate. Plates were then sealed with an adhesive strip and incubated at room temperature for 2 hours.
6. Aspiration/washing was repeated as in step 2
7. Detection antibody (100 µl) was added to each well and plates were covered with an adhesive strip and incubated at room temperature for 2 hours.
8. Aspiration/washing was repeated as in step 2
9. Streptavidin-HRP was made up (50 µl strep + 9950 µl distilled water) and 100 µl added to each well. Plates were covered with an adhesive strip and incubated at room temperature for 20 min out of direct light.
During this time 12.5 ml TMB (substrate solution) was taken from the fridge (kept in tinfoil as it is light sensitive) and was allowed to reach room temperature.
10. Aspiration/washing was repeated as in step 2
11. TMB (100 µl) was added to each well and plates were left to incubate for 20 min at room temperature (and out of direct light) until colour began to develop.
12. The reaction was stopped by adding 50 µl of stop solution (2N sulphuric acid, H₂SO₄) to each well.

13. The optical density of plates was determined using a plate reader at 450nm.

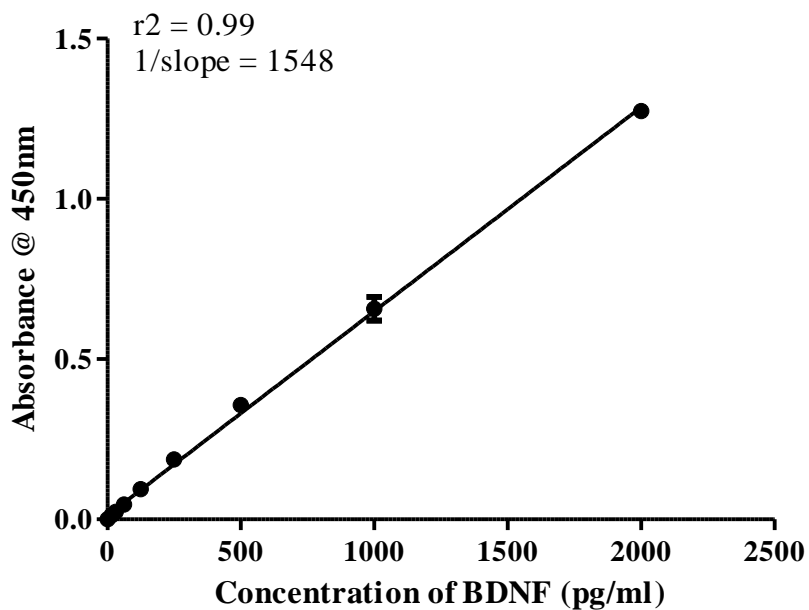
The values obtained were entered into a Microsoft Excel® spreadsheet, then a standard curve (0–2000 pg/ml) was plotted (Fig. 2.13) by subtracting the average absorbance at 0 pg/ml from the other standard averages.

The slope was obtained using Graphpad Prism® software. Using the slope and absorbance reading the concentration of BDNF in each brain sample in terms of pg / ml was calculated ($\text{Concentration} = \text{Absorbance} \times 1/\text{Slope}$). BDNF was expressed as pg/mg tissue protein.

Table 2.5: Serial dilution of brain-derived neurotrophic factor standards in reagent diluent

BDNF Standard (pg/ml)	Volume of Stock required (μ l)	Volume of Reagent Diluent required (μ l)	Total Volume of standard (μ l)
2000	500	0	500
1000	500 of 2000 pg/ml	500	1000
500	500 of 1000 pg/ml	500	1000
250	500 of 500 pg/ml	500	1000
125	500 of 250 pg/ml	500	1000
62.5	500 of 125 pg/ml	500	1000
31.25	500 of 62.5 pg/ml	500	1000
15	500 of 31.25 pg/ml	500	1000
0	0	500	500

BDNF, brain-derived neurotrophic factor

**Figure 2.13:** Brain-derived neurotrophic factor standard curve. Concentration BDNF (pg/ml), data expressed as mean \pm standard deviation ($n = 2$ replicates), created in GraphPad Prism®.

2.8 Statistical analyses

All data were analysed using SPSS statistical software (version 18). Normal distribution was established by Levene's test. One-way ANOVAs were carried out in all experiments to assess baseline strain and housing differences and dose-response effects within housing, sex or strain groups in pharmacology studies. *Post-hoc* SNK and Tukey tests were carried out to explore pair-wise comparisons. Two-way ANOVAs were used to assess main and interaction effects in all studies, repeated measures ANOVAs were also conducted in each study to analyse body weight, MWM performance and/or drug effects over time.

Chapter 3

Effects of Housing on Baseline and Drug-Induced Behavioural Responses in the Male Rat

Chapter 3

Results chapter 1

Effects of housing on baseline and drug-induced behavioural responses in the male rat

3.1 Introduction

The provision of EE can influence the young rat's development. Pre-weaning (i.e. prior to PND 21, see table 3.1) EE can enhance neural development in the somatosensory cortex (Fernandez et al., 1998) and auditory cortex (Cai et al., 2009), as well as reducing dominant play behaviours (Magalhaes et al., 2007) and subsequent responsiveness to stress (Welberg et al., 2006). A number of protocols implement a period of EE just after weaning (Fig. 1.2); post-weaning EE increases exploration and improves habituation to a novel arena (Zimmermann et al., 2001, Brenes et al., 2009) compared to rats housed in IC or SC conditions. The effects of post-weaning EE on anxiety-like behaviours in other tests are inconsistent, with some reports of increases (Pena et al., 2009a, Pena et al., 2006), or no effect on (Brenes et al., 2009) open arm entries and open arm time in the EPM when compared to IC and SC controls. Similarly, EE during adolescence had no effect on latency to enter the bright side of the light/dark box in one study (Hellemans et al., 2005) but reduced latency to enter the bright side compared to SC and IC controls in another (Schrijver et al., 2002). When compared to SC and IC, rats housed in EE from weaning have been shown to spend more time swimming and less time immobile in the FST (Brenes et al., 2008, Brenes et al., 2009), which were correlated with neurochemical alterations; i.e. climbing and swimming behaviour correlated positively, while immobility time correlated negatively with NA and 5HT levels in the hippocampus of EE rats (Brenes et al., 2009). This would suggest that EE housing helped to improve rats' coping skills and display more a more active strategy (i.e. trying to escape) when faced with such a stressor as the FST (Magalhaes et al., 2004).

Table 3.1: Rat age at various stages of development and later life

Stage:	Age
Pre – weaning:	< PND 21
Post – weaning:	> PND 21
Early adulthood – Adulthood:	6 weeks – 12 months old
Older adulthood - Aged:	> 12 months old

PND, Post natal day

Post-weaning EE has also improved performance in tests of learning and memory, with reduced latencies to locate the platform in the MWM compared to SC controls (Schrijver et al., 2002, Helleman et al., 2004), significantly greater preference for the novel object in the NOR test compared to SC (Escorihuela et al., 1995) and greater recognition of spatial displacement of an object compared to IC controls (Schrijver et al., 2002). These results reflect improvements in brain plasticity due to the stimulation provided by EE rearing (van Praag et al., 2000). EE and SC rearing from weaning resulted in greater LTP induction in the CA1 area of the hippocampus (Lu et al., 2003), increased newborn cells in the sub-granule cell layer of dentate gyrus and evidence of superior memory skills in the MWM (Lu et al., 2003, Nilsson et al., 1999) when compared to IC controls. Furthermore, increased spine density of parietal neurons due to EE housing were associated with superior problem-solving skills in the radial arm maze and memory in the MWM compared to IC and SC controls (Leggio et al., 2005). Thus EE conditions can enhance plasticity in the rat brain and improve cognitive functioning when compared to rats reared in an impoverished environment.

The behavioural and neuronal changes observed after EE rearing are not confined to young animals. EE has been shown to enhance cortical structure from prenatally developing rats to those over 2 years of age (Diamond, 1988, Diamond, 2001) and the ability of EE to promote plasticity in the brain prompted investigation of the ability of EE to attenuate cognitive decline associated with age-related deficits. Findings have been inconsistent however, possibly due to challenges such as rat longevity and high mortality rates when studying aged rats (approximately 600 days old). Notably a lower mortality rate for 27-month-old rats housed in EE was

reported compared to IC controls (Lores-Arnaiz et al., 2006). Increased hippocampal neurogenesis (Segovia et al., 2006), increased cell body area and dendritic processes (Soffie et al., 1999, Lores-Arnaiz et al., 2006) have been found in aged rats housed in EE conditions compared to controls. However cognitive tests of aged rats do not always reflect these findings as EE did not improve performance in the Morris maze (Segovia et al., 2008) or in a matching-to-sample task (Soffie et al., 1999) compared to IC controls.

Similar to its effects when provided post-weaning, EE in adulthood has been shown to reduce anxiety-like behaviour in the EPM and defensive burying tests compared to SC controls (Leal-Galicia et al., 2007), and reduced latencies in the MWM compared to IC controls (Pham et al., 1999a, Harris et al., 2009), coupled with increased neurotrophic factor concentration in various brain regions (Pham et al., 1999a) and hippocampal neurogenesis (Leal-Galicia et al., 2007). EE implementation immediately post-weaning has reduced immobility time compared to IC and SC controls (Brenes et al., 2009, Green et al., 2010) in the FST. However, few studies have investigated the effects of early adulthood EE on male rats' behaviour in the FST, and those which have report somewhat inconsistent results. Long Evans and SD rats (housed in EE conditions for 6 weeks from approximately 3 months of age) displayed significantly more climbing and swimming behaviours in the FST compared to SC controls (Konkle et al., 2010). However Wistar rats aged 2-2.5 months old showed no effect of EE or SC housing on activity in the FST (Veena et al., 2009). Furthermore, few studies of early adulthood housing effects employ SC and IC control groups to account for any effects of social enrichment alone on behaviour.

The aim of the present study therefore was to investigate the effects of EE and IC housing during early adulthood on baseline and drug-induced behaviours in a number of common behavioural pharmacology tests, compared to SC controls.

3.2 Methods

3.2.1 Animals

Seventy-two male SD rats (150-170 g on arrival) were housed in groups of 4 for four days acclimatisation, then randomly assigned to one of three housing conditions; IC, SC (4/cage) or EE (4/enriched cage).

The IC and SC animals were housed in standard-sized cages while the EE animals were housed in larger cages with enrichment objects and toys (see section 2.2.2). All animals were maintained in their respective housing conditions for four weeks before behavioural testing began and throughout the test period (total 9 weeks, see figure 3.1) and all rats were weighed weekly.

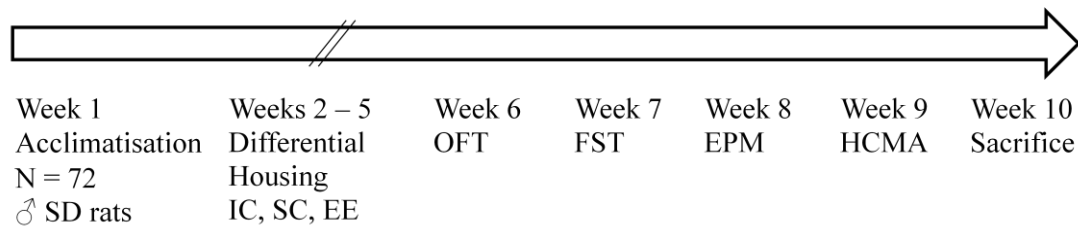


Figure 3.1: Experimental timeline. SD, Sprague Dawley; IC, isolated conditions; SC, standard group conditions; EE, environmentally enriched conditions; OFT, open field test; FST, forced swim test; EPM, elevated plus maze; HCMA, homecage monitoring apparatus.

3.2.2 Drugs

See section 2.1 for details and drug sources.

DZP (1.25, 2.5 and 5 mg kg⁻¹, i.p.) was administered 30 minutes prior to EPM testing. DMI (2.5, 5 and 10 mg kg⁻¹, i.p.) was administered 24, 5 and 1 hour prior to the 5-minute FST. AMP (0.75, 1.5, 3, and 3.75 mg kg⁻¹, s.c.) was administered immediately prior to homecage monitoring.

3.2.3 Open Field Test

See section 2.3.2

3.2.4 Elevated Plus Maze

See section 2.3.3

3.2.5 Forced Swim Test

See section 2.3.4

3.2.6 Homecage activity monitoring

Homecage activity was monitored over 1 hour. See section 2.3.5 HCMA

3.3 Statistical analysis

See section 2.8

3.4 Results

3.4.1. Body Weight, Food and Water consumption (Figure 3.2, Table 3.2)

Regarding body weight, there were significant main effects of housing [$F(2, 69) = 6.82, p < 0.01$], week of study [$F(8, 552) = 772.71, p < 0.001$] and a significant week x housing interaction [$F(16, 552) = 1.36, p < 0.05$] on body weight. All animals gained weight over the 9 week housing and testing period, rats housed in EE were significantly lighter than both SC and IC after the first week of differential housing, for the remaining weeks the group housed (SC and EE) were significantly lighter than IC animals. Overall body weight gain (i.e. body weight on week 9 – body weight on week 1) was also significantly greater in IC rats [$F(2, 68) = 5.75, p < 0.01$] compared to SC; there was no difference between EE and SC rats in weight gain.

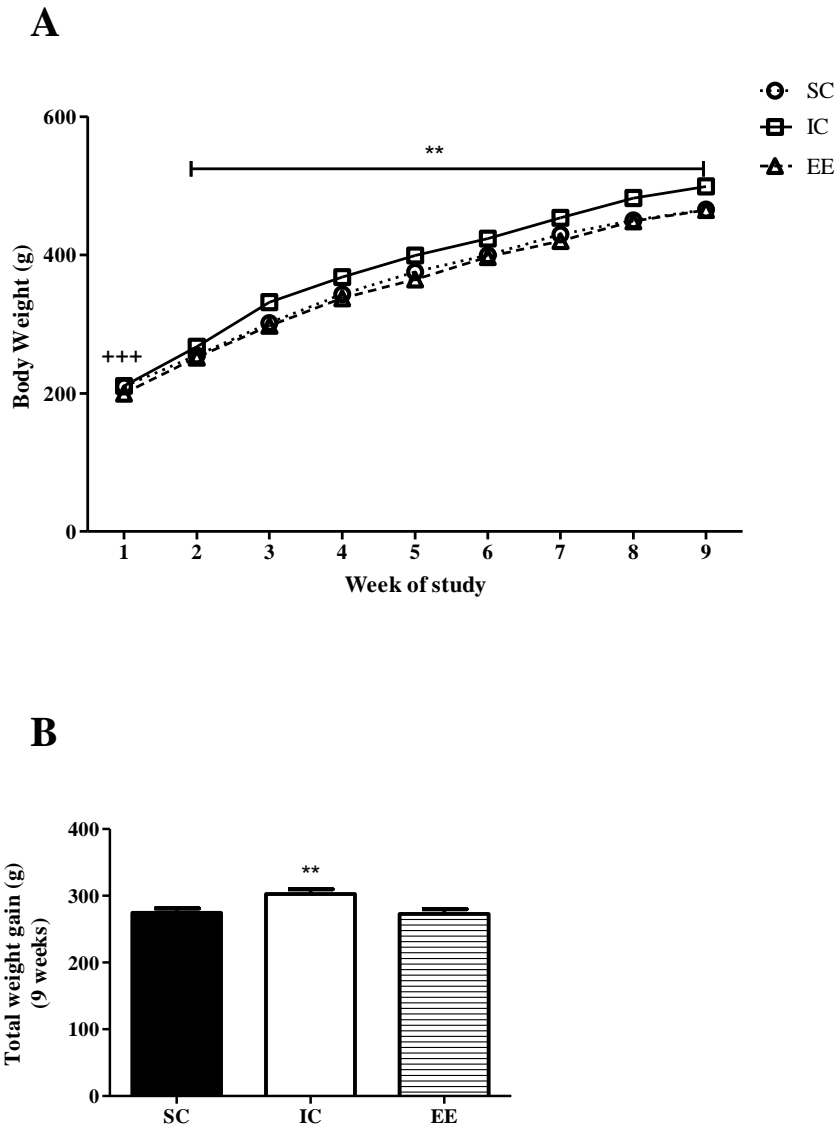


Figure 3.2: The effects of isolation and enriched housing conditions on body weight. The effects of isolated (IC) and enriched (EE) housing on body weight (A) and weight gain (B) compared to standard (SC) conditions over the 9-week housing and testing period. Data expressed as mean + SEM, $n = 24$ per group, +++ $p < 0.001$ EE vs. SC, ** $p < 0.01$ IC vs. SC.

With regards to food consumption, significant effects of time [$F(6, 90) = 16.96, p < 0.001$] and housing condition [$F(2, 15) = 20.90, p < 0.001$], and a significant time x housing interaction [$F(12, 90) = 3.64, p < 0.01$] were seen in weekly food consumption during the 7-week differential housing and testing period. Food consumption remained consistent in the SC group during the 7-week observation period. There was a significant increase in the IC group during weeks 1-3 ($p < 0.001$) and 5-7 ($p < 0.01$) compared to SC-housed rats. EE rats had reduced food consumption compared to IC but were not significantly different to SC.

Significant main effects of time [$F(6, 90) = 24.87, p < 0.001$] and housing condition [$F(2, 15) = 8.33, p < 0.01$] and a significant time x housing interaction [$F(12, 90) = 21.14, p < 0.001$] were seen in weekly water consumption during the 7-week differential housing and testing period. EE rats displayed significantly reduced water consumption on weeks 1, 2 and 4 (all $p < 0.05$) and IC rats had significantly increased water consumption on weeks 3 and 6 ($p < 0.001$) compared to SC rats.

Table 3.2: The effects of isolation and enriched housing conditions on food and water consumption

Housing Condition	Week of study						
	1	2	3	4	5	6	7
	Food consumption (g)						
SC	200 ± 4	190 ± 3	192 ± 3	187 ± 4	195 ± 3	190 ± 3	197 ± 3
IC	224 ± 6***	221 ± 6***	242 ± 10***	198 ± 5	211 ± 6**	210 ± 8**	225 ± 9**
EE	195 ± 6	190 ± 2	196 ± 2	176 ± 2	184 ± 3	179 ± 2	195 ± 2
	Water consumption (ml)						
SC	187 ± 7	191 ± 5	187 ± 3	180 ± 4	190 ± 5	187 ± 6	193 ± 6
IC	193 ± 10	179 ± 9	242 ± 1***	203 ± 13	212 ± 13	255 ± 13***	206 ± 18
EE	165 ± 3*	167 ± 4*	166 ± 4	154 ± 4*	171 ± 5	172 ± 6	181 ± 5

Standard group (SC), isolation (IC) and environmental enrichment (EE) housing conditions effects on water consumption over a 7-week differential housing period. Data expressed as mean ± SEM, $n = 6$ per group, *** $p < 0.001$, $p < 0.05$ vs. SC.

3.4.2 Open Field Test (Figure 3.3)

In the OFT, housing conditions had no effect on distance moved [$F(2, 21) = 1.83, p > 0.05$]. Similarly, entries to [$F(2, 21) = 3.30, p = 0.057$] and time spent [$F(2, 21) = 3.30, p = 0.057$] in the inner arena were also not significantly different between the IC and SC or EE and SC groups, although they were close to the 0.05 level of significance.

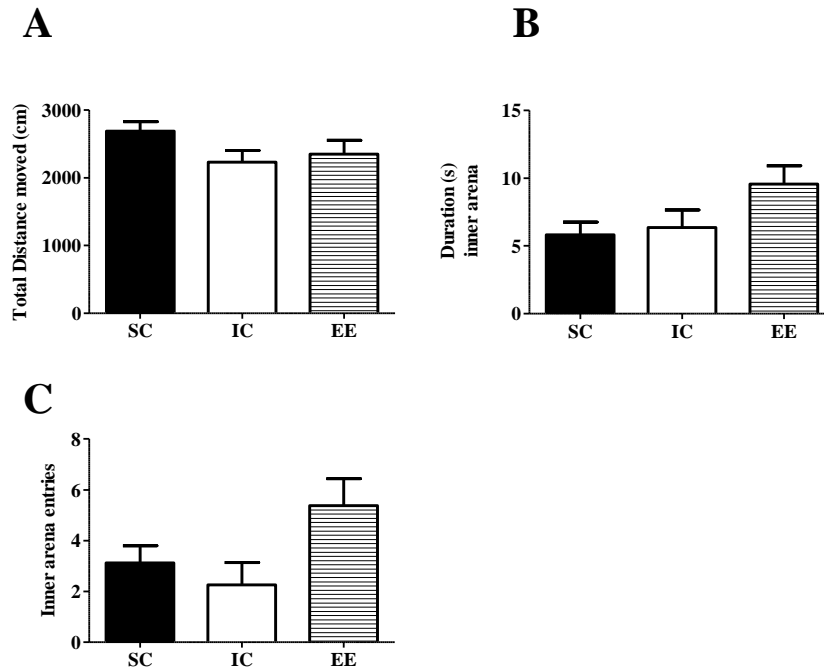


Figure 3.3: The effects of isolation and enriched housing conditions on activity in the open field test. Distance moved (A), time spent in the inner arena (B) and inner arena entries (C) by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 8$ per group).

3.4.3 Elevated Plus Maze

Baseline Behaviour (Figure 3.4)

There was no housing effect on distance moved [$F(2, 15) = 1.55, p > 0.05$] or open arm entries [$F(2, 15) = 1.68, p > 0.05$] for saline controls in the EPM. There was an effect of housing on percent time spent in the open arms [$F(2, 15) = 4.56, p < 0.05$], *post-hoc* tests showed that IC rats had significantly lower percent open arm time than SC and EE groups, who did not differ from one another.

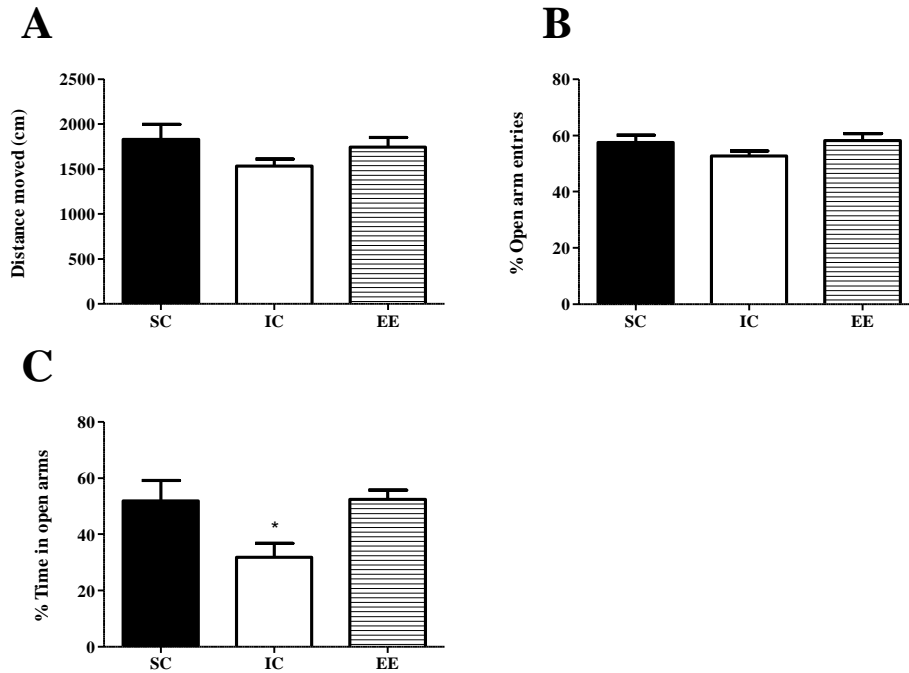


Figure 3.4: The effects of isolation and enriched housing conditions in the elevated plus maze. Distance moved (A), percent open arm entries (B) and percent open arm time (C) by saline-treated rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$ vs. SC.

Diazepam Effects (Figure 3.5)

There was a significant a significant drug effect on distance moved [$F(3, 60) = 17.90$, $p < 0.001$] in the EPM. One-way ANOVAs revealed significant drug effects for all three groups and *post-hoc* tests showed that the 5 mg kg^{-1} dose reduced distance moved for rats in IC ($p < 0.05$), SC ($p < 0.01$) and EE ($p < 0.001$) compared to saline controls; indicating a sedative effect of the drug at this dose for rats in all conditions. There were no significant housing [$F(2, 60) = 0.11$, $p > 0.05$] or housing x drug interaction [$F(6, 60) = 1.07$, $p > 0.05$] effects on distance moved.

There was a significant drug effect on percent open arm entries [$F(3, 60) = 4.85$, $p < 0.01$]. When one-way ANOVAs were applied there was a significant drug effect on percent open arm entries for SC [$F(3, 20) = 4.46$, $p < 0.05$], *post-hoc* tests showed an increase in percent open arm entries in response to 5 mg kg^{-1} DZP compared to saline controls ($p < 0.05$). No significant drug effects were observed with IC [$F(3, 20) = 0.23$, $p > 0.05$] or EE [$F(3, 20) = 0.61$, $p > 0.05$] housing. There was no

significant main effect of housing [$F(2, 60) = 2.36, p > 0.05$] or housing \times dose interaction [$F(6, 60) = 0.74, p > 0.05$] on percent open arm entries.

A significant main effect of drug was also found for percent time in the open arms [$F(3, 60) = 3.25, p < 0.05$] of the EPM. One-way ANOVA results showed a significant drug effect on percent open arm entries with IC [$F(3, 20) = 3.31, p < 0.05$]; *post-hoc* tests showed a significant increase in percent open arm time in response to 2.5 and 5 mg kg^{-1} DZP compared to saline controls ($p < 0.05$). However no dose effects were seen for the SC [$F(3, 20) = 1.79, p > 0.05$] and EE ($F(3, 20) = 0.13, p > 0.05$) groups. There were no main housing [$F(2, 60) = 1.58, p > 0.05$] or housing \times dose interaction effects [$F(6, 60) = 1.28, p > 0.05$] on percent open arm time.

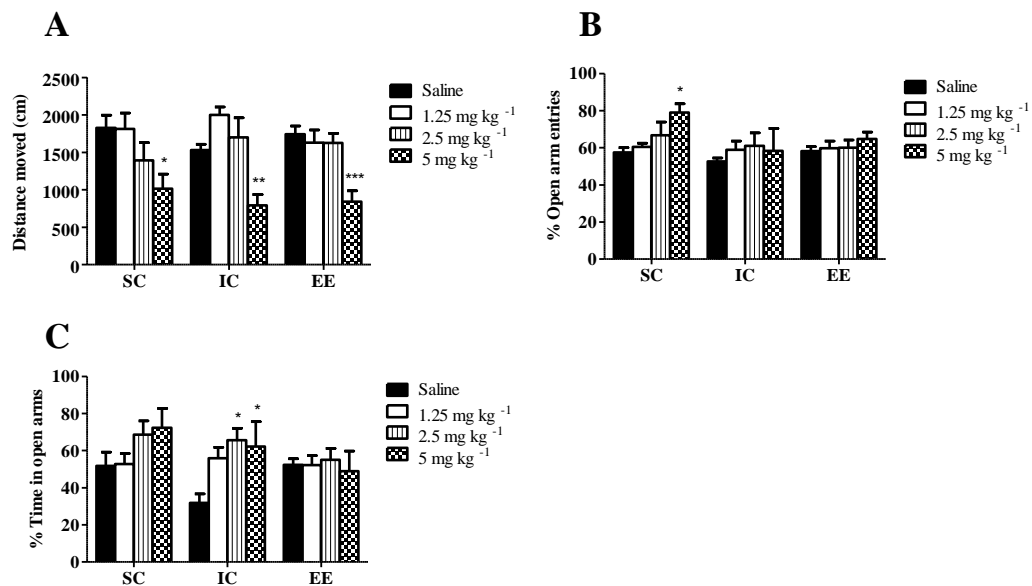


Figure 3.5: Diazepam and housing effects in the elevated plus maze. The effects of DZP (1.25, 2.5, 5 mg kg^{-1} , i.p.) on distance moved (A) percent open arm entries (B) and percent open arm time in the EPM by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$, ** $p < 0.01$ vs. saline controls in the same housing condition.

3.4.4 Forced Swim Test

Baseline Behaviour (Figure 3.6)

In the FST, time spent immobile was not significantly different for saline controls in IC or EE compared to SC housing [$F(2, 14) = 0.38, p > 0.05$].

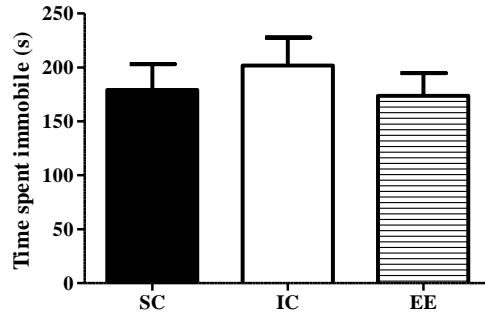


Figure 3.6: The effects of isolation and enriched housing conditions in the forced swim test. Time spent immobile by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 6$ per group).

Desipramine Effects (Figure 3.7)

Time spent immobile was not significantly affected by housing [$F(2, 58) = 2.17, p > 0.05$], drug [$F(3, 58) = 2.45, p > 0.05$] or housing x drug interaction [$F(6, 58) = 1.79, p > 0.05$] effects. When one-way ANOVAs were applied there was a significant drug effect with IC housing [$F(3, 20) = 3.60, p < 0.05$]. *Post-hoc* tests revealed a significant reduction in immobility time with 2.5 and 5 mg kg⁻¹ DMI ($p < 0.05$) compared to saline controls. No drug effects however were revealed for the SC [$F(3, 20) = 0.95, p > 0.05$] or EE [$F(3, 20) = 1.27, p > 0.05$] groups.

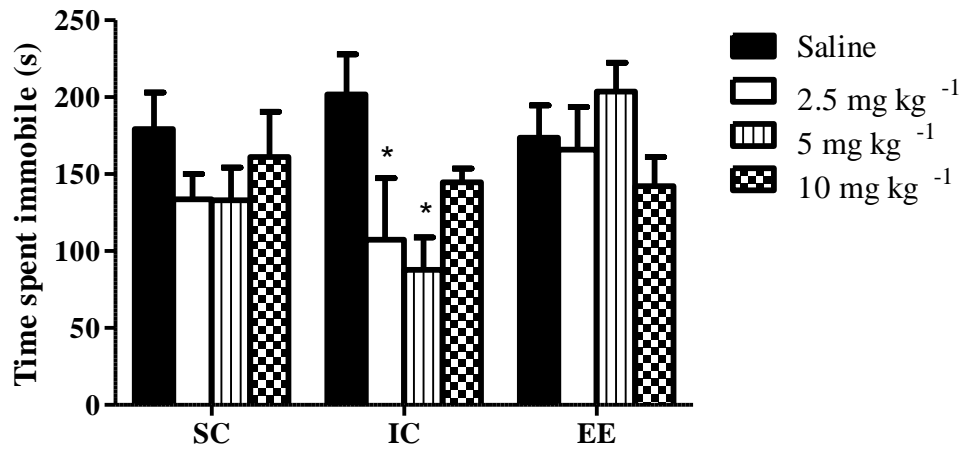


Figure 3.7: Desipramine and housing effects in the forced swim test. The effects of DMI (2.5, 5 and 10 mg kg⁻¹, i.p.) on time spent immobile in the forced swim test by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$ vs. IC saline control.

3.4.5 Homecage Monitoring

Baseline Behaviour (Figure 3.8)

Differential housing had no effect on baseline distance moved in the homecage in one hour [$F(2, 9) = 1.36$, $p > 0.05$].

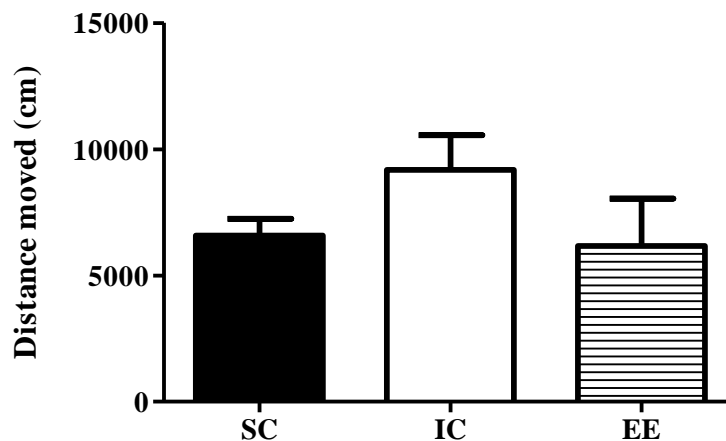


Figure 3.8: The effects of isolation and enriched housing conditions on distance moved in the homecage. Total distance moved (cm) by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 4$ per group).

Amphetamine Effects (Figures 3.9 and 3.10)

There was a significant drug effect on total distance moved in the HCMA for one hour after AMP administration [$F(4, 45) = 11.68, p < 0.001$]. When one-way ANOVAs were applied there were significant drug effects on locomotor activity for rats housed in SC [$F(4, 15) = 4.14, p < 0.05$], IC [$F(4, 15) = 4.78, p < 0.05$] and EE [$F(4, 15) = 5.92, p < 0.01$] conditions. *Post-hoc* tests revealed that the 1.5 and 3 mg kg⁻¹ doses significantly increased activity compared to saline controls for both the IC and EE groups ($p < 0.01$ and $p < 0.05$ for 1.5 and 3 mg kg⁻¹ respectively), while only the 1.5 mg kg⁻¹ dose significantly increased activity for SC rats ($p < 0.05$). There was no main effect of housing condition [$F(2, 45) = 1.41, p > 0.05$] or housing x drug interaction [$F(8, 45) = 1.34, p > 0.05$] effect on distance moved.

When data were expressed as distance moved relative to baseline (i.e. saline controls), there were significant main effects of housing condition [$F(2, 45) = 9.10, p < 0.001$] and drug [$F(4, 45) = 12.09, p < 0.001$], but no significant housing x drug interaction effect [$F(8, 45) = 1.42, p > 0.05$]. There were significant effects of AMP with SC [$F(4, 15) = 4.78, p < 0.05$], IC [$F(4, 15) = 4.14, p < 0.05$] and EE [$F(4, 15) = 5.92, p < 0.01$] housing conditions. The 1.5 mg kg⁻¹ dose significantly increased activity for all groups (IC: $p < 0.05$, SC and EE: $p < 0.01$) and the 3 mg kg⁻¹ dose significantly increased activity for the SC and EE conditions only ($p < 0.05$).

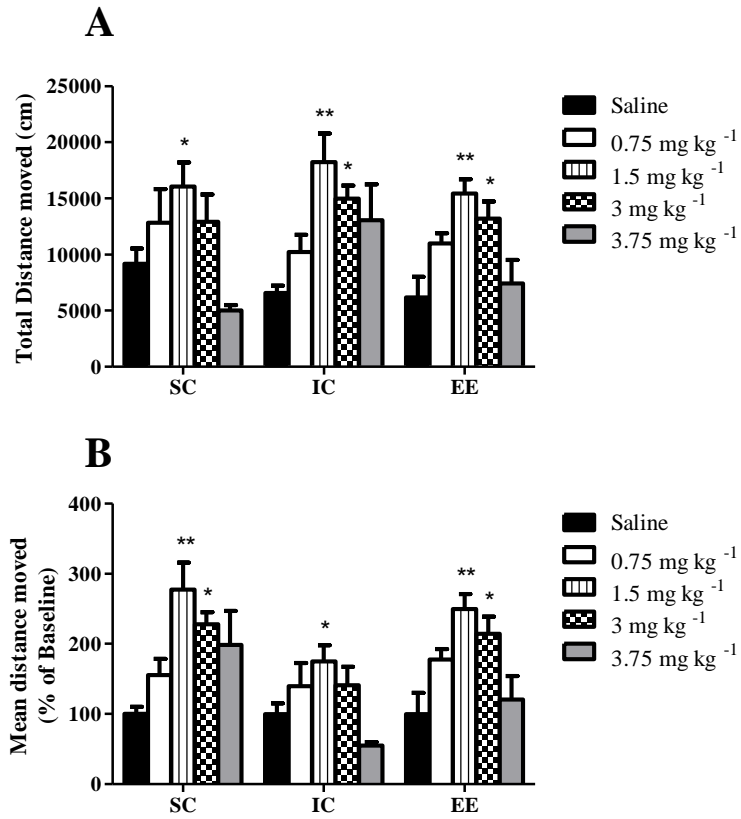


Figure 3.9: Amphetamine and housing effects on distance moved in the homecage. The effects of AMP (0.75, 1.5, 3 and 3.75 mg kg⁻¹, s.c.) on (A) total distance moved in the homecage over one hour and (B) distance moved in the homecage relative to baseline levels by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 4$ per group), * $p < 0.05$, ** $p < 0.01$ vs. saline controls in the same housing condition.

AMP effects on movement over 15-min timebins were analysed to assess its effects over time. There were significant main effects of drug and time on activity with SC (time: [$F(3, 45) = 11.83, p < 0.001$], drug: [$F(4, 15) = 4.78, p < 0.05$]) but there was no time x drug interaction effect [$F(12, 45) = 0.69, p > 0.05$]. There were significant main and interaction effects of drug and time with IC (time: [$F(3, 45) = 6.89, p < 0.001$], dose: [$F(4, 15) = 4.14, p < 0.05$], interaction: [$F(12, 45) = 2.03, p < 0.05$]) and EE (time: [$F(3, 45) = 17.39, p < 0.001$], dose: [$F(4, 15) = 7.69, p < 0.01$], interaction: [$F(12, 45) = 3.23, p < 0.01$]). The results of one-way ANOVA analyses are presented in figure 3.10. The EE group appeared to be most sensitive to the locomotor-enhancing effects of AMP, particularly during the first 30 min.

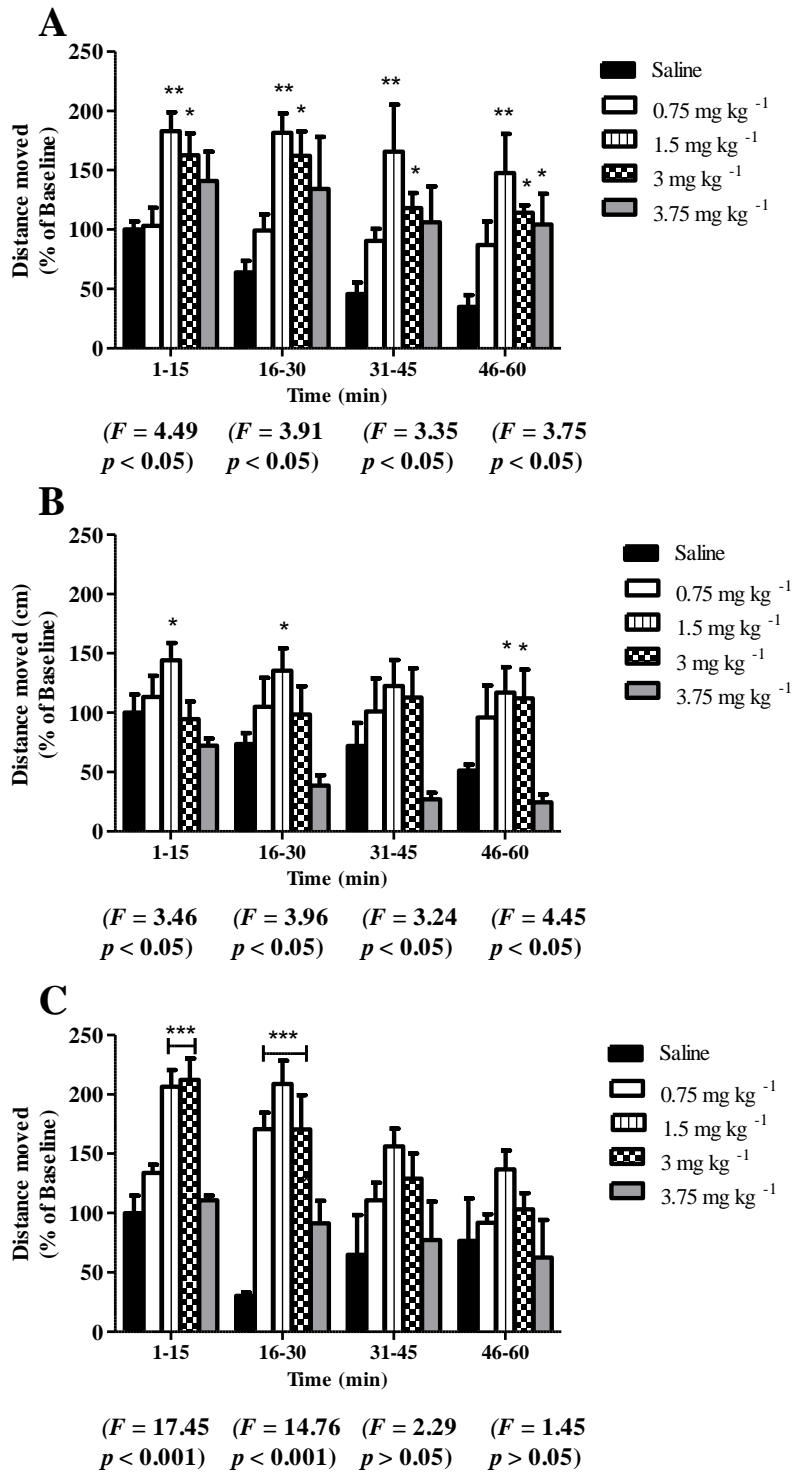


Figure 3.10: Amphetamine and housing effects on distance moved in the homecage over 15-min timebins. The effects of AMP (0.75, 1.5, 3, 3.75 mg kg⁻¹, s.c.) administration on distance moved in the homecage, expressed as percent of baseline control by rats housed in (A) standard (SC), (B) isolated (IC) and (C) environmentally enriched conditions (EE) over 15-min timebins. Data expressed as mean + SEM ($n = 4$ per group), $df(4, 15)$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. saline controls in the same housing condition, according to 1-way ANOVA ($df=4,15$).

3.5 Discussion

In the present study rats housed in isolated conditions gained significantly more weight than those housed in standard groups and those housed with environmental enrichment, which did not differ from one another. Analysis of food consumption showed this was due to the rats in isolated conditions eating more than those housed in standard conditions. In addition, the presence of cagemates provides constant opportunity for activity and interaction for the standard and enriched groups, compared to singly-housed rats. There was no effect of housing on locomotor activity in the open field test, though there was a trend for environmentally enriched animals to spend more time in the centre of the arena than those in isolated conditions. There was an effect of housing in the elevated plus maze as singly-housed rats had a lower percent open arm time than the socially-housed standard and enriched groups. The anxiolytic diazepam had no effect on elevated plus maze behaviour for enriched rats but increased percent open arm entries and percent open arm time for standard- and isolation-housed rats respectively. In the forced swim test there was no difference in baseline immobility. The tricyclic antidepressant desipramine reduced immobility time for rats housed in isolated conditions but had no effect on the two socially-housed groups. As with the open field test, there was no effect of environmental enrichment or isolation housing on locomotor activity in the homecage compared to standard-housed controls. Rats in all three groups displayed a dose-dependent increase in locomotor activity in response to amphetamine. When activity was expressed as a percent of baseline distance moved it was evident that amphetamine stimulated activity to a greater extent in rats housed with cagemates compared to singly-housed animals.

The finding that environmentally-enriched rats weighed less than isolated and group-housed rats during the experimental period is consistent with previous results (Moncek et al., 2004), but not with others who have reported no difference in weight when enriched were compared to standard controls (Munetsuna et al., 2011) and one study found that male SD rats housed in enriched conditions for 6 weeks gained more weight than those in isolation (Konkle et al., 2010). It may be that the provision of environmental enrichment in adulthood does not produce the effects seen when rats are housed post-weaning. For instance, Zaias and colleagues found

that post-weaning environmental enrichment for as little as one week resulted in lower body weight than group- and isolation-housed counterparts (Zaias et al., 2008). This effect of environmental enrichment on body weight is relatively enduring into adulthood and thus may reflect developmental changes in appetite or metabolism (Pena et al., 2009a). In the present study the finding that rats housed with enrichment ate less than the isolated group is likely due to increased opportunity for engaging in other activities. A recent study reported that rats housed with enrichment objects spent less time positioned beneath the food/water hoppers than those housed in barren cages (Abou-Ismaïl et al., 2010), though the authors did not measure food and water consumption, it may be that the provision of shelters allowed for rest periods in other parts of the cage that drew activity and attention away from the hoppers. There may be a “boredom” effect as opportunities for exercise and activity in environmental enrichment and interaction with cagemates in group-housed conditions are not available to isolated rats.

In the open field test there was no effect of housing on overall distance moved. This conflicts with others who have found that enriched housing for 2-3 month old male rats’ reduced locomotor activity (Segovia et al., 2008, Pham et al., 1999a) in the open field compared to singly-housed controls. However Pham (1999) and Segovia (2008) employed 12 and 3 months environmental enrichment respectively, compared to the 5 week exposure prior to the open field test in the present experiment. It is thought that enriched housing reduces hyperactivity in response to a novel environment, as is often seen in rats reared in social isolation (Zhao et al., 2009), and promotes more rapid habituation and adaptation to such situations (Zimmermann et al., 2001). In the present study however there was no social isolation-induced hyperactivity observed. Environmental enrichment tended to increase entries to the centre of the arena compared to isolated rats which may suggest that they were less fearful and motivated to explore more than singly-housed rats (Brenes et al., 2006).

According to a factor analysis report by Brenes and colleagues (2006), the provision of enriched housing is thought to be “protective” against depressive-like symptoms such as behavioural despair, which is assessed by immobility in the forced swim test (Brenes et al., 2006). Social isolation rearing has been shown to reduce hippocampal serotonin levels, with concurrent increased behavioural despair in the

forced swim test (Brenes and Fornaguera, 2008, Kuramochi and Nakamura, 2009), both of which are attenuated by antidepressant (fluoxetine) administration (Brenes and Fornaguera, 2009). In contrast however social isolation in the present study did not cause increased immobility; nor was there any effect of environmental enrichment on baseline immobility time in the forced swim test in the present study. This contrasts somewhat with Brenes and others who have shown rats in enriched housing conditions for up to 12 weeks to display decreased immobility, increased swimming (Brenes et al., 2009, Brenes et al., 2008), as well as increased climbing and diving (Brenes et al., 2008) compared to isolation- and standard group-housed controls. However shorter duration environmental enrichment protocols (i.e. 2-4 weeks duration) have been unsuccessful in finding any effect of environmental complexity on immobility in the forced swim test compared to isolation-housed (Koh et al., 2007) and group-housed controls (Cui et al., 2006, Veena et al., 2009). It may be that the relatively short period of housing (i.e. 5-6 weeks) prior to the forced swim test had an impact on the present results.

Though there was no effect of housing on baseline activity in the forced swim test, there was a differential response to the antidepressant desipramine in the present study. Only rats housed in social isolation displayed a significant dose-related decrease in immobility time compared to saline controls. Few studies have addressed environmental enrichment effects on responses to antidepressant drugs such as desipramine. The oral administration of fluoxetine over 34 days reduced immobility and increased swimming time for rats housed in isolation to levels equivalent to those observed in the socially-housed (3 rats/cage) group (Brenes and Fornaguera, 2009). Wongwitdecha and colleagues (2006) housed male Wistar rats from weaning age in isolated or group-housed (6 rats/cage) conditions. After 4 weeks of differential housing desipramine (5, 10 and 20 mg kg⁻¹) was administered 24, 5 and 1 hour prior to the forced swim test. They found that singly-housed rats were less immobile in the forced swim test than the socially-housed group at baseline, this contrasts with other studies demonstrating greater immobility by isolated animals and the authors attribute this baseline difference to isolation-induced hyperactivity. Immobility was scored (by an observer who was blind to experimental conditions) when the animal was motionless, in an upright position, making minimal movements required to keep their head above the water surface. The singly-housed

rats displayed reduced time spent immobile in response to all three desipramine doses employed, whereas the higher doses (10 and 20 mg kg⁻¹) were necessary to reduce immobility by the socially-reared groups (Wongwitdecha et al., 2006). In a similar study, post-weaning isolation or social housing conditions were employed for 4-8 weeks, then half of each group received 20 mg kg⁻¹ desipramine prior to the forced swim test. Among saline controls, rats in isolation displayed longer periods of immobility than those housed in social conditions. Desipramine reduced the immobility time for the singly-housed rats but did not significantly reduce immobility among the group-housed animals (Heritch et al., 1990). This blunted drug effect is in agreement with the present study; however desipramine (10 mg kg⁻¹) has been shown to reduce immobility time in the forced swim test for rats housed in isolation (McDermott and Kelly, 2008, Connor et al., 1998) pairs (Page et al., 1999) and in group (3/cage) conditions (Jama et al., 2008). In these studies however a single dose of desipramine was employed, moreover, rats were not maintained in their respective housing conditions for a number of weeks as the objective was to demonstrate desipramine effects, not housing condition effects. It may be that the effects of isolation / social rearing on the antidepressant effects of desipramine, as demonstrated in the present study, emerge over a number of weeks. As desipramine blocks noradrenaline reuptake into the nerve terminal, isolation rearing may alter noradrenaline transporter sites and affect the concentration of noradrenaline in the synaptic cleft. Moreover, Wongwitdecha and colleagues (2006) suggest that the decreased immobility observed in singly-housed animals, and their enhanced response to desipramine, may be reflective of a greater aversion or anxiogenic response compared to group-housed animals. This is consistent with a previous study carried out by the same group wherein isolated rats were unable to produce a normal noradrenergic response to an acute stressor due to enhanced α_2 autoreceptor function (Fulford and Marsden, 1997). The reason for a blunted response to desipramine in the socially-housed standard and enriched groups in the present study however is unclear.

Housing had an effect on percent time spent in the open arms of the elevated plus maze, both standard and environmental enrichment groups had increased percent open arm time compared to rats housed in isolation. This is in agreement with previous studies wherein adulthood enrichment has increased open arm time

and entries (Leal-Galicia et al., 2007, Sparling et al., 2010) compared to group- and isolation-housed controls. Rats housed in isolation are thought to be more fearful and anxious than those housed in social groups and this is often reflected in reduced percent open arm time and entries compared to rats housed with environmental enrichment (Hellemans et al., 2004) and in social groups (Weiss et al., 2004, Da Silva et al., 1996). In the present study rats housed with environmental enrichment displayed a blunted response to the benzodiazepine anxiolytic diazepam, while 5 mg kg⁻¹ increased percent open arm entries for group-housed rats only, and 2.5 and 5 mg kg⁻¹ increased percent open arm time for singly-housed rats only. There is a paucity of research into environmental enrichment interactions with anxiolytic drug effects using the elevated plus maze, however it is likely that the high baseline levels of percent open arm time and entries in the elevated plus maze blunted any effect of diazepam for the environmental enrichment group. Furthermore, the sensitivity of the elevated plus maze to anxiolytic drug effects is based on the animals' baseline aversion to the open arms, which can be affected by methodological variables including lighting and closed arm features (Violle et al., 2009). In a previous study, diazepam (3 mg kg⁻¹), significantly increased percent open arm entries, compared to saline controls, for rats housed in isolated conditions but had no effect on those in standard group conditions (Da Silva et al., 1996), suggesting differences in pharmacodynamics resulting in a greater sensitivity of rats housed in isolation to the drug effects. However in a social interaction test, singly-housed rats were less sensitive to the anxiolytic effects of diazepam than socially-housed rats (Wongwitdecha and Marsden, 1996), furthermore, isolation rearing was associated with reduced GABA_A receptor function (Serra et al., 2000), which suggests that rats housed without the potential for social interaction are less responsive to diazepam effects than group-housed counterparts. In the present experiment and in the absence of neurochemical analyses the differences in response to diazepam in the elevated plus maze may be attributed to differences in baseline open arm activity in the test.

As in the other tests there was no difference in baseline activity in the homecage in the present study. In previous studies the open field test or a similar novel arena is generally used for monitoring baseline locomotor activity. The arenas employed can vary greatly between laboratories in size and physical characteristics, furthermore, a disadvantage of using a novel arena to monitor baseline activity is that

locomotor levels may be an artefact of the animal's response to an unfamiliar environment (Stanford, 2007). The advantage of homecage monitoring in the present study is that rats are habituated to the cage prior to behavioural observation, thus activity levels recorded may be more reflective of impulsive behaviour in a familiar environment. Amphetamine increased activity relative to baseline levels for rats in all three conditions. Administration of 1.5 mg kg^{-1} amphetamine produced peak hyperactivity for all three groups and this was the only dose to significantly increase locomotor activity for rats housed in isolated conditions compared to saline controls, while the next highest dose, 3 mg kg^{-1} , sustained a significant hyperactivity response for the socially-housed standard and enriched groups. The magnitude of response was also greater in the group-housed and environmental enrichment rats; 1.5 mg kg^{-1} amphetamine increased isolated rats' activity to almost 200% of the baseline level; however the same dose increased the standard and enriched groups' activity to almost 300% and 250% of baseline distance moved, respectively. Furthermore, when the data were analysed over 15-min intervals, rats from environmental enrichment conditions displayed the greatest response of the three groups to 1.5 and 3 mg kg^{-1} doses of amphetamine during the first 30 min, the magnitude of response is slightly lower for standard group-housed and lower again for isolation-housed rats. This is consistent with past research wherein acute amphetamine ($0.5 - 2 \text{ mg kg}^{-1}$, s.c.) increased line crosses (Bowling and Bardo, 1994) and rearing activity (Bardo et al., 1995) to a greater extent for SD male rats reared in enriched conditions than those in groups without enrichment and those housed in isolation. In addition, rats housed in isolation from post-weaning failed to show place preference to amphetamine compared to group-housed rats (Wongwitdecha and Marsden, 1995), while 0.5 mg kg^{-1} (i.p.) amphetamine produced significantly greater conditioned place preference in rats housed with environmental enrichment than their singly-housed counterparts (Bowling and Bardo, 1994). The reason for the enhanced locomotor response observed after environmental enrichment is not fully understood. Bardo and colleagues (1995) suggest differences between enriched and isolated rats in pharmacodynamics may contribute to the differences as *in vitro* analysis showed no difference in amphetamine-induced dopamine release in the nucleus accumbens or striatum between rats housed with environmental enrichment or in barren cages in isolation (Bardo et al., 1995). The mesolimbic reward pathway in the brain is also regulated by the glutamatergic system, as amphetamine-induced increased glutamate

in the nucleus accumbens was greater in rats exposed to environmental enrichment than singly-housed rats, this may account in part for the enhanced hyperactive response of enriched animals observed following amphetamine (Rahman and Bardo, 2008). However, though environmental enrichment may enhance the effects of acute amphetamine, it reduces locomotor sensitisation to repeated drug exposure (Bardo et al., 1995) and reduces amphetamine self-administration (Bardo et al., 2001), thus environmental enrichment is in fact protective against drug abuse vulnerability (Stairs and Bardo, 2009). Bardo and colleagues reared rats in differential conditions from weaning (PND 21) (Bardo et al., 1995, Bowling and Bardo, 1994, Bardo et al., 2001), however it is interesting that the altered response they report is evident even when animals are exposed to isolated and enriched conditions in early adulthood, and thus it is not dependent on post-weaning exposure.

The provision of environmental enrichment, or social isolation, during young adulthood only had an effect on baseline activity in the elevated plus maze as singly-housed rats had reduced percent open arm time compared to the other two groups. Housing conditions affected drug-induced behaviours as environmental enrichment blunted responsiveness to diazepam in the elevated plus maze, while rats housed in isolation were the only group to show reduced immobility in response to desipramine in the forced swim test. Consistent with previous studies environmental enrichment and group-housing produced a greater sensitivity to the locomotor-stimulating effects of amphetamine compared to isolated conditions. Overall the results highlight the importance of housing environment on rat behaviour and particularly its possible effects on behavioural responses to psychotropic drugs, which may influence results and their interpretation.

Chapter 4

Effects of Housing on Baseline and Drug-Induced Behavioural Responses and Brain Monoamine Concentrations in Male and Female Rats

Chapter 4

Results chapter 2

Effects of housing on baseline and drug-induced behavioural responses and brain monoamine concentrations in male and female rats

4.1 Introduction

Behavioural pharmacology can be influenced by many variables including sex and environmental conditions. Male and female rats differ in behavioural (Alstott and Timberlake, 2009), physiological (Bakos et al., 2009) and neurochemical (Castner et al., 1993, Bisagno et al., 2003) states. Sex differences exist in locomotor activity (Brotto et al., 2000, Olivier et al., 2008), response to stressors (Brown and Grunberg, 1995, Conrad et al., 2004) and in tests of memory and learning (D'Hooge and De Deyn, 2001, Dalla and Shors, 2009). Females generally have higher levels of activity than males in the OFT and EPM tests (Olivier et al., 2008, Duncko et al., 2001), which can interfere with the sensitivity of these tests in assessing anxiety-like behaviours (Johnston and File, 1991b). Sex differences also impact animal models of depression as females do not respond to learned helplessness as males do (Dalla et al., 2008b), on the other hand, females are more vulnerable to the CMS model of depression than males (Dalla et al., 2005). In behavioural studies of learning and memory males have been shown by some to perform better than females in the MWM (D'Hooge and De Deyn, 2001, Harris et al., 2009) and some classical and operant conditioning paradigms (Dalla and Shors, 2009).

Environmental factors such as housing conditions can also impact on behaviour (Zimmermann et al., 2001, Schrijver et al., 2002), physiological (Konkle et al., 2010) and neurochemical (Hall, 1998, Brenes and Fornaguera, 2008) states. Rats are social animals and the impact of social housing as a model of social support has been investigated in studies of stress and coping responses of male and female rats. It was found that housing females with same-sex cagemates improved stress coping for females, but not for males; social housing increased stress sensitivity for males while isolation was stressful for females (Westenbroek et al., 2003b). If housing has differential effects on male and female animals, such differences ought to be identified to avoid reporting false conclusions regarding sex or housing effects

in response to drugs or other variables of interest. Moreover, biochemical alterations brought about by housing conditions may be valuable for manipulating and controlling indices of stress in order to more accurately assess the effects of drugs or other interventions (Belz et al., 2003, Brown and Grunberg, 1995).

Sex and housing interact and impact on HPA axis function, at baseline and in response to stressors. A number of studies have investigated this interaction and some have shown females have greater plasma ACTH (Pena et al., 2009a), CORT and larger adrenal glands (Bakos et al., 2009) than males. EE housing has been shown to reduce baseline CORT and ACTH in males and females compared to IC controls (Belz et al., 2003) and attenuate stress- or novelty-induced increases in CORT for males and females (Belz et al., 2003, Pena et al., 2009a). Another body of research has reported disparate rehabilitative effects of EE housing following brain injury. Male rats appear to suffer greater neurological deficits following traumatic brain injury (Wagner et al., 2005) and stroke (Saucier et al., 2007, Saucier et al., 2010) compared to females, and EE effects were greater on female neuronal markers of recovery than males (Wagner et al., 2005, Chen et al., 2005, Bakos et al., 2009). However regarding of functional recovery from brain damage, males appear to benefit more from EE effects as assessed by reduced latencies in the MWM compared to their female counterparts (Wagner et al., 2002, Saucier et al., 2010).

Studies of sex and housing effects thus tend to focus on stress response and/or rehabilitation. In a social interaction test, males housed in EE showed increased exploration time towards a stranger rat than group-housed controls whereas no difference was found between EE females and controls (Pena et al., 2006). The authors suggest that these findings indicate that patterns of social interaction in male rats have greater sensitivity to enrichment than females. It may be that male rats are more affected by social stress than are females (Brown and Grunberg, 1995). Male rats are possibly more aggressive than females and the establishment of the dominance hierarchy when EE is introduced or when novel objects and configurations are added to the environment may be more disruptive and stressful to males than to females. In the OFT, the effects of the separate elements of social and physical enrichment on open field activity in male and female SD rats were analysed. Rats were housed in isolation or in social groups, with or without physical enrichment objects. Thus some animals were housed in isolation in barren

cages, while others were housed in isolation with physical enrichment. Similarly a third group were housed with social enrichment in barren cages, while others were housed with social and physical enrichment. OFT acclimation and baseline activity was carried out prior to assignment to housing conditions and again on 3 occasions during differential housing. By the end of the experiment it was found that social EE reduced OFT activity to a greater extent than physical EE or a combination of social and physical EE. Overall their results suggest that over time the importance of social EE may be more salient than physical to both males and females, as assessed by habituation to the OFT, and that EE effects on OFT activity were greater for male rats than females (Elliott and Grunberg, 2005).

EE effects on rats in behavioural tests, neurochemistry and neuroanatomy have been well documented; however the majority of these studies have solely employed male rats (Fig. 1.4) and few studies have investigated sex differences in response to IC and EE housing compared to an SC group. Moreover, there is a paucity of data regarding neurochemical effects of housing and sex. The aim of the present study therefore was to assess any sex differences in commonly used behavioural tests for rats housed in IC, SC and EE conditions and investigate any sex or housing differences in monoamine levels in the brain.

4.2 Materials and Methods

4.2.1 Animals

Seventy-two male ($n = 36$) and female ($n = 36$) Sprague Dawley rats aged approximately 40 days old (weighing 100-150g on arrival) were housed in groups of 4 for four days acclimatisation, then randomly assigned to one of three housing conditions; IC, SC (4/cage) or EE (4/enriched cage).

The IC and SC animals were housed in standard cages while the EE animals were housed in larger cages with toys (see section 2.2.2). All animals were maintained in their respective housing conditions for four weeks before behavioural testing began and throughout the test period (total 9 weeks, see figure 4.1) and all rats were weighed weekly.

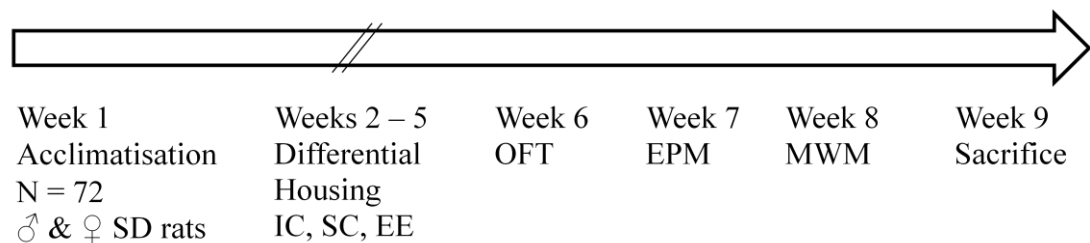


Figure 4.1: Experimental timeline. Timeline of experimental design; SD, Sprague Dawley; IC, isolated conditions; SC, standard group conditions; EE, environmentally enriched conditions; OFT, open field test; EPM, elevated plus maze; MWM, Morris water maze.

4.2.2 Open Field Test

See section 2.3.2

4.2.3 Elevated Plus Maze

See section 2.3.3

4.2.4 Morris Water Maze

See section 2.3.6

4.2.5 Determination of monoamine levels using high performance liquid chromatography

At the end of the 9-week differential housing period the experiment was ended and animals were sacrificed (section 2.5). The whole brains were cut and tissue sample punches taken as per section 2.5.2. HPLC analysis was carried out as described in

section 2.6 to determine concentrations of NA, DA, 5HIAA and 5HT. Results are expressed as nanograms per gram fresh weight of tissue.

4.3 Statistical Analysis

See section 2.8. Sex comparisons were made using unpaired t-test and Mann-Whitney U analyses.

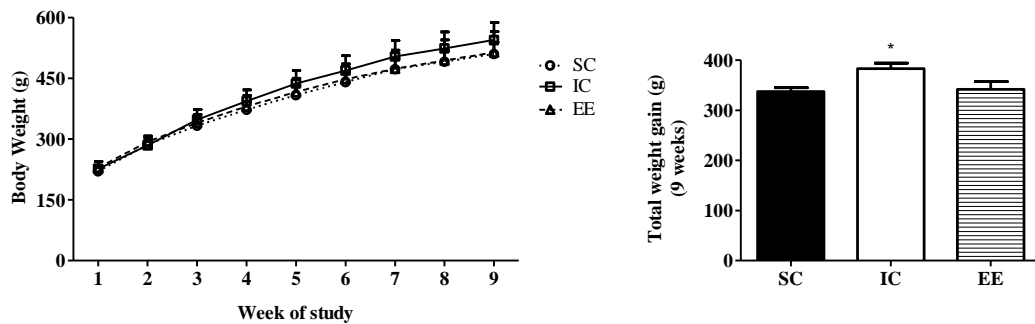
4.4 Results

4.4.1 Body Weight (Figure 4.2)

There was a significant main effect of time [$F(8, 264) = 1419.98, p < 0.001$] and a significant time x housing interaction [$F(16, 264) = 3.08, p < 0.001$], but no main housing effect [$F(2, 33) = 2.01, p > 0.05$] on male rats' body weight over the 9-week differential housing and testing period. One-way ANOVAs revealed there were no significant effects of IC or EE housing on body weight compared to the SC group throughout the study. When body weight gain over the 9 weeks (i.e. weight at week 9 – weight at week 1) was analysed there was a significant housing effect [$F(2, 33) = 4.41, p < 0.05$]. *Post-hoc* test showed that male rats housed in IC gained significantly more weight than those in SC or EE ($p < 0.05$).

For female rats' body weight there were significant effects of time [$F(8, 264) = 759.75, p < 0.001$], housing [$F(2, 33) = 6.83, p < 0.01$] and housing x time interaction [$F(16, 264) = 2.40, p < 0.001$] over the 9-week rearing and testing period. When one-way ANOVAs were applied there were significant housing effects at each week. *Post-hoc* tests showed the female rats in EE were significantly lighter than those housed in SC throughout the differential housing period ($p < 0.05$). EE rats were also significantly lighter than those in IC (during weeks 1 – 7). When overall weight gain over the 9 weeks was analysed however there was no significant effect of housing conditions [$F(2, 33) = 0.96, p > 0.05$].

Males



Females

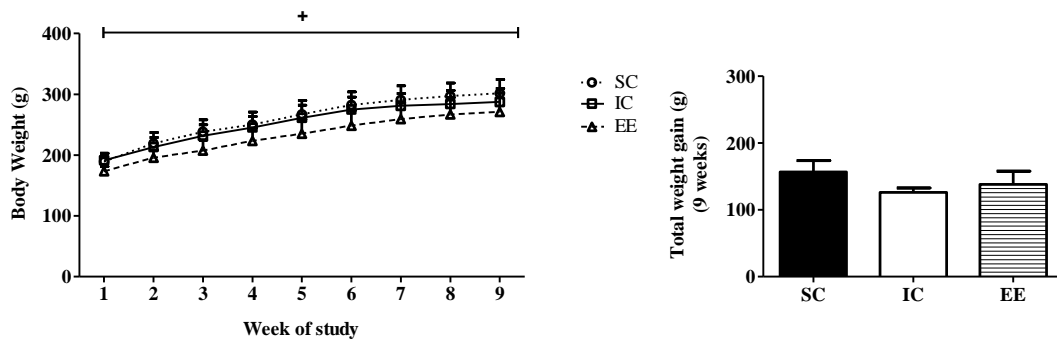


Figure 4.2: The effects of isolation (IC) and enriched (EE) housing on body weight and overall weight gain compared to standard (SC) conditions for male and female rats over the 9-week housing and testing period. Data expressed as mean + SEM, $n=12$ per group, * $p < 0.05$ compared to SC, + $p < 0.05$ EE vs. SC.

4.4.2 Open Field Test (Figure 4.3)

There was a significant overall sex effect on total distance moved [$F(1, 42) = 11.84$, $p < 0.01$] and on distance moved in the inner arena [$F(1, 42) = 5.13$, $p < 0.05$] of the OFT according to 2-way ANOVAs. There were no significant effects of housing conditions on total distance moved [$F(2, 42) = 0.29$, $p > 0.05$] or distance moved in the inner arena [$F(2, 42) = 0.62$, $p > 0.05$]. Nor were there significant housing x sex interaction effects on total distance moved [$F(2, 42) = 0.95$, $p > 0.05$] or distance moved in the inner arena [$F(2, 42) = 1.63$, $p > 0.05$]. For time spent in the inner arena [$F(1, 42) = 10.41$, $p < 0.01$] and entries made to the inner arena [$F(1, 42) = 6.24$, $p < 0.05$] significant sex effects were also observed. There was no significant effect of housing conditions on inner arena time [$F(2, 42) = 2.12$, $p > 0.05$] or

entries [$F(2, 42) = 0.62, p > 0.05$], nor were there housing x sex interaction effects on inner arena time [$F(2, 42) = 0.12, p > 0.05$] or entries [$F(2, 42) = 2.16, p > 0.05$].

One-way ANOVAs of distance moved revealed there were no significant effects of housing conditions (males [$F(2, 21) = 0.17, p > 0.05$], females [$F(2, 21) = 2.29, p > 0.05$]). Distance moved in the inner arena (males [$F(2, 21) = 0.47, p > 0.05$], females [$F(2, 21) = 1.31, p > 0.05$]), time spent in the inner arena (males [$F(2, 21) = 1.41, p > 0.05$], females [$F(2, 21) = 0.98, p > 0.05$]) and inner arena entries (males [$F(2, 21) = 0.45, p > 0.05$], females [$F(2, 21) = 1.72, p > 0.05$]) were not significantly affected by IC or EE housing conditions.

Sex differences in distance moved were revealed for SC [$t(14) = 2.23, p < 0.05$] and EE [$t(14) = 2.61, p < 0.05$], as female rats housed in SC and EE conditions showed greater movement than males, while there was no difference in total distance moved between male and female rats housed in IC [$t(14) = 1.19, p > 0.05$]. Distance moved in the inner arena was significantly greater for EE females compared to their male counterparts [$t(14) = 2.93, p < 0.05$], however there were no sex difference in inner arena distance moved with SC [$t(14) = 1.59, p > 0.05$] or IC [$t(14) = 0.36, p > 0.05$] conditions. EE females spent significantly more time in the inner arena compared to EE males [$t(14) = 2.58$], while there was no differences between males and females in SC [$U = 15, p > 0.05$] and IC [$t(14) = 1.03, p > 0.05$]. Entries made to the inner arena were significantly higher for female rats housed in SC [$t(14) = 2.26, p < 0.05$] compared to males. There was no sex difference in inner arena entries for IC [$t(14) = 0.03, p > 0.05$] or EE [$t(14) = 1.96, p > 0.05$].

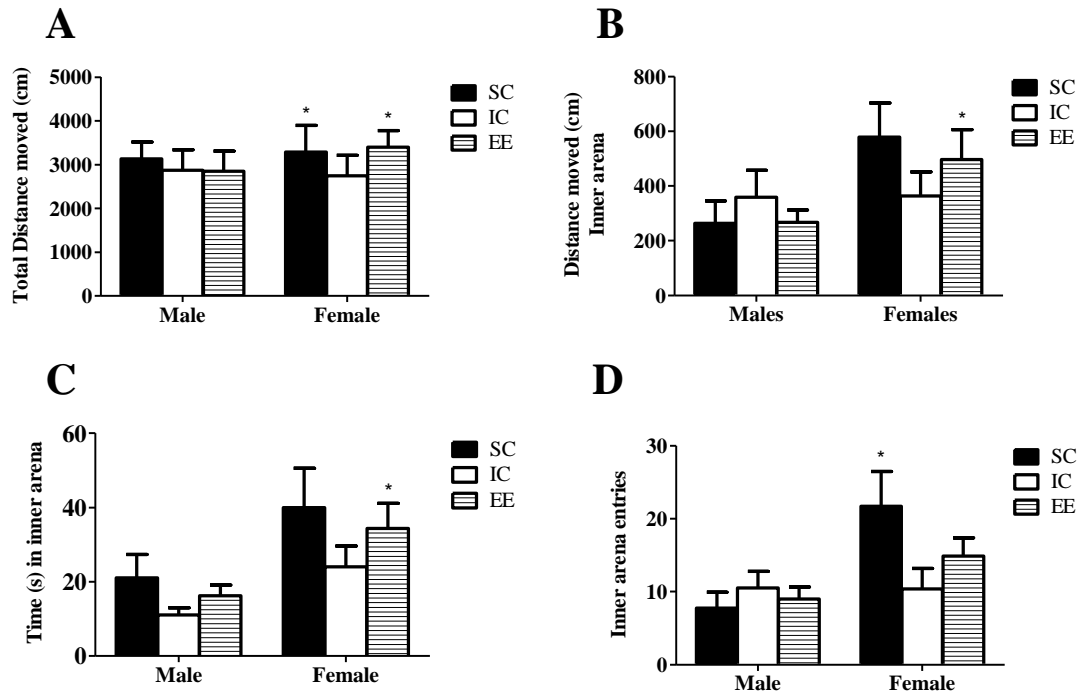


Figure 4.3: The effects of isolation and enriched housing conditions and sex in the open field test. Total distance moved (cm) (A), distance moved (cm) in the inner arena (B), time spent in the inner arena (C) and inner arena entries (D) by male and female rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 8$ per group), * $p < 0.05$ vs. males in the same housing condition.

4.4.3 Elevated Plus Maze (Figure 4.4)

Two-way ANOVAs of distance moved in the EPM revealed there were no significant main effects of housing [$F(2, 42) = 1.19, p > 0.05$] or sex [$F(1, 42) = 0.61, p > 0.05$], nor was there a housing x sex interaction effect [$F(2, 42) = 0.57, p > 0.05$]. There were no effects of housing conditions or sex on percent open arm entries (housing: [$F(2, 42) = 0.35, p > 0.05$], sex: [$F(1, 42) = 0.32, p > 0.05$], housing x sex: [$F(2, 42) = 1.54, p > 0.05$]) or percent open arm time (housing: [$F(2, 42) = 1.04, p > 0.05$], sex: [$F(1, 42) = 0.61, p > 0.05$], housing x sex: [$F(2, 42) = 3.23, p > 0.05$]) in the EPM.

There were no effects of housing conditions on distance moved (males: [$F(2, 21) = 0.19, p > 0.05$], females: [$F(2, 21) = 1.80, p > 0.05$]), percent open arm entries (males: [$F(2, 21) = 3.15, p = 0.06$], females: [$F(2, 21) = 1.85, p > 0.05$]) or percent

time spent in the open arms (males: [$F(2, 21) = 4.10, p > 0.05$], females: [$F(2, 21) = 0.58, p > 0.05$]) according to one-way ANOVAs.

There were no significant sex difference in distance moved (SC: [$t(14) = 0.26$], IC: [$t(14) = 0.47$], EE: [$t(14) = 1.14$]), percent open arm entries (SC: [$t(14) = 0.32$], IC: [$t(14) = 0.00$], EE: [$t(14) = 1.52$]) or percent open arm time (SC: [$t(14) = 2.10$], IC: [$t(14) = 1.51$], EE: [$t(14) = 0.75$]) in the EPM (all $p > 0.05$).

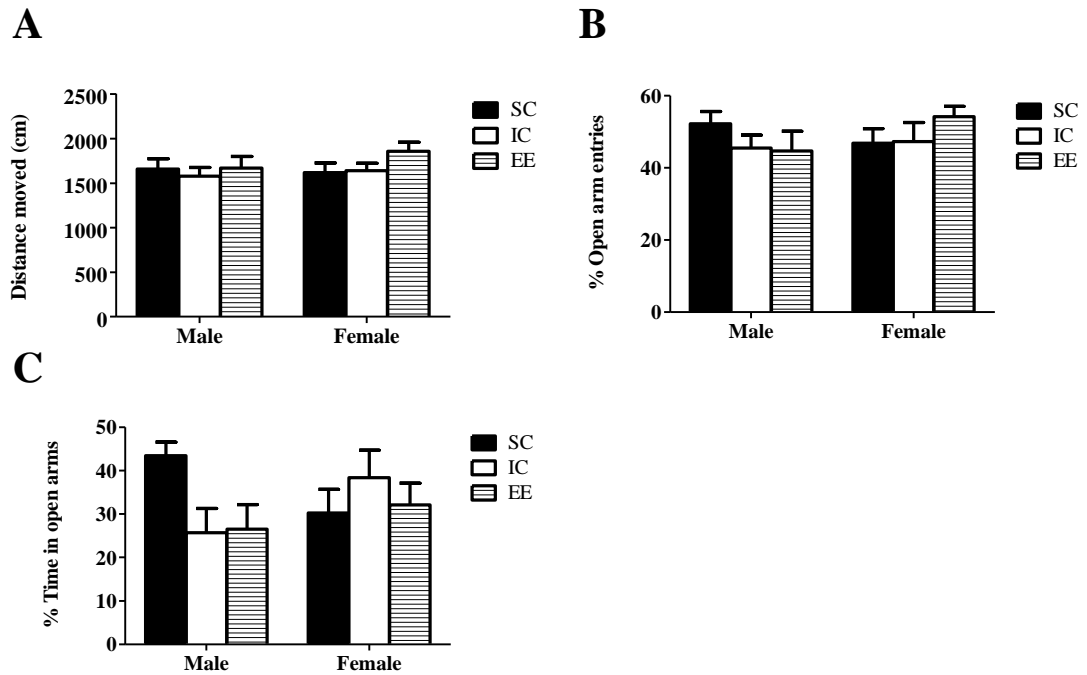


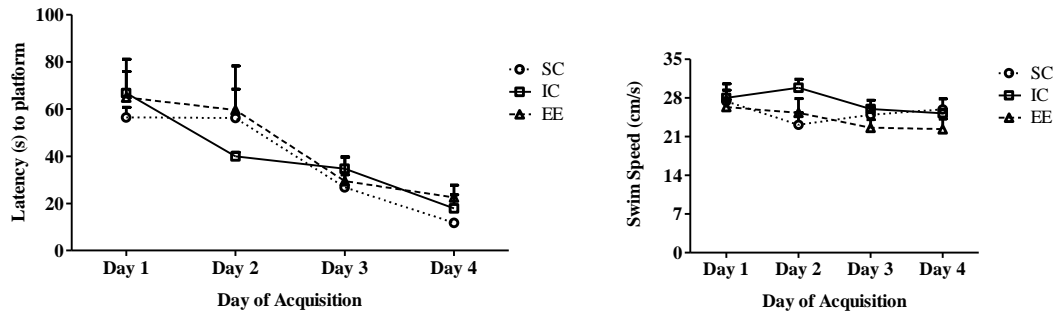
Figure 4.4: The effects of isolation and enriched housing conditions and sex in the elevated plus maze. Distance moved (A), percent open arm entries (B) and percent time spent in the open arms by male and female rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 8$ per group).

4.4.4 Morris Water Maze (Figures 4.5 and 4.6)

Regarding latencies to reach the escape platform in the MWM there were significant main effects of time for both male [$F(3, 27) = 14.49, p < 0.001$] and female rats [$F(3, 27) = 19.62, p < 0.001$], as all rats learned the platform location over the 4-day acquisition period. There was no effect of IC or EE housing on latencies for male [$F(2, 9) = 0.40, p > 0.05$] or female rats [$F(2, 9) = 2.31, p > 0.05$], nor were there any

significant housing x time interaction effects for males [$F(6, 27) = 0.56, p > 0.05$] or females [$F(6, 27) = 0.27, p > 0.05$]. When swim speed was analysed there were no significant housing condition effects (males: [$F(2, 9) = 1.25, p > 0.05$], females: [$F(2, 9) = 0.51, p > 0.05$]), time (males: [$F(3, 27) = 1.99, p > 0.05$], females: [$F(3, 27) = 1.46, p > 0.05$]) or housing x time interaction (males: [$F(6, 27) = 1.69, p > 0.05$], females: [$F(6, 27) = 1.69, p > 0.05$]).

Males



Females

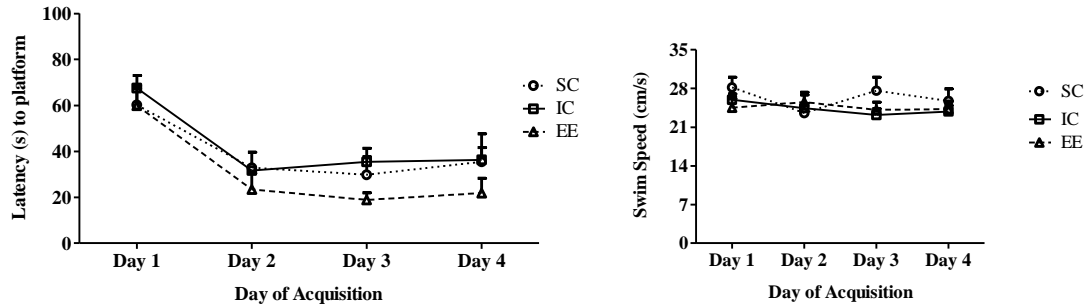


Figure 4.5: The effects of isolation and enriched housing conditions on latencies and swim speed in the Morris water maze acquisition. Latency to locate the platform and swim speed in the acquisition phase of the Morris maze for male and female rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 4$ per group).

In the MWM probe trial there were no overall main housing [$F(2, 18) = 2.51, p > 0.05$] or sex [$F(1, 18) = 4.17, p > 0.05$] effects on time spent in the target quadrant, nor was there any housing x sex interaction effect [$F(2, 18) = 1.81, p > 0.05$]. When one-way ANOVAs were carried out on time spent in the target quadrant there was no significant effect of housing for male [$F(2, 9) = 3.92, p = 0.06$] or female [$F(2, 9) = 0.08, p > 0.05$] rats. Time spent in the target quadrant was not significantly different between male and female rats in IC [$t(6) = 1.12$], SC [$t(6) = 0.24$] or EE [$t(6) = 2.42$] conditions.

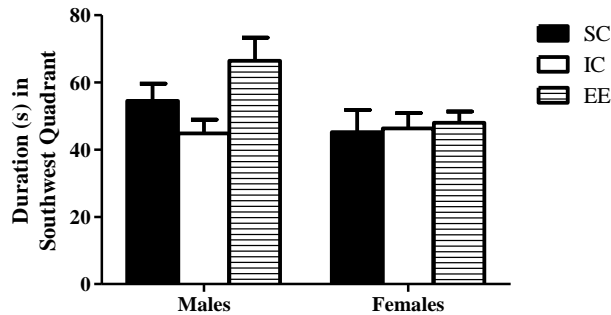


Figure 4.6: The effects of isolation and enriched housing conditions and sex in the Morris water maze probe trial. Time spent in the target (SW) quadrant during the Morris water maze probe trial by male and female rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 4$ per group).

4.4.5 Determination of monoamine levels using high performance liquid chromatography

Two-way ANOVAs were carried out to assess sex and housing effects on monoamine levels in the frontal cortex (Table 4.1), striatum (Table 4.2), amygdala (Table 4.3) and hippocampus (Table 4.4). One-way ANOVAs were applied to further investigate housing effects on male and female groups and t-tests (or Mann-Whitney U) were used to identify sex differences within the IC, SC and EE housing groups. There were significant sex and housing effects seen in all brain regions and sex x housing interaction effects were seen in all but the striatum and hippocampus. Levels of 5HT, 5HIAA and 5HT turnover were most affected by sex and housing conditions.

In the frontal cortex (Table 4.1), there was a housing effect for female rats. NA was significantly higher in EE females compared to SC. There were significant housing effects on 5HT as both male and female rats housed in IC had significantly lower 5HT than their SC counterparts. For 5HIAA there was a significant housing effect as males in IC had significantly lower 5HIAA than males in SC; there was no such housing effect observed for females. Regarding sex effects, females in SC housing had significantly lower 5HT, NA and DA concentration than females of the same housing condition. Female rats housed in EE had significantly lower 5HIAA and those in IC had lower NA compared to male rats in the same housing conditions. No sex differences were seen for IC and EE housing groups.

Table 4.1: Effects of isolation and enriched housing conditions and sex on monoamine levels in the rat frontal cortex

	Frontal Cortex					
Males	NA	DA	5HIAA	5HT	5HIAA/5HT	
SC	167 ± 15	174 ± 48	317 ± 32	257 ± 25	1.24 ± 0.07	
IC	127 ± 13	134 ± 17	207 ± 24*	111 ± 13***	1.96 ± 0.24*	
EE	173 ± 15	195 ± 25	312 ± 22	209 ± 13	1.50 ± 0.08	
Females						
SC	125 ± 6†	140 ± 8†	242 ± 23	171 ± 10††	1.41 ± 0.11	
IC	99 ± 16†	178 ± 42	226 ± 33	72 ± 24++	1.76 ± 0.57	
EE	190 ± 11++	191 ± 11	248 ± 9†	227 ± 30	1.18 ± 0.13	
Sex Effect	<i>F</i>	2.74	0.01	3.82	4.71	0.28
	<i>p</i>	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05
	<i>df</i>	1, 30				
Housing Effect	<i>F</i>	13.70	1.86	4.35	24.97	2.55
	<i>p</i>	< 0.001	> 0.05	< 0.05	< 0.001	> 0.05
	<i>df</i>	2, 30				
Sex x Housing	<i>F</i>	2.82	1.60	2.10	3.23	0.46
	<i>p</i>	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05
	<i>df</i>	2, 30				

SC, standard group condition; IC, isolated condition; EE, environmental enrichment condition; NA, noradrenaline; DA, dopamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid, 5HIAA/5HT, serotonin turnover. Data expressed as mean neurotransmitter concentration (ng / g tissue) ± SEM ($n = 6$ per group). Asterisks denote significant housing effects for male rats, * $p < 0.05$, *** $p < 0.001$ compared to SC males; crosshairs denote significant housing effects for female rats, ++ $p < 0.01$ compared to SC females; daggers denote significant sex differences, † $p < 0.05$, †† $p < 0.01$ compared to male rats in the same housing condition.

In the striatum (Table 4.2) housing condition effects showed that both male and female rats in IC had significantly lower DA, 5HIAA and 5HT levels than those in SC. Female rats in EE housing had significantly greater 5HT levels than their male counterparts.

Table 4.2: Effects of isolation and enriched housing conditions and sex on monoamine levels in the rat striatum

	Striatum					
Males	NA	DA	5HIAA	5HT	5HIAA/5HT	
SC	104 ± 12	2195 ± 221	448 ± 23	275 ± 35	1.71 ± 0.13	
IC	89 ± 16	927 ± 155***	295 ± 33**	125 ± 18*	2.43 ± 0.14	
EE	155 ± 12	1618 ± 201	497 ± 25	205 ± 43	1.79 ± 0.39	
Females						
SC	124 ± 39	2109 ± 124	470 ± 31	316 ± 29	1.51 ± 0.08	
IC	68 ± 10	1139 ± 171+	306 ± 34++	115 ± 28+++	1.88 ± 0.41	
EE	153 ± 16	2343 ± 439	494 ± 25	337 ± 24†	1.49 ± 0.08	
Sex Effect	<i>F</i>	0.55	2.22	0.18	4.69	2.89
	<i>p</i>	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05
	<i>df</i>	1, 30				
Housing Effect	<i>F</i>	3.91	13.36	25.50	19.30	2.98
	<i>p</i>	< 0.05	< 0.001	< 0.001	< 0.001	> 0.05
	<i>df</i>	2, 30				
Sex x Housing	<i>F</i>	1.09	1.55	0.09	2.72	0.27
Housing	<i>p</i>	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
	<i>df</i>	2, 30				

SC, standard group condition; IC, isolated condition; EE, environmental enrichment condition; NA, noradrenaline; DA, dopamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid, 5HIAA/5HT, serotonin turnover. Data expressed as mean neurotransmitter concentration (ng / g tissue) ± SEM (*n* = 6 per group). Asterisks denote significant housing effects for male rats, * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001 compared to SC males; crosshairs denote significant housing effects for female rats, + *p* < 0.05, ++ *p* < 0.01, +++ *p* < 0.001, compared to SC females; daggers denote significant sex differences, † *p* < 0.05 compared to male rats in the same housing condition.

In the amygdala (Table 4.3) one-way ANOVAs revealed NA levels were reduced in both male and female rats housed in IC compared to those in SC. 5HT concentration was reduced in EE male rats compared to those in SC. Similar to the striatum females in EE conditions had significantly greater 5HT than males in EE.

Table 4.3: Effects of isolation and enriched housing conditions and sex on monoamine levels in the rat amygdala

	Amygdala					
Males		NA	DA	5HIAA	5HT	5HIAA/5HT
SC		193 ± 15	801 ± 84	372 ± 18	245 ± 12	1.52 ± 0.04
IC		72 ± 27**	497 ± 83	184 ± 40	ND	ND
EE		213 ± 9	598 ± 116	335 ± 68	153 ± 38*	1.56 ± 0.55
Females						
SC		219 ± 36	687 ± 76	422 ± 21	262 ± 13	1.62 ± 0.10
IC		89 ± 16+	622 ± 126	186 ± 77	ND	ND
EE		194 ± 35	807 ± 148	340 ± 76	296 ± 42†	1.27 ± 0.28
Sex Effect	<i>F</i>	0.16	0.68	0.17	7.28	0.09
	<i>p</i>	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05
	<i>df</i>	1, 30			1, 20	
Housing Effect	<i>F</i>	16.446	1.58	7.71	0.95	0.25
	<i>p</i>	< 0.001	> 0.05	> 0.05	> 0.05	> 0.05
	<i>df</i>	2, 30			1, 20	
Sex x Housing	<i>F</i>	0.45	1.81	0.12	4.46	0.40
Housing	<i>p</i>	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05
	<i>df</i>	2, 30			1, 20	

SC, standard group condition; IC, isolated condition; EE, environmental enrichment condition; NA, noradrenaline; DA, dopamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid, 5HIAA/5HT, serotonin turnover, ND, not detectable. Data expressed as mean neurotransmitter concentration (ng / g tissue) ± SEM (*n* = 6 per group). Asterisks denote significant housing effects for male rats, ** *p* < 0.01 compared to SC males; crosshairs denote significant housing effects for female rats, + *p* < 0.05 compared to SC females, daggers denote significant sex differences, † *p* < 0.05 compared to male rats of the same housing condition.

In the hippocampus (Table 4.4) there was a significant housing effect on NA for male rats as greater levels NA levels were seen in EE compared to SC. Male rats housed in SC had lower 5HT than females, while males in EE had greater NA than females. Housing effects were seen for 5HT as male rats in EE had higher 5HT than males in SC, while IC female rats had lower 5HT than SC controls.

Table 4.4: Effects of isolation and enriched housing conditions and sex on monoamine levels in the rat hippocampus

	Hippocampus					
Males		NA	DA	5HIAA	5HT	5HIAA/5HT
SC		127 ± 10	170 ± 12	283 ± 19	66 ± 5	4.94 ± 1.01
IC		106 ± 11	110 ± 14	271 ± 35	43 ± 10	2.34 ± 0.16
EE		164 ± 10*	211 ± 31	271 ± 20	118 ± 10***	4.35 ± 0.28
Females						
SC		122 ± 23	184 ± 12	273 ± 17	119 ± 11†	3.35 ± 0.83
IC		139 ± 16	146 ± 13	358 ± 23+	42 ± 9+	2.28 ± 0.39
EE		123 ± 12†	240 ± 30	279 ± 22	133 ± 27	5.95 ± 1.51
Sex Effect	<i>F</i>	0.14	2.59	2.22	4.69	0.00
	<i>p</i>	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05
	<i>df</i>	1, 30				
Housing	<i>F</i>	1.16	11.84	1.77	21.22	6.65
Effect	<i>p</i>	> 0.05	< 0.001	> 0.05	< 0.001	< 0.01
	<i>df</i>	2, 30				
Sex x	<i>F</i>	3.11	0.16	2.43	2.50	2.01
Housing	<i>p</i>	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
	<i>df</i>	2, 30				

SC, standard group condition; IC, isolated condition; EE, environmental enrichment condition; NA, noradrenaline; DA, dopamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid, 5HIAA/5HT, serotonin turnover. Data expressed as mean neurotransmitter concentration (ng / g tissue) ± SEM (*n* = 6 per group). Asterisks denote significant housing effects for male rats, * *p* < 0.05, *** *p* < 0.001 compared to SC males; crosshairs denote significant housing effects for female rats, + *p* < 0.05 compared to SC females, daggers denote significant sex differences, † *p* < 0.05 compared to male rats of the same housing condition.

4.5 Discussion

In the present study male rats housed in environmental enrichment conditions gained significantly less overall body weight than males who were singly housed and enriched females had lower body weights over the differential housing period than their standard group- and isolation-housed counterparts. The difference in body weight between females in standard group and environmental enrichment conditions suggests a greater effect of the combined social and physical enrichment compared to social enrichment alone, but this was only the case for females. Previously social (Westenbroek et al., 2004) and enriched (Sparling et al., 2010) housing has reduced body weight for females, similarly both social and environmental enrichment conditions have reduced male rats' weight gain (Zaias et al., 2008). This is likely due to greater opportunity for activity provided by social interaction, enrichment objects and the presence of cagemates also vying for food. Pena and colleagues investigated the enduring effects of environmental enrichment on body weight gain and other measures. Male and female Sprague Dawley rats were housed in enriched or standard conditions from postnatal day 21 for 12 weeks, and then all animals were housed in standard group conditions throughout behavioural testing. Environmental enrichment reduced body weight for both males and females compared to controls; however 4 weeks after the cessation of enriched housing this difference had disappeared for females. On the other hand post-weaning enrichment effects on body weight were enduring for male rats as they maintained a reduced body weight compared to controls for up to 44 weeks (Pena et al., 2009a).

Consistent with previous studies (Duncko et al., 2001, Weiss et al., 2004), it was found that both standard group- and enriched-housed female rats were more active than males in the open field test and were more active in the centre of the open field test than males. This might suggest that females are less anxious than male rats, being more likely to explore the centre of the arena, however, as with the elevated plus maze, Fernandes (1999) cautions against such inferences as central activity in the holeboard test provided a measure of anxiety for males but was not a valid measure of anxiety for female rats (Fernandes et al., 1999). Notably there was no significant sex difference between rats housed in isolated conditions. This was unexpected as previously female rats display greater distance moved in the open field test than males regardless of isolation or enriched housing conditions (Weiss et

al., 2004). Pre-weaning social deprivation has been shown to reduce open field activity for female rats (Rees et al., 2006) however isolation housing in early adulthood reduced open field crossings compared to socially-housed controls for male rats but not for females (Douglas et al., 2003). Notably the female estrous cycle can also impact on open field behaviour, the administration of estrogen to ovariectomised rats increased activity and resulted in greater percent time in the centre of the open field test; while progesterone administration inhibited this anxiolytic effect (Hiroi and Neumaier, 2006). Likewise females in the proestrous phase experiencing peak estrogen levels make more central crossings than those in diestrous or male rats in the open field test (Frye and Walf, 2002). Environmental enrichment (or social isolation) housing had no effect on locomotor activity in the open field test in the present study. This is in agreement with Zimmermann (2001) who also showed no difference in fields crossed in the open field test by rats housed in enriched, standard or isolated conditions, however theirs was an “unforced” test (Zimmermann et al., 2001) compared to a forced test in the present study. Del Arco and colleagues (2007) also failed to find a difference in distance moved by rats in enriched or isolated housing conditions in a 60-minute open field test (Del Arco et al., 2007a). On the other hand, this study contrasts with Brenes and colleagues (2008) who showed that rats housed in enriched conditions made fewer open field test crossings than both standard and isolated controls following 1, 4, 9 or 11 weeks of differential housing (Brenes et al., 2008). Brenes employed a 10-minute open field test procedure and used a dim red bulb to illuminate the arena, the present study employed a 5-minute duration and the arena was brightly lit using a white bulb, which may have influenced behavioural differences between the two studies (Valle, 1970).

In the elevated plus maze, there were no effects of sex on distance moved or on open arm time or entries. Previously, females have shown greater overall activity (Pena et al., 2006) and greater percent open arm entries and time (Johnston and File, 1991b, Imanaka et al., 2008) than males. Traditionally these parameters are associated with reduced anxiety; however open arm behaviours in the elevated plus maze may not be valid indices of anxiety for females and males equally. Based on findings from a factor-analysis study, Fernandes and colleagues (1999) suggested that behaviour of female rats in the elevated plus maze is characterised by activity

more so than anxiety. Therefore, the test is less sensitive to the effects of anxiolytic agents on females as on males, but more sensitive to changes in general activity in females than in males (Fernandes et al., 1999). Moreover, the elevated plus maze has been shown to be sensitive to estrous cycle variations. Female rats in proestrous and estrous phases of the cycle have shown increased percent open arm time and entries compared to diestrous rats, who show a similar anxiety profile to males in the elevated plus maze (Mora et al., 1996, Marcondes et al., 2001). Estrous cycle phase was not determined in the present study, however the absence of any difference in open arm activity compared to males suggests it is possible that the female rats in the present study were in diestrous or metestrous cycle phases.

Environmental enrichment effects on anxiety-like behaviours in the elevated plus maze have been somewhat inconsistent but enrichment has increased open arm time and entries for female (Galani et al., 2007, Pena et al., 2006) and male (Hellemans et al., 2004, Pena et al., 2006) rats. On the other hand one study found no significant difference in time spent on, or entries to open arms between enriched, isolated and group-housed rats; the rats housed in enriched conditions made significantly more closed-arm entries than both control groups, which might suggest increased anxiety (Brenes et al., 2008). The authors suggest procedural factors accounting for this including elevated anxiety due to bright lighting and tail-marking rats. It is possible that the lighting in the present study was too high to detect any subtle changes brought about by housing conditions, though the same testing conditions have been successfully used to detect anxiolytic drug effects in the past (McDermott and Kelly, 2008). In the present study there was a non-significant trend toward a housing effect for male rats as those housed in standard group conditions had moderately greater percent open arm time than isolated and enriched rats. However this did not reach significance and whether or not this was indicative of reduced anxiety for this group is unclear.

Sex differences in spatial tasks and maze performance have been documented (D'Hooge and De Deyn, 2001, Brandeis et al., 1989); however in the present study there was no difference between male and female rats in latency to locate the platform on any of the four days of Morris water maze acquisition. Harris and colleagues (2009) suggest that impaired Morris water maze performance by both male and female rats was due to the acute stress posed by the test situation, as males

and females did not differ in latencies or in thigmotactic behaviour (i.e. swimming around close to the periphery of the maze, indicative of anxiety in the arena) (Harris et al., 2009). Female estrous hormones may contribute to female Morris water maze performance as intact females have increased latencies compared to ovariectomised females (Daniel et al., 1999). On the other hand, others have found no difference in Morris water maze performance at defined stages of the estrous cycle (Berry et al., 1997). One explanation for the similar performance of males and females in the present study may be in the females' use of cues in the test. In the present study, a number of visual cues remained constant throughout the Morris water maze procedure and once the rat was released into the pool the experimenter moved so she was not in the rat's view. Roof and Stein (1999) suggested that when females have landmark cues in the Morris water maze they perform at a level equivalent to males. In a series of experiments they demonstrated that when the location of a salient visual cue was varied, females swam over greater path lengths to reach the platform than males; however when the visual cue remained in a constant position, females performed as well as males, regardless of release point or platform position (Roof and Stein, 1999). Thus Morris water maze task methodology can affect learning and memory, and possibly impact on female rats' performance.

There was no effect of isolated or enriched housing condition on latency to the platform in the Morris water maze, this is in contrast with Nilsson and colleagues (1999) who employed a similar protocol to that used in the present experiment and found that 8 weeks environmental enrichment housing reduced latencies on acquisition training days 3 and 4 compared to singly-housed controls (Nilsson et al., 1999). On the other hand, 3 months enrichment did not significantly reduce path length for female Long Evans rats compared to standard group-housed controls (Harati et al., 2009). In agreement with (Pereira et al., 2008) there was an effect of housing conditions for males in the probe trial however as male rats housed in enriched conditions spent more time in the target quadrant than isolated male rats, but there was no significant difference when compared to the standard control group. It is important to note that the majority of environmental enrichment studies employing the Morris water maze are rehabilitation-based; wherein environmental enrichment is employed to alleviate the cognitive deficits associated with brain

injury, aging or stroke. It may be therefore that any housing effects on intact healthy rats are slight and not exposed in the present protocol.

Previous studies have demonstrated that female rats have greater serotonin levels than male rats in brain regions such as the prefrontal cortex (Staiti et al., 2011), striatum (Moraes et al., 2004) and hypothalamus (Schwarz et al., 2005), while others found no difference between male and female rats in serotonin levels in the frontal cortex (Kheirandish et al., 2005) hippocampus or striatum (Wallinga et al., 2011). It is likely that the various ages and rat strains employed contribute to these conflicting results. Sex differences in serotonin in the present experiment were evident as females housed in enriched conditions had higher serotonin levels than males in the striatum and amygdala. However housing conditions and sex had interactive effects on serotonin levels as females in isolated conditions had lower serotonin levels in the same region compared to singly-housed males. Similarly in the striatum and amygdala, sex differences were only significant in the rats housed in enriched conditions. Thus environmental conditions, enriched or impoverished, influence the serotonergic system in the rat brain.

In the present study male and female rats housed in isolated conditions had lower levels of serotonin in the frontal cortex and striatum compared to those housed in standard group conditions. This is somewhat in agreement with Brenes and colleagues (2009) who found environmental enrichment housing to increase serotonin in the frontal cortex and hippocampus compared to isolated and group-housed controls (Brenes et al., 2009). Isolation housing can attenuate amphetamine-induced serotonin release in the frontal cortex (Dalley et al., 2002) and can alter serotonin receptor binding and responsiveness in rats compared to socially-housed controls (for review see (Lapiz et al., 2003)). In the present study, enriched housing reduced serotonin in the amygdala, and increased serotonin in the hippocampus of male rats compared to standard non-enriched controls. Male rats in enriched housing have displayed increased serotonin receptor mRNA in the dorsal hippocampus compared to those housed in isolation (Rasmuson et al., 1998). The current experiment also found male rats in isolation had reduced 5-Hydroxyindoleacetic acid levels in the frontal cortex and striatum compared to the standard social groups. Previously Heidbreder (2000) showed reduced 5-Hydroxyindoleacetic acid in the nucleus accumbens of isolated male rats compared to standard controls (Heidbreder

et al., 2000). However in contrast to the present results Brenes and others (2008) showed 5-Hydroxyindoleacetic acid in the prefrontal cortex to be significantly higher in male rats housed in isolated conditions compared to those in enriched and standard social conditions (Brenes et al., 2008). For females, those in isolation had reduced 5-Hydroxyindoleacetic acid in the striatum, but elevated levels in the hippocampus compared to group-housed females. The reason for this sex difference is unclear but may somewhat reflect innate differences in male and female serotonin levels in the brain (Duchesne et al., 2009).

There were fewer sex differences in noradrenaline and dopamine concentrations compared to serotonin; however female rats had lower noradrenaline in the frontal cortex and hippocampus and lower levels of dopamine in the frontal cortex. As with serotonin these sex differences were dependent on housing condition. Others have suggested there are no sex differences in noradrenaline and dopamine levels of group-housed Long-Evans, Sprague-Dawley and Wistar rat strains (Staiti et al., 2011, Kheirandish et al., 2005, Papaioannou et al., 2002). In the present study, for the most part, rats in standard social housing had greater monoamine concentrations in the various brain regions, compared to those housed in isolation. As the enriched and standard groups did not generally differ from one another it may be said that social isolation was reducing monoamine levels. However in the present study behavioural tests did not reflect this.

Housing conditions also affected dopamine and noradrenaline in the rat brain. Compared to rats in standard conditions, both male and female rats housed in isolation had reduced dopamine in the striatum. This contrasts with others who found isolated conditions had no effect on striatal dopamine levels (Jones et al., 1992). Analysis of noradrenaline revealed reduced levels in the amygdala of male and females housed in isolation compared to both socially-housed controls. Environmental enrichment housing significantly increased hippocampal noradrenaline for males compared to both those in isolated and standard conditions. Fulford and Marsden (1997) have carried out a number of studies on the effects of social isolation on the noradrenergic system in the rat brain; they suggest isolation induces enhanced function of adrenoceptors in the dorsal hippocampus, which results in decreased functional responsiveness of hippocampal noradrenergic nerve terminals (Fulford and Marsden, 1997). In contrast to the present results, previously

there was no effect of isolation, standard or enriched housing on noradrenaline concentration in the prefrontal cortex reported (Brenes et al., 2008). In addition, male rats housed in environmental enriched and standard group conditions had significantly greater noradrenaline levels in the striatum compared to isolated controls (Brenes et al., 2008).

Overall there were sex differences in the open field test and an effect of housing for males in the Morris water maze probe trial. Social isolation seemed to have the greatest effect on monoamine levels in the brain regions studied. The reported effects of both isolation and enriched housing conditions on neurotransmitters are often fraught with inconsistencies due to differences in housing procedures, strain differences and various the methodological approaches including post-mortem tissue analysis, *in vivo* microdialysis or *in vitro* ligand binding experiments. Regardless of methodology however it can be said that housing conditions do impact on rats' neurochemistry.

Chapter 5:

Effect of Early Life Housing Manipulation on Baseline and Drug-Induced Behavioural Responses and Neurochemistry in the Male Rat

Chapter 5

Results chapter 3

Effect of early life housing manipulation on baseline and drug-induced behavioural responses and neurochemistry in the male rat

5.1 Introduction

Early-life exposure to EE has been employed to demonstrate plasticity in neural structure and function (Diamond, 1988); in contrast, post-weaning social isolation results in aberrant neural and behavioural development including neophobia, aggression and reduced social interaction, decreased cortical and hippocampal plasticity (Robbins et al., 1996, Fone and Porkess, 2008). EE increases cortical thickness and dendritic growth (Diamond, 1988), alters development in brain regions such as the auditory (Engineer et al., 2004), visual (Sale et al., 2004) and sensory cortex (Coq and Xerri, 1998), in addition to promoting neural plasticity via markers such as neurotrophic factors and synaptic strengthening (Pham et al., 1999a, Pham et al., 1999b, Nithianantharajah and Hannan, 2006). Rearing rats in a complex environment has also been shown to reduce anxiety and improve ability to cope with stress (Fernandez-Teruel et al., 2002), improve habituation to novel environments (Zimmermann et al., 2001) and accelerate learning and memory (Schrijver et al., 2002, Larsson et al., 2002).

As yet, no defined protocol exists for the implementation of EE and the age at which rats are exposed to EE varies from one laboratory to another (Fig. 1.2), however, many introduce rats to EE housing conditions immediately after weaning as a rat's full synaptic density is not yet accomplished at this point. The rat hippocampus is sensitive to the plastic effects of training and experience throughout adulthood (Rosenzweig and Bennett, 1996); up to 85% of granule cells in the dentate gyrus are generated postnatally, and the greatest proliferation is seen during PND 20-30 (Altman and Das, 1965) and can continue to be generated in adulthood (Kaplan and Hinds, 1977, Kaplan and Bell, 1984). Likewise monoamine neurotransmitter levels continue to change postnatally (Moll et al., 2000, Benes et al., 2000) and the plastic effects of EE rearing extend to modify monoaminergic neurotransmitter systems via neurotransmitter levels, transporter levels and receptor densities. The

dopaminergic system is extensively analysed due to its role in the mesolimbic “reward” pathway and rearing environment has been shown to impact on rats’ responses to drugs of abuse. Rats housed in social isolation display abnormal DA activity and behavioural characteristics which model clinical symptoms of schizophrenia such as hyperactivity, attention and cognitive deficits (Watson et al., 2011, Fone and Porkess, 2008, King et al., 2009, Bianchi et al., 2006). On the other hand, EE also impacts on monoamine levels. EE housing (6–12 weeks) reduced D₁ DA receptor density (Del Arco et al., 2007a), reduced DAT expression (Zhu et al., 2005) and function (Zhu et al., 2004) in the PFC compared to IC controls. The serotonergic and noradrenergic systems are also affected by EE rearing; compared to both IC and SC controls EE rats exhibited the highest level of 5HT in the PFC and the highest level of striatal NA (Brenes et al., 2008). Furthermore, adult SD rats housed in EE for thirty days had significantly greater hippocampal 5HT_{1A} receptor expression and binding compared to IC controls (Rasmuson et al., 1998).

In addition to, and perhaps in parallel with effects on neurotransmitter expression, EE has been shown to affect neurotrophic factors, such as BDNF, glial-derived neurotrophic factor (GDNF), and NGF. These proteins are integral to neuronal proliferation and synaptic transmission (Kang and Schuman, 1995) and are likely to mediate the effects of the experience on brain structures and function (Branchi et al., 2004). Exposure to EE has significantly increased NGF in the hippocampus, basal forebrain, hindbrain (Pham et al., 1999a, Ickes et al., 2000), and in the entorhinal and visual cortices compared to IC controls (Pham et al., 1999b). Year-long EE also increased BDNF levels in the basal forebrain, cortex, hippocampus and hindbrain; in addition to increased NT₃ in the basal forebrain and cortex, compared to IC controls (Ickes et al., 2000).

In behavioural tests, EE rearing has been found to decrease the time spent immobile in the FST compared to both IC and SC (3/cage) controls, which suggests that EE reduces behavioural despair (Brenes et al., 2008). This coping behaviour displayed by EE rats and the reduced swimming and climbing behaviours in IC-reared animals were associated with altered neurotransmitter levels in the prefrontal cortex and ventral striatum (Brenes and Fornaguera, 2008). In tests of locomotor activity such as the OFT EE rats have consistently displayed significantly less locomotor activity (Elliott and Grunberg, 2005, Segovia et al., 2008, De Bartolo et

al., 2008) and more rapid habituation (Pham et al., 1999b, Zimmermann et al., 2001, Del Arco et al., 2007a) compared to IC and SC controls, which indicates better adaptability to a novel environment compared to controls. In the EPM inconsistent EE effects have been reported; some have found that EE rearing increased the percent time spent on the open arms and the percent of open arm entries on the EPM (Galani et al., 2007), which would suggest an overall anxiolytic effect of EE. Others however have reported that EE had no effect on open arm time or entries to the open arms (Brenes et al., 2009). Studies often employ the MWM to assess memory deficits following brain damage and the rehabilitative effects of EE housing. However, in studies of spatial learning for non-damaged rats, those housed in EE displayed more rapid spatial learning and better memory in the MWM (i.e. reduced escape latencies) than those housed in IC (Pham et al., 1999a, Nilsson et al., 1999). In one study, EE rats reached peak performance as early as day 3 of trials, in which it took approximately 10 seconds to reach the escape platform and this was not significantly different to their fastest time of approximately 7 seconds, which was reached by day 6. It took SC animals 6 days of training to reach the same performance level (Schrijver et al., 2002).

Few studies have addressed the effects of EE rearing from weaning on behavioural pharmacology test responses in early adulthood. The aim of this study therefore was to investigate baseline and drug-induced responses shown by rats in the FST, EPM, OFT, MWM and homecage activity levels following differential rearing conditions from weaning. Post-mortem monoamine neurotransmitter and BDNF levels in various brain regions were also assayed to determine any effects of post-weaning EE or IC rearing.

5.2 Methods

5.2.1 Animals

Seventy-two male SD rats born in the laboratory were weaned on PND 21 and randomly assigned to one of three housing conditions, IC, SC and EE. IC and SC rats were housed singly or in groups of four in standard-sized cages, whilst the EE group was housed in groups of four in larger cages enriched with a variety of toys/enrichment objects (see section 2.2.2). All animals were maintained in their respective housing environments for five weeks prior to beginning behavioural testing and throughout testing (total of approximately 9 weeks, see table 5.1) and all rats were weighed weekly. A counter-balanced design was employed wherein animals were randomly assigned to one of six groups ($n = 4/\text{housing condition in each group}$), each group was exposed to each behavioural test, but on different days. The aim of using this counter-balanced design was to avoid any confounding effects of test day on behavioural performance. The OFT and EPM tests were carried out between 08:00 and 12:00, while the FST pre-test and testing were carried out at 13:00-14:00 and the homecage monitoring tests were carried out from 08:00 to approximately 16:00, depending on the number of animals for testing on a given day.

Table 5.1: Experimental outline

PND	Experimental Progress
21	Weaning, differential housing (IC, SC, EE)
60	Behavioural testing
88	Sacrifice

IC, isolated conditions; SC, standard conditions; EE, environmentally enriched conditions.

5.2.2 Drugs

See section 2.1 for details and drug sources.

DZP (0.625, 1.25 and 2.5 mg kg⁻¹, i.p.) was administered 30 minutes prior to EPM testing. DMI (2.5, 5 and 10 mg kg⁻¹, i.p.) was administered 24, 5 and 1 hour prior to the 5-minute FST. AMP (0.2, 0.5, 1, 2 and 5 mg kg⁻¹, s.c.) was administered immediately prior to homecage monitoring.

5.2.3 Open field test

See section 2.3.2

5.2.4 Elevated Plus Maze

See section 2.3.3

5.2.5 Forced Swim Test

See section 2.3.4

5.2.6 Homecage activity monitoring

Homecage activity was monitored over 2 hours. See section 2.3.5, HCMA

5.2.7 Morris Water Maze

See section 2.3.6

5.2.8 Determination of monoamine levels using high performance liquid chromatography

Upon completion of the experiment rats were sacrificed by decapitation, the brain was removed from the skull and frozen on dry ice before being stored at -80 °C. The hypothalamus, frontal cortex, striatum, hippocampus and cerebellum were later dissected as per section 2.5.1.

HPLC analysis was carried out as described in section 2.6 to determine concentrations of NA, DA, 5HIAA and 5HT. Results are expressed as nanograms per gram fresh weight of tissue.

5.2.9 Determination of BDNF levels using ELISA

BDNF levels in the frontal cortex, striatum, hippocampus and cerebellum were assayed using the ELISA as outline in section 2.7. BDNF levels were expressed as pg/mg tissue protein.

5.3 Statistical analysis

See section 2.8. Kruskal-Wallis tests were carried out where appropriate to analyse housing effects on monoamine levels.

5.4 Results

5.4.1 Body Weight (Figure 5.1)

Regarding weekly body weight, there were significant main effects of housing [$F(2, 69) = 3.18$, $p < 0.05$], week [$F(8, 552) = 5860.66$, $p < 0.001$] and a significant housing \times week interaction effect [$F(16, 552) = 3.76$, $p < 0.001$]. One-way ANOVAs revealed rats housed in IC were significantly heavier than those in both SC and EE conditions during weeks 3-6 of differential housing ($p < 0.05$). However, when overall weight gain (i.e. weight at week 9 – weight at week 1) was analysed there was no effect of IC or EE housing conditions [$F(2, 69) = 0.81$, $p > 0.05$].

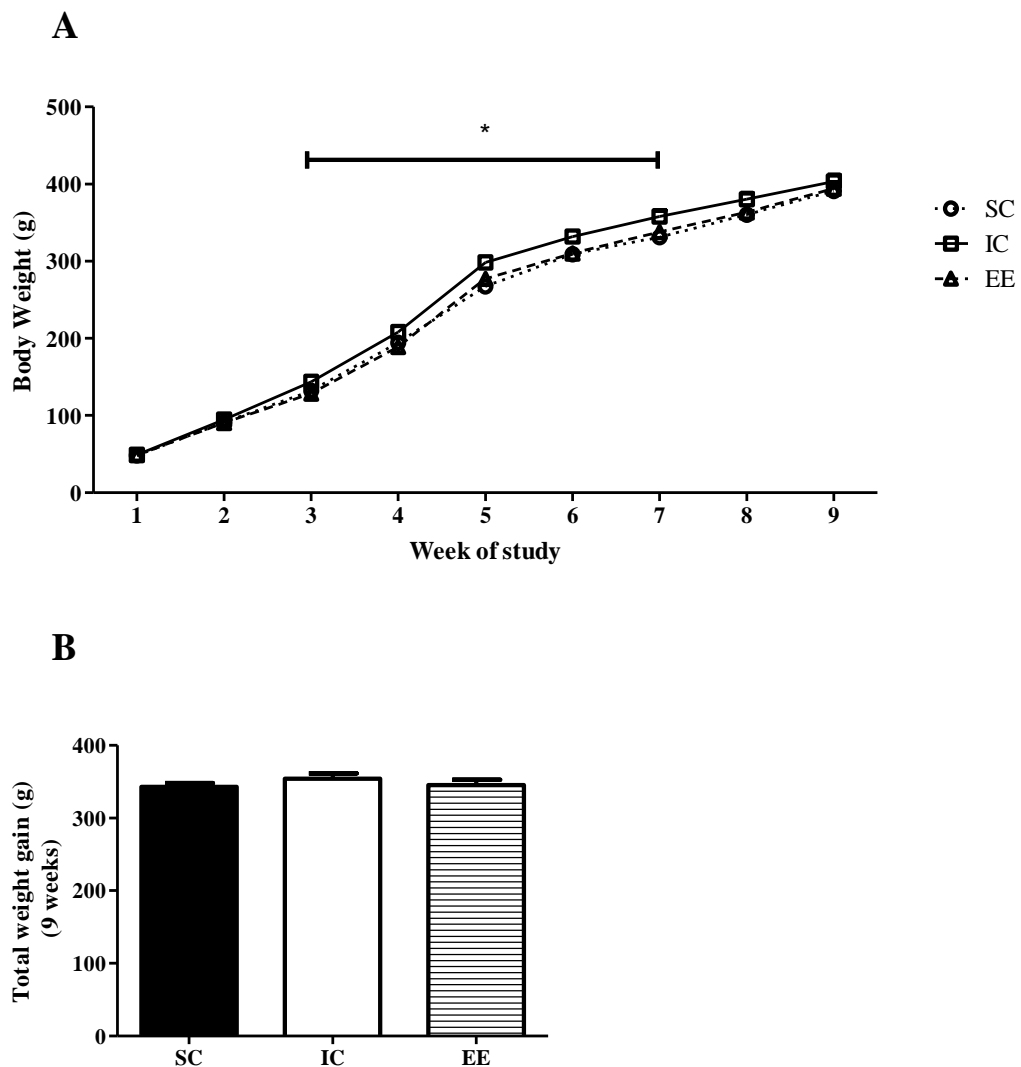


Figure 5.1: The effects of isolation and enriched housing conditions on body weight. The effects of standard (SC), isolated (IC) and enriched (EE) housing conditions on body weight (A) and weight gain (B) over the 9-week housing and testing period. Data expressed as mean \pm SEM, $n = 24$ per group, * $p < 0.05$ IC vs. SC.

5.4.2 Open Field Test (Figure 5.2)

There was a significant effect of housing condition on total distance moved in the OFT [$F(2, 21)$, $p < 0.05$], *posthoc* tests revealed that rats housed in EE moved significantly less than those in SC ($p < 0.05$). There was no effect of housing conditions on distance moved in the inner arena [$F(2, 21) = 5.57$, $p > 0.05$]. There were however significant differences in entries made to the inner arena [$F(2, 21) = 6.41$, $p < 0.01$] and time spent in the inner arena [$F(2, 21) = 5.57$, $p < 0.05$]. *Post-hoc* tests showed that rats housed in EE made fewer entries and spent less time in the inner arena compared to their SC housed counterparts.

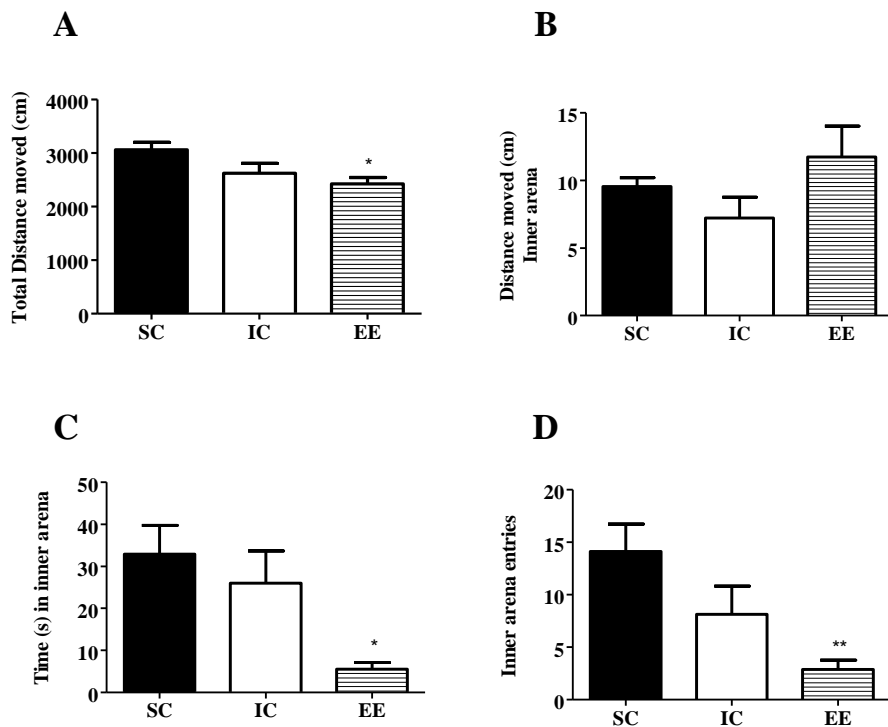


Figure 5.2: The effects of isolation and enriched housing conditions in the open field test. Total distance moved (A) distance moved in the inner arena (B), time spent in (C) and entries made to the inner arena (D) by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 8$ per group), * $p < 0.05$, ** $p < 0.01$ vs. SC.

5.4.3 Elevated Plus Maze

Baseline Behaviour (Figure 5.3)

Distance moved [$F(2, 15) = 0.17, p > 0.05$], percent open arm entries [$F(2, 15), p > 0.05$] or percent open arm time [$F(2, 15) = 1.65, p > 0.05$] were not affected by IC or EE housing conditions among saline control in the EPM.

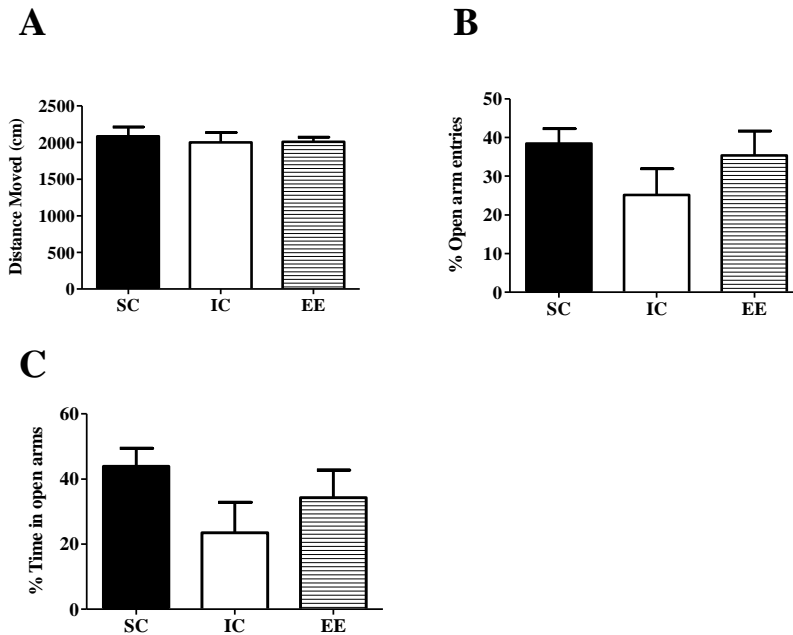


Figure 5.3: The effects of isolation and enriched housing conditions in the elevated plus maze. Distance moved (A), percent open arm entries (B) and percent open arm time (C) by saline-treated rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 6$ per group).

Diazepam Effects (Figure 5.4)

Regarding distance moved in the EPM there was no significant housing [$F(2, 60) = 0.022, p > 0.05$], drug [$F(3, 60) = 6.03, p > 0.05$] or housing x drug interaction effects [$F(6, 60) = 0.60, p > 0.05$]. For percent open arm entries there were no significant effects of housing conditions [$F(2, 60) = 1.39, p > 0.05$] or drug [$F(3, 60) = 2.71, p > 0.05$], nor was there a drug x housing interaction effect [$F(6, 60) = 0.60, p > 0.05$]. However significant housing [$F(2, 60) = 4.90, p < 0.05$] and drug [$F(3, 60) = 3.43, p < 0.05$] effects were found for percent open arm time, but there was no significant interaction [$F(6, 60) = 0.45, p > 0.05$].

One-way ANOVAs for distance moved revealed a significant DZP effect for SC rats [$F(3, 20) = 3.24, p > 0.05$], however *post-hoc* tests showed there was no drug effect on distance moved when compared to saline controls. Rats housed in EE displayed a significant drug response [$F(3, 20) = 4.67, p < 0.05$] as 2.5 mg kg⁻¹ DZP significantly reduced distance moved in the EPM for EE rats compared to saline-treated controls ($p < 0.05$). There was no significant drug effect on distance moved for IC rats [$F(3, 20) = 0.61, p > 0.05$]. No significant effects of DZP were revealed for percent open arm entries (SC: [$F(3, 20) = 1.31, p > 0.05$], IC: [$F(3, 20) = 1.59, p > 0.05$], EE: [$F(3, 20) = 0.86, p > 0.05$]) or percent open arm time (SC: [$F(3, 20) = 2.00, p > 0.05$], IC: [$F(3, 20) = 1.85, p > 0.05$], EE: [$F(3, 20) = 1.77, p > 0.05$]).

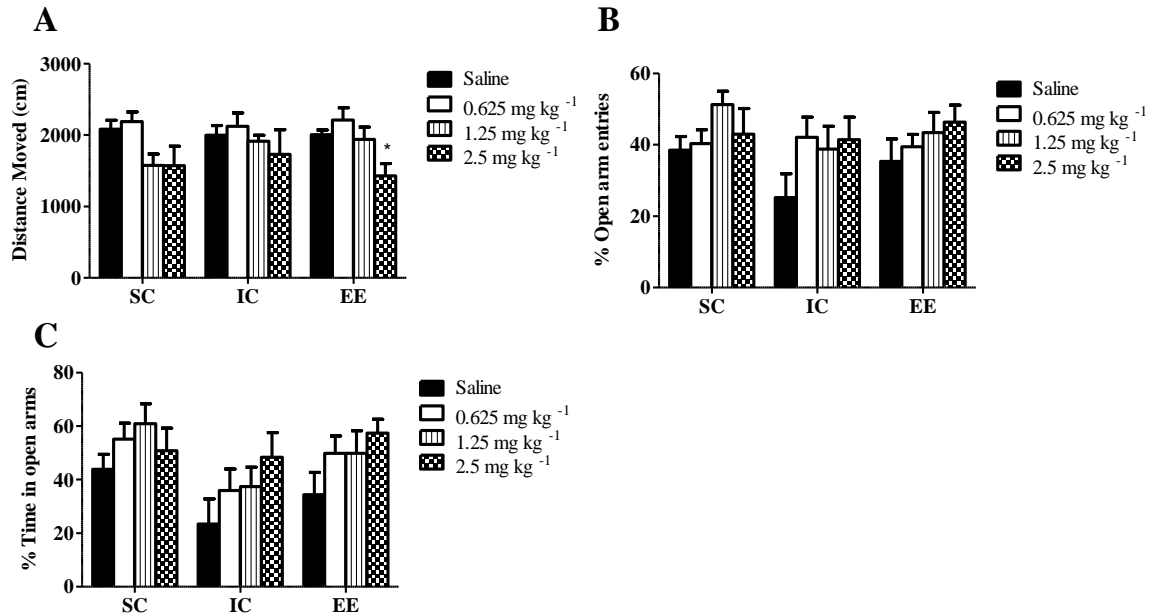


Figure 5.4: Diazepam and housing effects in the elevated plus maze. The effects of DZP (0.625, 1.25, 2.5 mg kg⁻¹, i.p.) on distance moved (A), percent open arm entries (B) and percent open arm time (C) in the EPM by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$, vs. EE saline control.

5.4.4 Forced Swim Test

Baseline Behaviour (Figure 5.5)

Baseline immobility time was not affected by IC or EE housing [$F(2, 15) = 0.69$, $p > 0.05$].

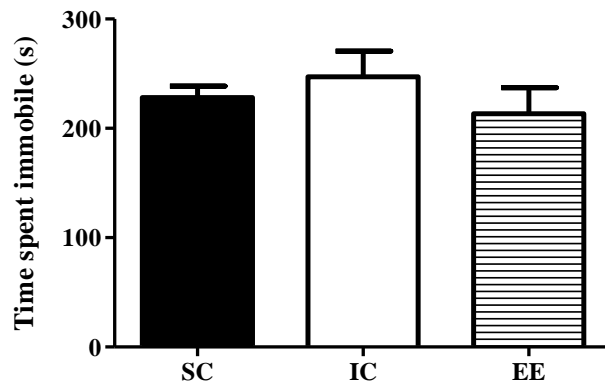


Figure 5.5: The effects of isolation and enriched housing conditions in the forced swim test. Time spent immobile by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 6$ per group).

Desipramine Effects (Figure 5.6)

Two-way ANOVA of time spent immobile in the FST revealed no significant overall drug effects [$F(3, 60) = 1.77$, $p > 0.05$], housing conditions [$F(2, 60) = 0.14$, $p > 0.05$] or drug x housing interaction [$F(6, 60) = 1.19$, $p > 0.05$] effects.

One way ANOVAs showed a significant drug effect in time spent immobile for the IC group as the 0.5 mg kg^{-1} and 10 mg kg^{-1} DMI doses reduced immobility time compared to IC saline controls ($p < 0.05$). However no such significant drug effects were found for SC [$F(3, 20) = 0.82$, $p > 0.05$] and EE groups [$F(3, 20) = 0.52$, $p > 0.05$].

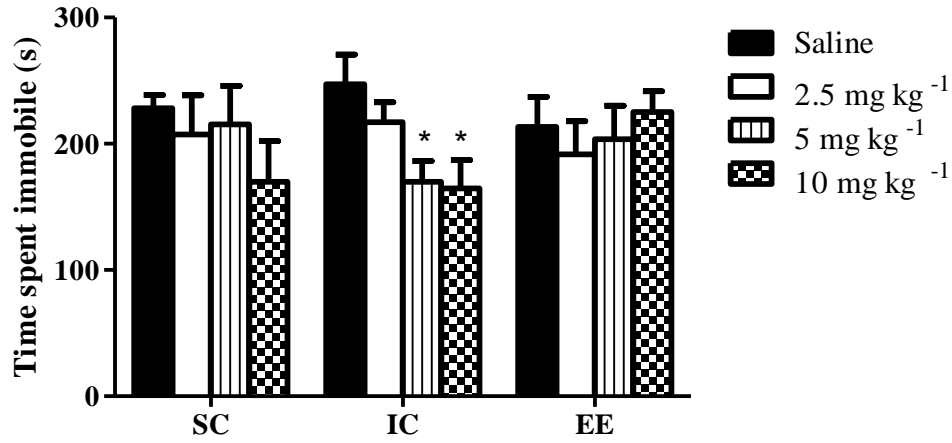


Figure 5.6: Desipramine and housing effects in the forced swim test. The effects of DMI (2.5, 5 and 10 mg kg⁻¹, i.p.) on time spent immobile in the forced swim test by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$ vs. IC saline control.

5.4.5 Homecage Monitoring

Baseline Behaviour (Figure 5.7)

One-way ANOVA showed baseline total distance moved in the homecage was not significantly altered by IC or EE housing conditions [$F(2, 9) = 0.76, p > 0.05$].

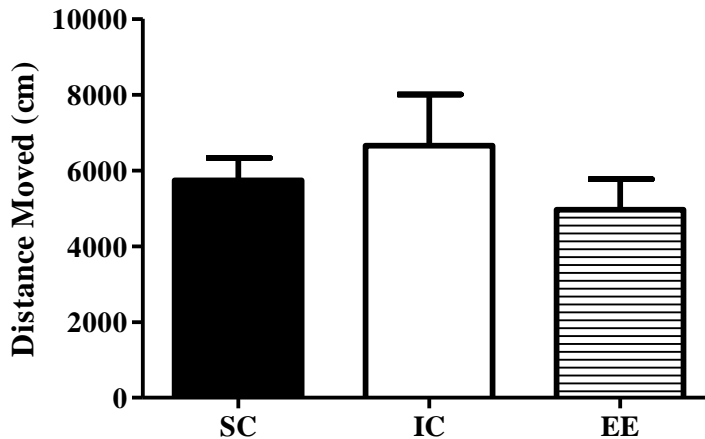


Figure 5.7: The effects of isolation and enriched housing conditions on homecage activity. Total distance moved in the homecage over a 2-hour monitoring period by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 4$ per group).

Amphetamine Effects (Figures 5.8 and 5.9)

Total distance moved in the HCMA was analysed by two-way ANOVA, there was a significant drug effect [$F(5, 54) = 23.99, p < 0.001$] but no main housing effect [$F(2, 54) = 2.48, p > 0.05$] or housing x drug interaction [$F(10, 54) = 0.61, p > 0.05$] effect over the 2-hour monitoring period. One-way ANOVAs showed that rats from all three housing conditions displayed significant drug effects (SC: [$F(2, 18) = 7.59, p < 0.001$], IC: [$F(2, 18) = 6.53, p < 0.01$], EE: [$F(2, 18) = 16.21, p < 0.001$]). *Post-hoc* tests revealed a significant increase in activity for the IC group in response to the 1 and 2 mg kg⁻¹ AMP doses while the 0.5, 1 and 2 mg kg⁻¹ doses increased distance moved for the SC and EE groups, compared to saline controls.

When distance moved data was expressed as a percent of saline baseline controls the same dose-response pattern emerged. There was a significant drug effect [$F(2, 54) = 24.20, p < 0.001$] but no significant housing [$F(2, 54) = 2.67, p = 0.08$] or housing x drug interaction effects [$F(10, 54) = 0.75, p > 0.05$]. One-way ANOVAs demonstrated that significant AMP effects for SC [$F(2, 18) = 7.59, p < 0.001$], IC [F

(2, 18) = 6.33, $p < 0.01$] and EE F (2, 18) = 13.77, $p < 0.001$] persisted when baseline activity levels were normalised.

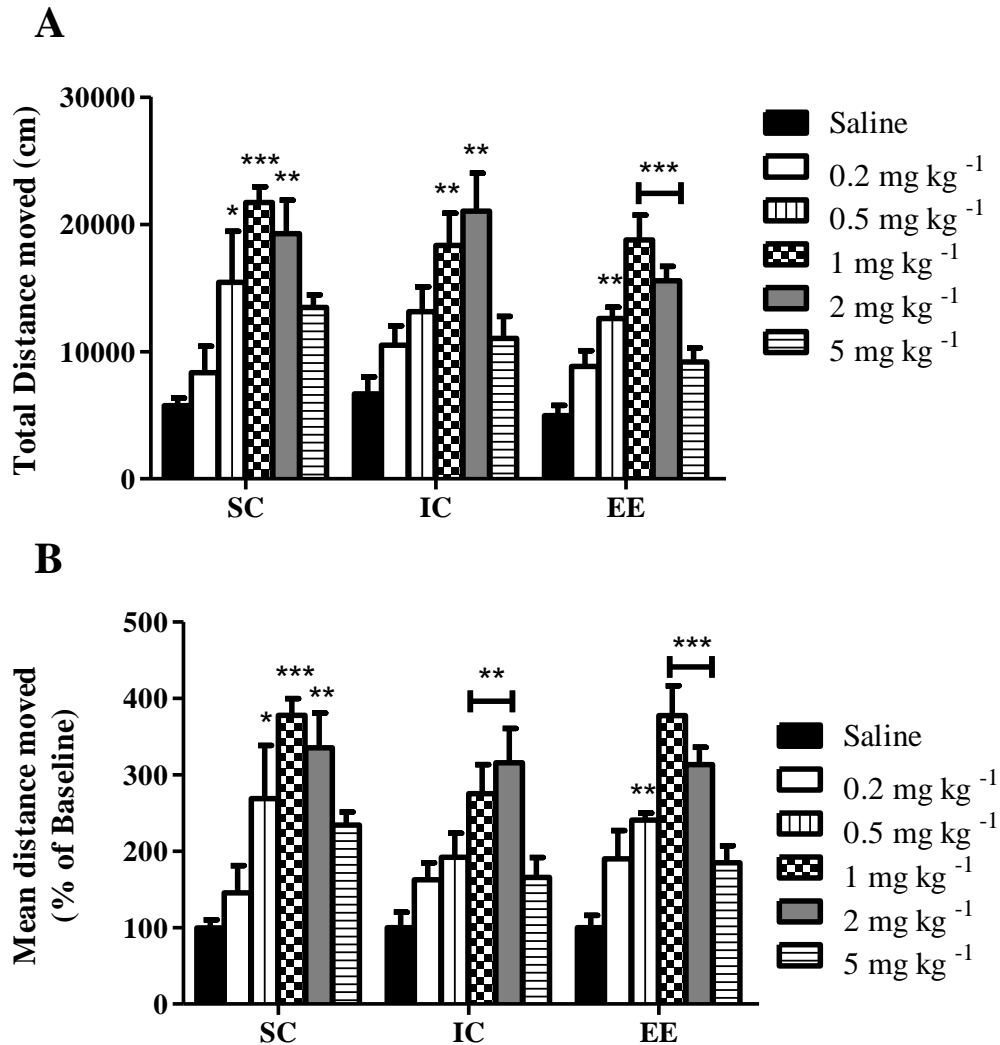


Figure 5.8: Amphetamine and housing effects on distance moved in the homecage. The effects of AMP (0, 0.2, 0.5, 1, 2, 5 mg kg⁻¹, s.c.) on total distance moved (A) and distance moved expressed as percent of baseline control (B) in the homecage by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 4$ per group), *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. appropriate saline controls.

To assess temporal effects of AMP on distance moved the 2-hour monitoring period was analysed over six 20-minute timebins. For SC rats there were significant time [$F(5, 90) = 32.83, p < 0.001$] and drug effects [$F(5, 18) = 7.59, p < 0.01$], but no significant interaction [$F(25, 90) = 1.58, p > 0.05$] on distance moved. As depicted in figure 5.9, SC rats showed maximal locomotor response to 1 mg kg⁻¹ during the first hour, thereafter peak activity was seen in response to 1 and 2 mg kg⁻¹ doses, the SC group also showed sensitivity to the 0.5 mg kg⁻¹ AMP dose. For IC rats, significant time [$F(5, 90) = 17.38, p < 0.001$] and drug [$F(5, 18) = 6.53, p < 0.01$] effects, without a significant interaction [$F(25, 90) = 0.48, p > 0.05$] were also observed. Rats housed in IC displayed significantly increased activity in response to 1 and 2 mg kg⁻¹ AMP from 20–100 minutes, with the 2 mg kg⁻¹ dose producing peak increased activity. Rats housed in EE showed significant time [$F(5, 90) = 50.32, p < 0.001$], drug [$F(5, 18) = 16.21, p < 0.001$] and significant time x drug interaction effects [$F(25, 90) = 1.99, p < 0.05$]. EE rats were sensitive to the locomotor effects of all AMP doses during the first 20 minutes and were the only group to have significantly increased activity following 0.2 mg kg⁻¹ AMP. Rats in EE conditions displayed maximal locomotor response to the 1 mg kg⁻¹ dose. One-way ANOVA results are presented in figure 5.9.

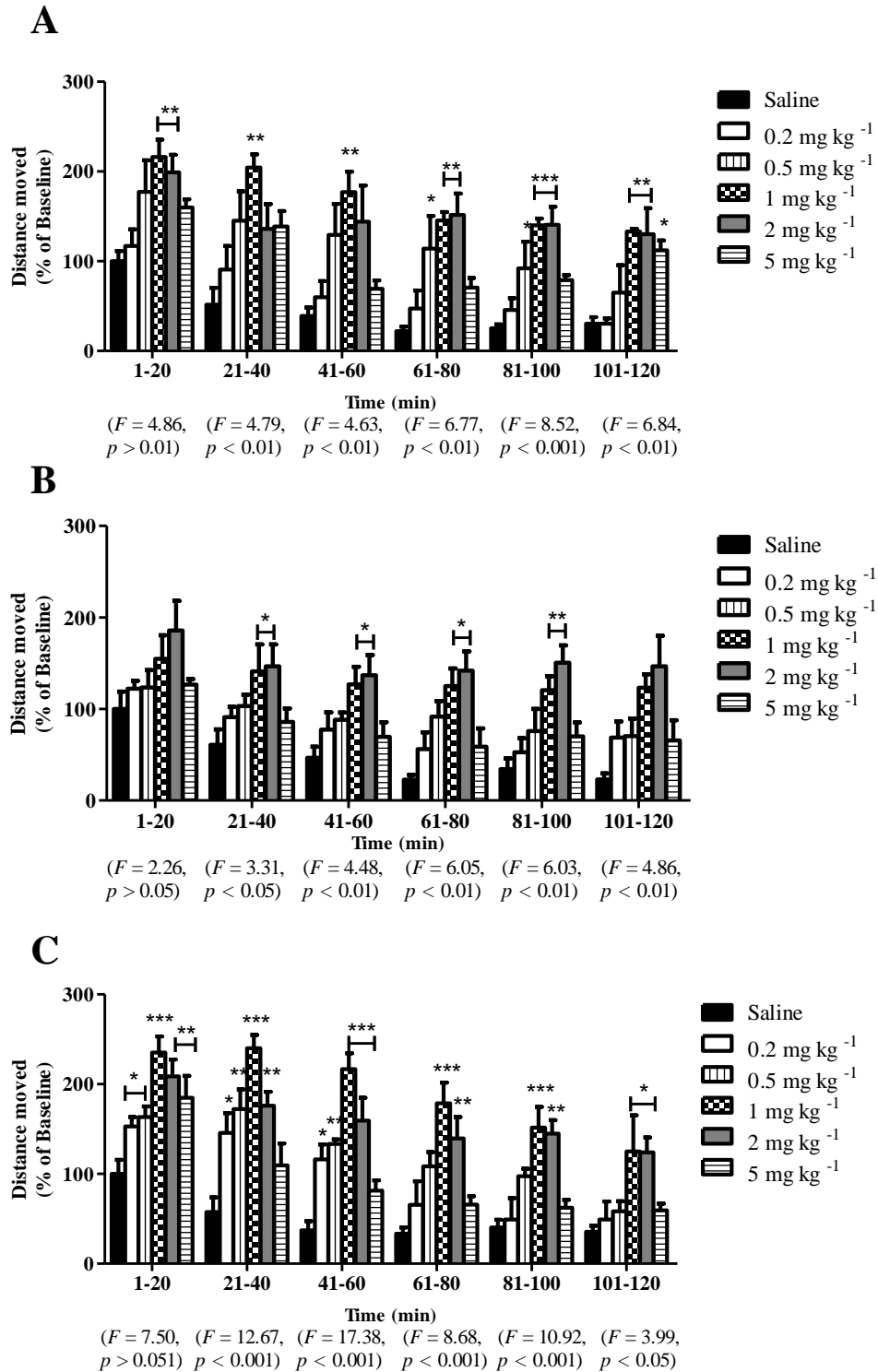


Figure 5.9: Amphetamine and housing effects on distance moved in the homecage over 20-minute timebins. The effects of AMP (0, 0.2, 0.5, 1, 2 or 5 mg kg⁻¹, s.c.) administration on distance moved in the homecage, expressed as percent of baseline control by rats housed in standard (SC) (A), isolated (IC) (B) and environmentally enriched (EE) (C) conditions. Data expressed as mean + SEM ($n = 4$ per group), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. appropriate saline controls according to 1-way ANOVA ($df = 5, 18$).

5.4.6 Morris Water Maze (Figure 5.10)

Latencies in the MWM showed a significant effect of time [$F(3, 63) = 25.33, p < 0.001$] as all rats learned the platform location during the 4-day acquisition phase of the MWM. There was no effect of housing on MWM latencies [$F(2, 21) = 2.99, p > 0.05$], nor was there any housing x time interaction effect [$F(6, 63) = 1.12, p > 0.05$]. There was a significant effect of housing [$F(2, 21) = 6.65, p < 0.01$] and time [$F(3, 63) = 3.73, p < 0.05$] on swim speed as the EE group swam more slowly than the SC group on days 2 and 4 (both $p < 0.05$). There was no housing effect on time spent in the target quadrant during the probe trial [$F(2, 21) = 0.64, p > 0.05$].

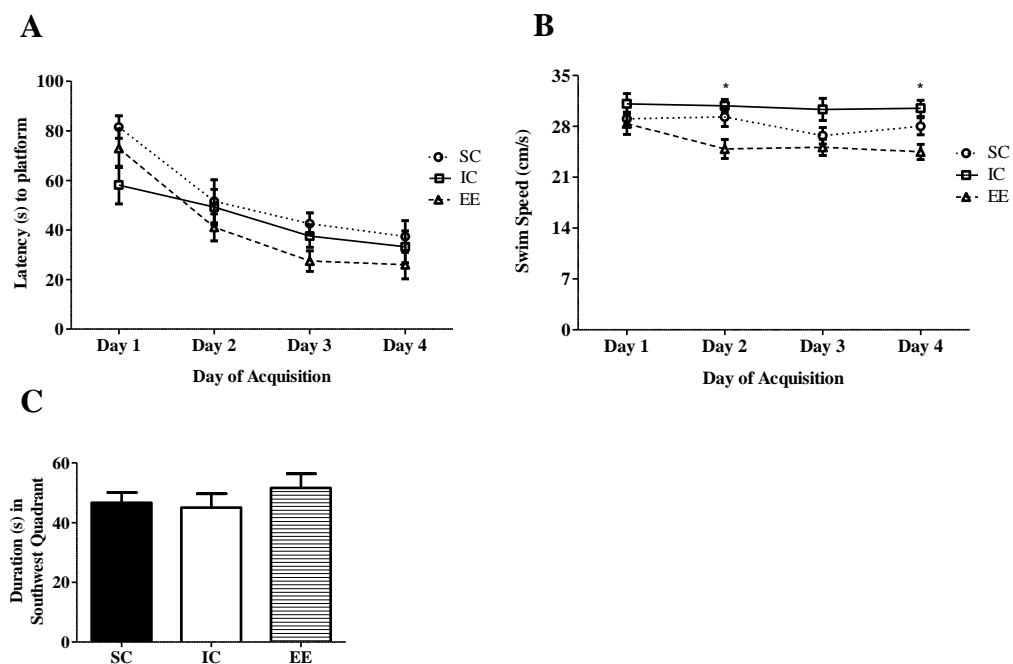


Figure 5.10: The effects of isolation and enriched housing conditions in the Morris water maze. Latency to locate the platform (A) and swim speed (B) in the acquisition phase, and time spent in the target quadrant in the probe trial (C) of the MWM by rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM ($n = 8$ per group), * $p < 0.05$ EE vs. SC.

5.4.7 Determination of monoamine levels using high performance liquid chromatography (Table 5.2)

There were no significant housing effects on monoamine levels in the frontal cortex, striatum, hippocampus or cerebellum, descriptive statistics and ANOVA results are presented in table 5.2.

Table 5.2: Effects of housing on monoamine neurotransmitter levels in the rat brain

		NA	DA	5HIAA	5HT	5HIAA/5HT
		Frontal Cortex				
SC		212 ± 17	52 ± 5	369 ± 38	340 ± 23	1.07 ± 0.05
IC		346 ± 20	63 ± 13	451 ± 36	353 ± 52	1.40 ± 0.17
EE		199 ± 22	86 ± 45	341 ± 29	275 ± 22	1.27 ± 0.15
Housing Effect	<i>F</i>	1.32	0.41	2.73	1.38	1.64
	<i>p</i>	> 0.05				
	<i>df</i>	2, 18				
		Striatum				
SC		207 ± 9	3938 ± 538	435 ± 42	307 ± 16	1.44 ± 0.02
IC		184 ± 32	2664 ± 389	408 ± 50	251 ± 35	1.70 ± 0.20
EE		212 ± 51	3652 ± 567	311 ± 44	250 ± 39	1.31 ± 0.13
Housing Effect	<i>F</i>	0.18	1.78	2.03	0.93	1.42
	<i>p</i>	> 0.05				
	<i>df</i>	2, 18				
		Hippocampus				
SC		270 ± 13	17 ± 8	443 ± 28	255 ± 15	1.77 ± 0.15
IC		329 ± 27	34 ± 17	521 ± 68	426 ± 193	1.61 ± 0.26
EE		302 ± 25	43 ± 2	357 ± 34	298 ± 59	1.31 ± 0.13
Housing Effect	<i>F</i>	1.68	1.44	3.08	0.88	1.49
	<i>p</i>	> 0.05				
	<i>df</i>	2, 18				
		Cerebellum				
SC		149 ± 8	ND	102 ± 8	96 ± 52	1.89 ± 0.12
IC		191 ± 27	ND	105 ± 8	53 ± 12	1.84 ± 0.25
EE		162 ± 21	ND	74 ± 6	34 ± 12	1.40 ± 0.15
Housing Effect	<i>F</i>	1.76		1.30†	1.02	1.68
	<i>p</i>	> 0.05				
	<i>df</i>	2, 18				
		Hypothalamus				
SC		958 ± 120	174 ± 25	554 ± 62	322 ± 58	1.76 ± 0.15
IC		974 ± 140	254 ± 96	243 ± 95	381 ± 83	2.04 ± 0.26
EE		922 ± 118	153 ± 21	430 ± 49	236 ± 31	1.91 ± 0.22
Housing Effect	<i>F</i>	0.76	0.64†	1.36†	1.37	0.44
	<i>p</i>	> 0.05				
	<i>df</i>	2, 14				

SC, standard group condition; IC, isolated condition; EE, environmental enrichment condition; NA, noradrenaline; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; 5-HIAA / 5-HT, serotonin turnover; ND, not detectable. Data expressed as mean neurotransmitter concentration (ng / g tissue) ± SEM ($n = 5 - 7$ per group), † demotes Kruskal Wallis analysis (χ^2)

5.4.8 Brain-Derived Neurotrophic Factor Concentration (Figure 5.11)

There were no differences between IC, SC and EE- reared rats in BDNF levels in the frontal cortex [$F(2, 18) = 0.68, p > 0.05$], striatum [$F(2, 18) = 0.58, p > 0.05$], hippocampus [$F(2, 18) = 0.78, p > 0.05$] and cerebellum [$F(2, 18) = 0.97, p > 0.05$].

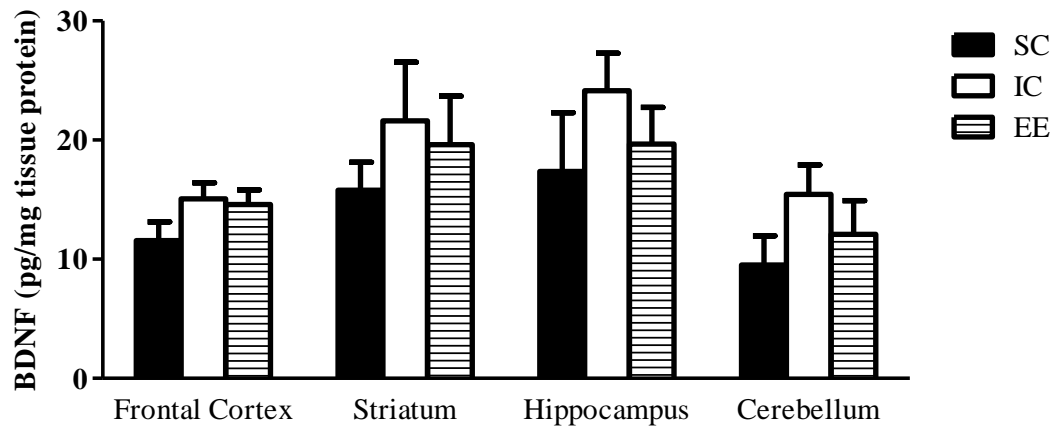


Figure 5.11: The effects of isolation and enriched housing conditions on levels of brain-derived neurotrophic factor in the rat brain. BDNF levels in the frontal cortex, striatum, hippocampus and cerebellum for rats housed in standard (SC), isolated (IC) and environmentally enriched (EE) conditions. Data expressed as mean + SEM pg/mg tissue protein ($n = 7$ per group).

5.5 Discussion

Post-weaning differential housing had no effect on baseline behaviours in the present study. Rats housed in isolated conditions displayed a clear response to the antidepressant desipramine in the forced swim test, while increasing dose had no effect on immobility for standard group and enriched rats. Both group and enriched rats displayed a greater sensitivity to 1 mg kg⁻¹ amphetamine than singly-housed rats. In the elevated plus maze diazepam did not significantly increase percent open arm time or entries.

Singly-housed rats were significantly heavier than those housed in groups of four in standard conditions or with environmental enrichment, which did not differ from one another, during weeks 3-6 of differential housing. This would suggest that the presence of cagemates influenced body weight during these weeks. Previous studies have shown post-weaning environmental enrichment reduced body weight (Hellemans et al., 2004) and weight gain (Pena et al., 2009b), others have observed no effects of post-weaning enrichment on body weight compared to standard controls (Angelucci et al., 2009) or increased weight gain for adult rats provided with environmental enrichment for 8 hours of the light phase, for 5 days a week compared to isolated controls (Konkle et al., 2010). Divergent results are likely to reflect difference in enrichment protocols, the types of controls employed and rat strain differences. The lower body weight seen in socially housed rats compared to those in isolation may be due to factors such as competition for food, increased activity, decreased food consumption, as shown by Zaias and colleagues (Zaias et al., 2008) and factors such as social stress and altered food intake patterns as a result of early rearing environment (Brown and Grunberg, 1996).

Rats housed in environmental enrichment conditions moved significantly less in the open field test than those in standard conditions. This is somewhat in agreement with Brenes and colleagues (2009) who showed male Sprague-Dawley rats housed in enriched conditions from postnatal day 28 for 5 weeks had significantly lower distance moved than isolated rats in a 10-minute open field test (Brenes et al., 2009). However no effect of isolation conditions were observed in the present study, Brenes (2009) employed a dimly-lit open field test while in the present study a bright open field arena was used, which may reduce distance moved and impair the identification of subtle differences (Valle, 1970). The reduced inner

arena entries and time spent in the inner arena displayed by the enriched rats in the present study may be reflective of their overall reduced movement compared to standard-housed controls.

Housing condition had no effect on baseline open arm time and open arm entries in the elevated plus maze. This contrasts with the findings of Galani and colleagues (2007) who showed female Long Evans rats (12 weeks old) housed in enrichment for 30 days displayed increased percent open arm time and entries compared to pair-housed controls (Galani et al., 2007). However the results of the present experiment are in agreement with those of Brenes and colleagues (2009) who found male Sprague Dawley rats (postnatal day 28) housed in isolation, standard (3/cage) or enriched conditions (12/cage) for 4–5 weeks prior to elevated plus maze testing did not differ in percent open arm time or percent open arm entries (Brenes et al., 2009). As light intensity is a factor in plus maze activity it may be that the lux on the open arms of the present study (90 lux) and that carried out by Brenes (200 lux) were too high to encourage open arm activity (Violle et al., 2009). Diazepam failed to significantly increase percent open arm time or entries for any housing group in the present study. Few studies have addressed the effects of environmental enrichment on reactivity to diazepam, one study compared Wistar rats housed in isolation or standard conditions (6/cage) from postnatal day 30 to postnatal day 60 in elevated plus maze activity following 1 mg kg⁻¹ diazepam. They found no drug effect on percent open arm time for either group but that diazepam significantly increased percent open arm entries for rats in isolated housing conditions compared to saline controls, while there was no effect on the standard-housed group (Da Silva et al., 1996), however a high baseline level of percent open arm entries made by the social-housed group is likely to have suppressed any drug effect in their study. The absence of a significant dose-response effect for diazepam in the elevated plus maze in the present study may be due to relatively small sample sizes and inter-sample variation within the isolated group.

There was no significant effect of housing on immobility in the forced swim test for the baseline groups. This contrasts with previous studies wherein environmental enrichment reduced immobility time and increased swimming (Brenes et al., 2008, Magalhaes et al., 2004) compared to singly- and group-housed controls. Brenes and colleagues housed male Sprague Dawley rats in isolation,

standard (3/cage) or enriched conditions (15/cage) from postnatal day 30 for 11 weeks before the forced swim test was carried out. In the present experiment the rats were housed in their respective conditions for approximately 5-7 weeks before the forced swim test, the difference in duration may have contributed to the divergent results. Likewise, the enrichment conditions employed may account for the absence of any baseline differences; the environmental enrichment cage employed by Brenes and colleagues (2008) was larger than those in the present study (Brenes, 120 cm length \times 70 cm width \times 100 cm height; present study, 54 cm length \times 38 cm width \times 19 cm height), thus creating a larger area for exploration, moreover, all rats of the enriched group ($n = 15$) were housed together in this cage. When desipramine effects in the forced swim test were analysed the singly-housed rats were the only group displaying significantly reduced immobility time in response to the drug. Few studies have investigated the effects of enriched housing on responsiveness to antidepressant drugs. Male Wistar rats (postnatal day 21) were housed in isolated or standard conditions (5-6/cage) for 4 weeks, followed by forced swim test exposure. Employing the same dose regime as the present study (desipramine i.p. administered 24, 5 and 1 hour prior to testing), 5-15 mg kg⁻¹ desipramine reduced immobility time and increased struggling time for both isolated and standard group conditions compared to saline-treated controls. However, the drug had a greater effect on the singly-housed rats than those housed in social groups (Wongwitdecha et al., 2006). The authors suggest that as desipramine acts by reducing noradrenaline and serotonin reuptake, the increased amine concentration coupled with increased sensitivity at adrenoceptors for rats housed in isolation (Fulford and Marsden, 1997) may contribute to their enhanced response to desipramine.

As expected, distance moved in the homecage increased with increasing amphetamine dose in the present study. Isolated rats displayed peak locomotor activity in response to 2 mg kg⁻¹ amphetamine, whereas the lower dose of 1 mg kg⁻¹ was sufficient to produce maximal locomotor response for both of the socially-housed groups. It has been shown previously that standard-housed animals displayed significantly increased distance moved following 1 mg kg⁻¹ amphetamine while singly-housed rats' activity was not significantly different to that of saline controls (Wang et al., 2010). Acute amphetamine (0.5–2 mg kg⁻¹) increased horizontal (Bowling and Bardo, 1994) and vertical activity (Bardo et al., 1995) to a greater

extent in rats housed in enriched conditions (for 5 weeks from weaning) compared to their singly-housed counterparts. While this would suggest that the rewarding effects of acute amphetamine are enhanced following environmental enrichment rearing, enrichment is in fact protective in reducing drug abuse vulnerability (Green et al., 2002, Bardo et al., 2001, Stairs et al., 2006). The enhanced response of environmentally enriched rats to acute amphetamine is thought to be due to differences in the dopaminergic system. Elevated activity levels by enriched rats were accompanied by greater decreases in the concentration of the dopamine metabolite 3,4-Dihydroxyphenylacetic acid in the nucleus accumbens than isolated rats (Bowling et al., 1993). However, when this was assessed in an *in vitro* slice preparation no difference between rats in isolated and enriched conditions was observed (Bardo et al., 1995). The authors suggest that the behavioural differences may therefore be due to pharmacokinetic differences in drug availability or pharmacodynamic factors in brain regions other than the nucleus accumbens (Bardo et al., 1995). *In vivo* microdialysis showed that enriched rats had greater amphetamine-induced glutamate release, but a non-significant elevation in drug-induced dopamine release in the nucleus accumbens (Bardo et al., 1995, Rahman and Bardo, 2008). Environmental-enrichment induced alterations in dopamine and dopamine transporter (Zhu et al., 2004) function in the medial prefrontal cortex may lead to increased dopamine release in the striatum or nucleus accumbens of rats housed in enriched conditions, contributing to the enhanced effect of acute amphetamine.

Isolation or enriched housing conditions had no effect on latencies in the Morris water maze. This contrasts with previous findings that rats housed with environmental enrichment had shorter escape latencies than standard group-housed (Schrijver et al., 2002) and isolation-housed controls (Nilsson et al., 1999). Methodological differences may contribute to the disparate findings as Schrijver and colleagues (2002) employed a different Morris maze protocol to that used in the present study (Schrijver et al., 2002), similarly Nilsson and colleagues (1999) studied female Long-Evans rats while male Sprague-Dawley were employed in the present study. The present results agree somewhat with two studies of brain damage wherein sham control rats in enriched or standard housing conditions did not differ in Morris water maze latencies. Male Wistar rats were housed in enrichment or

standard conditions (6-8/cage) during postnatal day 7–30, animals were then all housed in standard social conditions until behavioural testing on postnatal day 90. The results showed there was no effect of early environmental enrichment on adulthood Morris maze performance (Pereira et al., 2008). Likewise, male Wistar rats aged 7 weeks, housed in enriched or standard conditions (3/cage) for 10 weeks did not differ in latencies or distance swum in the Morris water maze (Gobbo and O'Mara, 2004).

In spite of differential amphetamine effects, there were no differences in dopamine levels in the brain detected by high performance liquid chromatography in the present study. Nor were there any effects of housing on any monoamine levels in the brain regions studied. Results of enrichment effects on tissue neurotransmitter levels have been ambivalent, post-weaning environmental enrichment has been shown by some to increase serotonin and noradrenaline levels in the hippocampus (Brenes et al., 2009) and increase serotonin in the prefrontal cortex (Brenes et al., 2008) compared to isolated and standard-housed (3/cage) controls. On the other hand, Galani and colleagues (2007) found no difference between enriched and standard (pair-housed) controls in dopamine levels in the hippocampus or cortex, nor did their high performance liquid chromatography analysis reveal any differences in noradrenaline levels in the ventral hippocampus or serotonin levels in the dorsal hippocampus (Galani et al., 2007). Furthermore, tissue levels of 5-Hydroxyindoleacetic acid were not different in the hippocampus or striatum of enriched, standard and isolation-housed rats (Brenes et al., 2008, Brenes et al., 2009). Some microdialysis studies have also reported no differences in basal dopamine levels in the prefrontal cortex of rats housed in enrichment and isolation (Segovia et al., 2008, Del Arco et al., 2007b). The method of neurotransmitter detection and methodological factors again play a role in the consistency of results across laboratories. The absence of a difference in tissue neurotransmitter levels in the present study correspond with the similar patterns of baseline behaviours displayed by rats in each of the three housing conditions.

The absence of any difference in brain-derived neurotrophic factor levels in the present study contrasts with previous findings of elevated levels in discrete brain regions following environmental enrichment compared to singly-housed (Ickes et al., 2000) and standard-housed controls (Angelucci et al., 2009). The duration of

environmental enrichment exposure however in the present study was much less than Ickes and colleagues (2000), who housed male Sprague-Dawley rats in isolation or enriched conditions from 2 to 14 months of age and reported increased brain-derived neurotrophic factor in the basal forebrain, cerebral cortex, hippocampus and hindbrain of enriched rats. Angelucci (2009) maintained male Wistar rats in enriched or standard (pairs) conditions from weaning for a 20-week period, they showed rats in environmental enrichment to have increased brain-derived neurotrophic factor levels in the cerebellum, frontal cortex and hippocampus, but reduced levels in the striatum, compared to pair-housed controls (Angelucci et al., 2009). It is worth noting however the aforementioned studies' enrichment protocols included running wheels and larger cages with a greater number of cagemates than in the present study. This is relevant as voluntary physical exercise can promote increased brain-derived neurotrophic factor levels in the rat brain (Oliff et al., 1998, Johnson and Mitchell, 2003).

Overall the results would suggest that post-weaning enriched or isolation housing had no impact on baseline behaviours, monoamine levels and brain-derived neurotrophic factor concentration in the brain during early adulthood in the present study. In response to common pharmacology tests, the effects of housing on drug responses were dependent on the test and drug employed, with rats housed with environmental enrichment and standard group-housed rats showing a blunted response to the antidepressant drug desipramine compared to the singly-housed group. In response to amphetamine, enriched rats appeared most sensitive to the locomotor-stimulating effects of increasing doses. The results of the present study suggest that modest enrichment, as provided in this study, has no impact on baseline behaviours or monoamine and brain-derived neurotrophic factor levels, thus it is suitable to implement as a commonplace husbandry practice, however caution must be taken when investigating responsiveness to psychotropic drugs. Similarly post-weaning isolation housing may cause alterations in the brain which result in enhanced or blunted responses to drugs. Finally, test methodology (for example the Morris water maze), the nature of the controls employed and moreover, the enrichment conditions provided, may impact considerably on results, particularly during the sensitive post-weaning period.

Chapter 6

Examination of Sex Differences in Baseline and Drug-Induced Behavioural Responses

Chapter 6

Results chapter 4

Examination of sex differences in baseline and drug-induced behavioural responses

6.1 Introduction

In the past female animals have been largely excluded in scientific research, with the exception of sex-specific reproductive behavioural investigations. Recently however increasing evidence documents sex influences on the brain and behaviour, both clinically and preclinically (Lynch, 2006). Due to the fact that the majority of scientific research is carried out using male animals only, sex has not always been regarded as an obvious influencing factor in data interpretation. The employment of males only however could impact negatively on pharmacological research as many CNS diseases such as schizophrenia (Hafner et al., 1994), substance abuse disorders (Lynch et al., 2002), anxiety and depressive disorders (Gorman, 2006, Linzer et al., 1996) show sexually dimorphic patterns of prevalence and/or severity. The exclusion of female rats from research studies is primarily due to the estrous cycle, which is thought to interfere with experimental manipulations by introducing variability. The rat cycle is much shorter than the human cycle, consisting of 4 to 5 days and the phases of the rat estrous cycle are described in detail in the general introduction (Fig. 1.9). Briefly, progesterone and estrogen levels are low during metestrous; in diestrous progesterone increases sharply and then drops in late diestrous on the same day. During proestrous, on day 3, estrogen levels increase followed by increased progesterone secretion. This triggers ovulation on day 4, during which hormones return to baseline levels when ovulation occurs, subsequently there is a brief peak of estrogen on the evening of estrous (Emanuele et al., 2002). Hormonal changes during the ovarian cycle can affect activity levels in the OFT (Tonelli et al., 2008, Frye and Walf, 2002), time spent on the open arms of the EPM (Sadeghipour et al., 2007), anxiety behaviours in the elevated T-maze (Gouveia et al., 2004), conditioned avoidance behaviour (Sfikakis et al., 1978) and even pain sensitivity (Frye et al., 1993). The alternatives proposed to avoid the estrous cycle effects are to

test/administer drugs during diestrous only, employ males only, or use a counterbalanced design to average any variations (Hughes, 2007).

Sex differences in many commonly-used behavioural tests have been reported. Female rats have displayed greater activity levels in the OFT (Duncko et al., 2001, Olivier et al., 2008), EPM (Pena et al., 2006, Pena et al., 2009b, Duchesne et al., 2009) and spend more time swimming in the FST (Kokras et al., 2009) than male rats. Females have also been shown to have greater percent time on the open arms and less time in the closed arms of the EPM compared to males (Weintraub et al., 2010, Johnston and File, 1991a), which is likely due to baseline activity differences rather than differences in anxiety levels between the sexes (Fernandes et al., 1999). Indeed male and female rats' responses to stressors have been described in detail (Weinstock, 2007, Sullivan et al., 2009), however sex differences and the magnitude of stress response is dependent on the nature of the stressor (Palanza, 2001). As a consequence, significant sex differences exist in rats' response to aversive stimuli and conditioning procedures, which impact on rates of learning and memory retention in cognitive tests. Female rats generally outperform males in the classic eye-blink conditioning, fear-potentiated startle and most operant conditioning tasks; whereas males perform better and are more resistant to extinction in classical fear-conditioning and taste aversion programs (Dalla and Shors, 2009).

Sex differences in baseline behaviour and activity levels can impact on study design and subsequent data interpretation. Moreover, sex differences in baseline activity level will likely impact on response to drug treatments. Females display greater sensitivity to psychomotor stimulants than males (Milesi-Halle et al., 2007, Bisagno et al., 2003) and this is thought to be mediated by ovarian hormones and sexually dimorphic DA systems in the brain (Castner et al., 1993). Female rats also display greater alcohol consumption (Juarez and Barrios de Tomasi, 1999), as well as greater self-administration of morphine (Cicero et al., 2003) and nicotine (Donny et al., 2000) than male rats. Similarly, clinical studies addressing sex differences in substance abuse disorders are growing as illicit drug use among women is increasing, according to the American Substance Abuse and Mental Health Services Administration. Moreover, recent evidence suggests that women may be more likely to misuse prescribed drugs to cope with emotional issues than men (Jamison et al., 2010). According to the World Health Organisation, affective disorders such as anxiety spectrum and depressive disorders affect more women than men, and women

are more likely to be prescribed anxiolytics and antidepressants than men (Simoni-Wastila, 1998). Differences between the sexes in response to such drugs, preclinically and clinically, are therefore important considerations in preclinical study design.

Just as sex differences exist in the use of and response to drugs of abuse, sexual dimorphisms can also affect responses to other psychotropic drugs; this is potentially important when employing animal models for screening drug efficacy. Preclinically, the tricyclic antidepressant clomipramine reduced immobility time in the FST for females of the FSL rat strain compared to saline controls, but not for the control SD strain (Kokras et al., 2009); similarly, the selective serotonin reuptake inhibitor (SSRI) fluoxetine had no effect on immobility or swimming behaviours in female albino rats (Lifschytz et al., 2006). The FST and learned helplessness models of depression thus may not be suitable models of depressive symptoms for female rats (Dalla et al., 2005, Dalla et al., 2008b); the CMS model may be more applicable as antidepressants have reversed some of the depressive symptoms of female rats in this model (Willner, 1997). The efficacy of anxiolytic drugs are often assessed using locomotor activity-dependent tests, as outlined previously; females have higher baseline activity levels which could affect interpretation of drug responses. Moreover, in anxiety tests female rats have displayed reduced anxiety-like patterns during the proestrous and estrous phases, compared with metestrous and diestrous phases (Marcondes et al., 2001, Mora et al., 1996). DZP (1 mg kg^{-1}) enhanced the acquisition of conditioned responses during the estrous phase of the cycle, but impaired acquisition in all other cycle phases compared to saline-treated controls (Diaz-Veliz et al., 2000), it may be that the therapeutic effects of benzodiazepines depend on sex and hormone cycle stage.

In light of such sex differences the purpose of the present study was to measure sex differences in baseline behaviours and how these affect responses to psychotropic drugs in behavioural pharmacology tests commonly-employed in drug efficacy screening.

6.2 Methods

6.2.1 Animals

Forty-eight male and female SD rats weighing 100-150g (approximately 6 weeks old on arrival) were singly-housed in standard plastic-bottomed cages (see section 2.2.2). Rats were handled daily for 5 days prior to behavioural testing. A counter-balanced design was used wherein animals were randomly assigned to one of four groups ($n = 6/\text{sex}$ in each group), each group was exposed to each behavioural test, but on different days. The aim of using this counter-balanced design was to avoid any confounding effects of test day and estrous cycle effects on behavioural performance. The OFT and EPM tests were carried out between 08:00 and 12:00, while the FST pre-test and testing were carried out at 13:00-14:00 and the homecage monitoring tests were carried out from 08:00 to approximately 16:00, depending on the number of animals for testing on a given day.

6.2.2 Drugs

See section 2.1 for details and drug sources

DZP (0.625, 1.25 and 2.5 mg kg⁻¹, i.p.) was administered 30 minutes prior to EPM testing. DMI (2.5, 5 and 10 mg kg⁻¹ i.p.) was administered 24, 5 and 1 hour prior to the 5-minute FST. AMP (0.2, 0.5, 1, 2 and 5 mg kg⁻¹ s.c.) and APO (0.75, 1.5 and 3 mg kg⁻¹ s.c.) were administered immediately prior to homecage monitoring.

6.2.3 Open Field Test

See section 2.3.2

6.2.4 Elevated plus maze

See section 2.3.3

6.2.5 Forced Swim Test

See section 2.3.4

6.2.6 Homecage activity monitoring

Homecage activity was monitored over 1 hour. See section 2.3.5 HCMA

6.3 Statistical Analyses

See section 2.8. Unpaired t-tests were carried out to assess sex differences in baseline activity levels.

6.4 Results

6.4.1 Open Field Test

Baseline Behaviour (Figure 6.1)

There was a significant sex difference in baseline activity in the OFT as females were significantly more active with greater distance moved [$t(10) = 2.32, p < 0.05$] and velocity [$t(10) = 2.52, p < 0.05$] compared to males. There were no significant differences between males and females in baseline entries to the inner arena [$t(10) = 1.55, p > 0.05$] or time spent in the inner arena [$t(10) = 1.40, p > 0.05$] of the OFT.

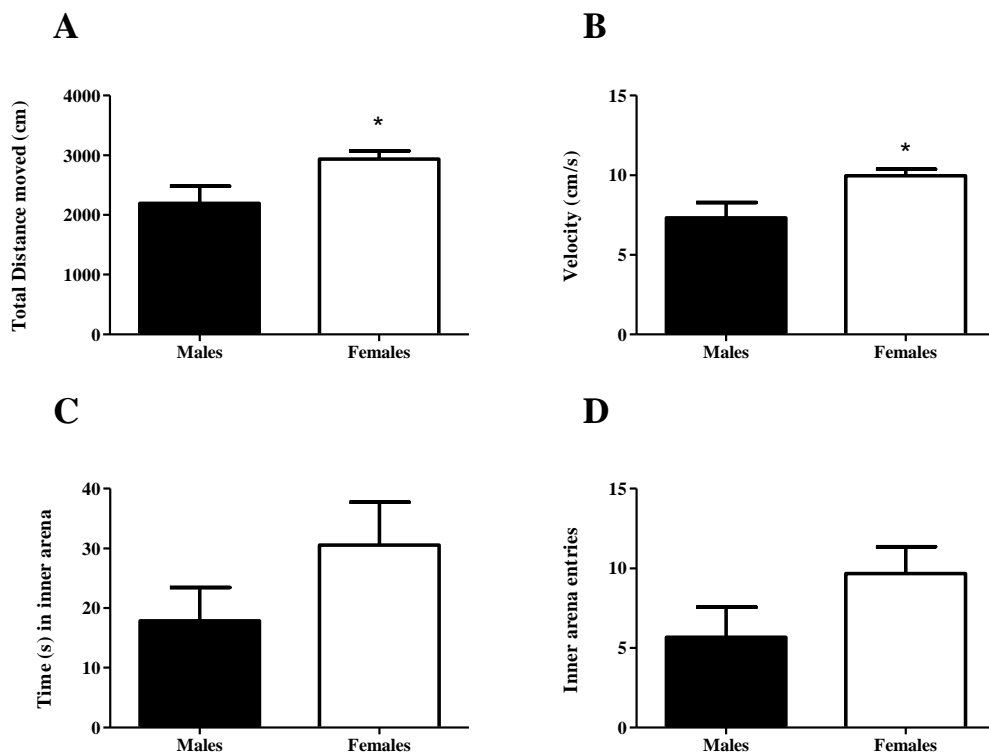


Figure 6.1: Sex effects in the open field test. Distance moved (A), velocity (B), time spent in the inner arena (C) and entries to the inner arena (D) made by male and female rats in the OFT. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$ vs. males.

Diazepam Effects (Figure 6.2)

Analysis of distance moved in the OFT revealed a significant sex effect following DZP administration [$F(1, 40) = 8.55, p < 0.01$], however there were no drug [$F(3, 40) = 2.05, p > 0.05$] or drug \times sex interaction [$F(3, 40) = 0.87, p > 0.05$] effects. There were no effects on inner arena entries (sex: [$F(1, 40) = 2.74, p > 0.05$], drug: [$F(3, 40) = 2.22, p > 0.05$], interaction: [$F(3, 40) = 0.47, p > 0.05$]) or time spent in the inner arena (sex: [$F(1, 40) = 1.13, p > 0.05$], drug: [$F(3, 40) = 2.83, p > 0.05$], interaction: [$F(3, 40) = 1.29, p > 0.05$]).

One-way ANOVAs for distance moved in the OFT showed there was no effect of DZP for male [$F(3, 20) = 0.58, p > 0.05$] or female rats [$F(3, 20) = 2.84, p = 0.06$]. For male rats there was no drug effect in inner arena entries [$F(3, 20) = 0.55, p > 0.05$] or inner arena time [$F(3, 20) = 0.64, p > 0.05$]. Females similarly displayed no drug effect in inner arena entries [$F(3, 20) = 2.53, p > 0.05$], however a significant drug effect was observed in females' time spent in the inner arena [$F(3, 20) = 3.47, p < 0.05$]. *Post-hoc* tests revealed that time spent in the inner arena was significantly reduced in the female group treated with 2.5 mg kg⁻¹ DZP compared to saline controls ($p < 0.05$).

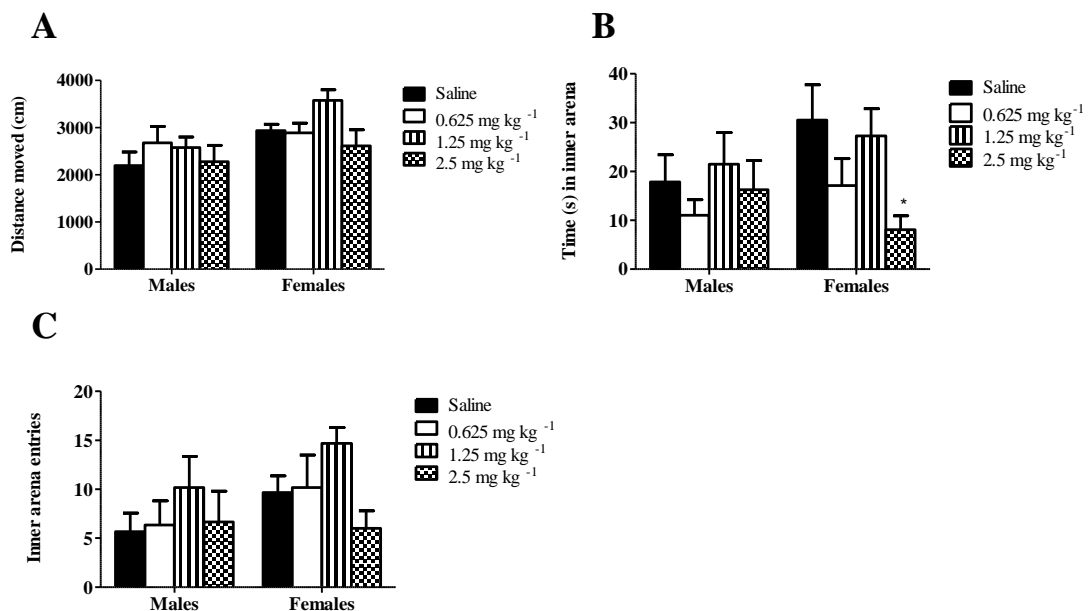


Figure 6.2: Diazepam and sex effects in the open field test. The effects of DZP (0.625, 1.25, 2.5 mg kg⁻¹, i.p.) on distance moved (A), time spent in the inner arena (B) and entries to the inner arena (C) by male and female rats in the OFT. Data expressed as mean + SEM ($n = 6$ per group). * $p < 0.05$ vs. the female saline control group.

6.4.2 Elevated Plus Maze

Baseline Behaviour (Figure 6.3)

Saline-treated females moved faster [$t(10) = 3.46, p < 0.01$] and made greater percent open arm entries than males in the EPM [$t(10) = 2.17, p < 0.05$]. There were no sex differences however in distance moved on the EPM [$t(10) = 1.31, p > 0.05$] or in percent time spent on the open arms [$t(10) = 1.36, p > 0.05$].

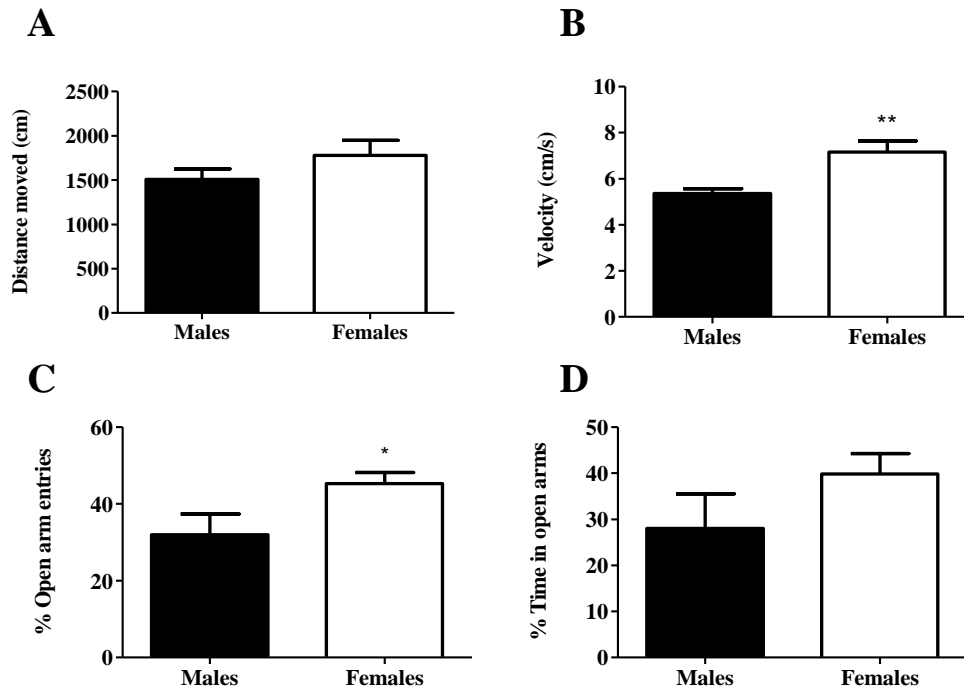


Figure 6.3: Sex effects in the elevated plus maze. Distance moved (A), velocity (B), percent open arm entries (C) and percent open arm time (D) by male and female rats in the EPM. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$, ** $p < 0.01$ vs. males.

Diazepam Effects (Figure 6.4)

Analysis of percent open arm entries revealed there were no significant overall sex [$F(1, 40) = 2.17, p > 0.05$] or drug [$F(3, 40) = 2.17, p > 0.05$] effects, nor was there a significant interaction effect [$F(3, 40) = 0.74, p > 0.05$] in the EPM. Similarly for percent open arm time in the EPM there were no significant overall sex [$F(1, 40) = 0.02, p > 0.05$], drug [$F(3, 40) = 2.04, p > 0.05$] or interaction [$F(3, 40) = 0.88, p > 0.05$] effects for male and female rats.

For male rats, one-way ANOVA of percent open arm time demonstrated a significant effect of DZP [$F(3, 20) = 3.93, p < 0.05$], but no drug effect was seen for percent open arm entries [$F(3, 20) = 1.65, p > 0.05$]. *Post-hoc* tests showed that the 1.25 mg kg^{-1} DZP dose significantly increased percent open arm time for male rats compared to saline controls. For females however percent open time [$F(3, 20) = 0.18, p > 0.05$] or entries [$F(3, 20) = 0.10, p > 0.05$] were not significantly altered by DZP.

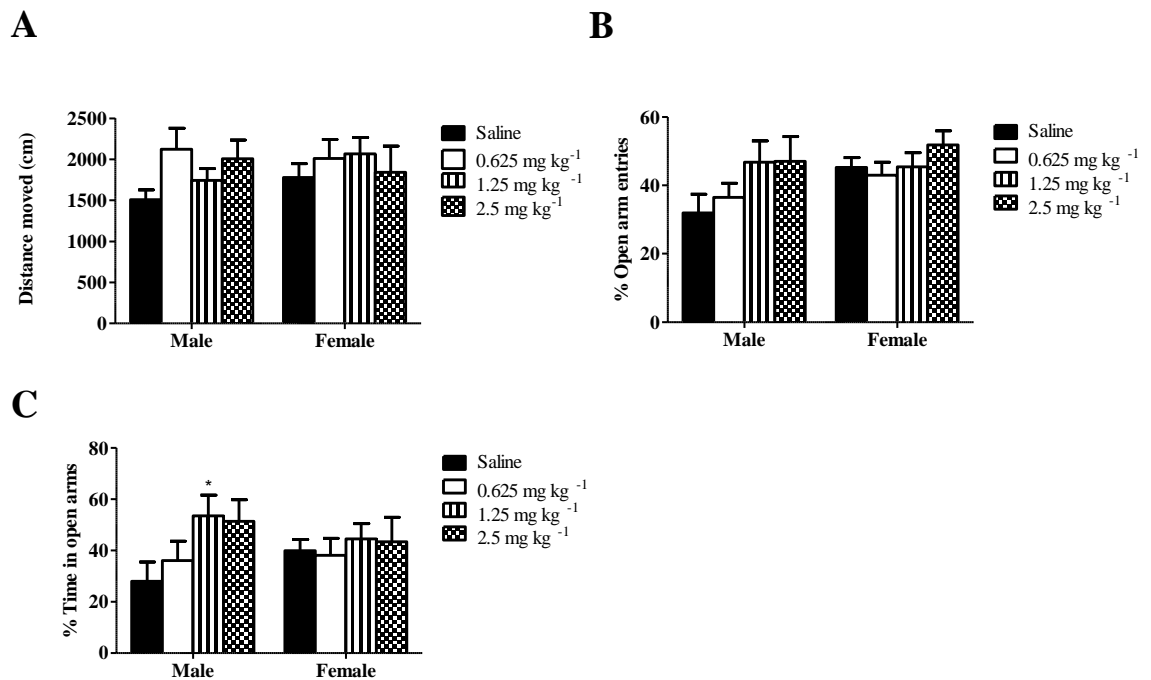


Figure 6.4: Diazepam and sex effects in the elevated plus maze. The effects of DZP (0.625, 1.25, 2.5 mg kg⁻¹, i.p.) on distance moved (A) percent open arm entries (B) and percent time spent in the open arms (C) of the elevated plus maze by male and female rats. Data expressed as mean + SEM ($n = 6$ per group) * $p < 0.05$ vs. the male saline control group.

6.4.3 Forced Swim Test

Baseline Behaviour (Figure 6.5)

Female rats spent significantly less time immobile [$t(10) = 3.25, p < 0.01$] and spent more time swimming [$t(10) = 3.42, p < 0.01$] in the FST compared to males.

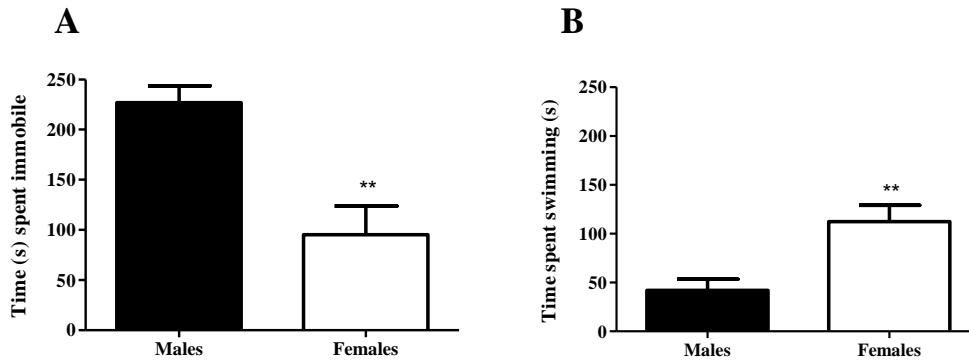


Figure 6.5: Sex effects in the forced swim test. Time spent immobile (A) and swimming (B) by male and female rats in the FST. Data expressed as mean + SEM ($n = 6$ per group), ** $p < 0.01$ vs. male rats.

Desipramine effects (Figure 6.6)

For time spent immobile in the FST there was a significant sex effect [$F(1, 40) = 11.70, p < 0.01$] but no overall main drug effect [$F(3, 40) = 1.46, p > 0.05$], or interaction [$F(3, 40) = 0.76, p > 0.05$] effect for male and female rats after DMI administration.

One-way ANOVAs for immobility time showed a significant drug effect for male rats [$F(3, 20) = 3.07, p < 0.05$] but not for females [$F(3, 20) = 0.12, p > 0.05$]. For male rats, 5 mg kg⁻¹ DMI significantly reduced immobility time compared to saline controls; however DMI had no effect on immobility time for female rats.

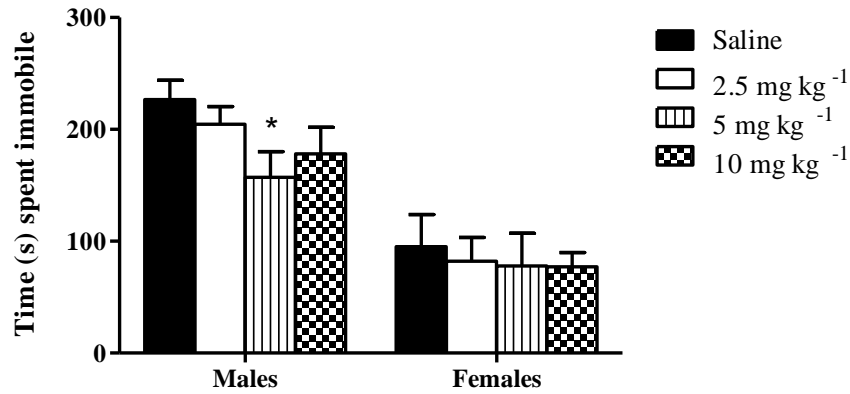


Figure 6.6: Desipramine and sex effects in the forced swim test. The effects of DMI (2.5, 5 and 10 mg kg⁻¹, i.p.) on time spent immobile in the FST by male and female rats. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$ vs. the male saline control group.

6.4.4 Homecage Monitoring

Baseline Activity (Figure 6.7)

Female rats displayed significantly greater distance moved [$t(6) = 3.31$, $p < 0.05$] and movement velocity [$t(6) = 3.31$, $p < 0.05$] in the homecage during the hour-long monitoring period compared to males.

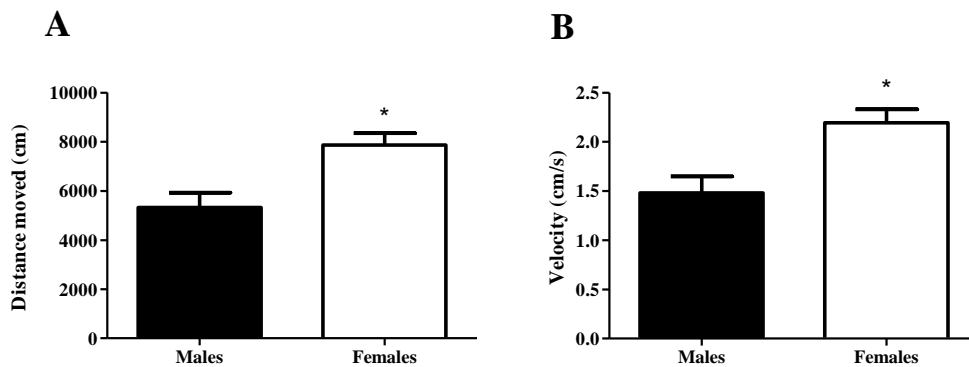


Figure 6.7: Sex effects on activity in the homecage. Distance moved (A) and velocity (B) by male and female rats in the homecage over 1-hour. Data expressed as mean + SEM ($n = 4$ per group), * $p < 0.05$ vs. male rats.

Amphetamine effects (Figures 6.8, 6.9 and 6.10)

Analysis of total distance moved in the homecage revealed were significant drug [$F(5, 36) = 11.35, p < 0.001$] and sex [$F(1, 36) = 50.54, p < 0.001$] effects, but no significant interaction [$F(5, 36) = 1.89, p > 0.05$]. One-way ANOVAs for distance moved revealed significant drug effects for male [$F(5, 18) = 5.57, p < 0.01$] and female rats [$F(5, 18) = 7.04, p < 0.001$]. Both males and females displayed significantly increased locomotor activity following 0.5, 1 and 2 mg kg⁻¹ AMP compared to saline controls.

To eliminate the effect of baseline sex differences in activity levels the distance moved was represented as a percent of saline baseline controls. There was a significant drug effect [$F(5, 36) = 10.65, p < 0.001$], but no sex [$F(1, 36) = 2.12, p > 0.05$] or drug x sex interaction effects [$F(5, 36) = 1.29, p > 0.05$]. When one-way ANOVAs were applied there were significant drug effects for males [$F(5, 18) = 5.18, p < 0.01$] and females [$F(5, 18) = 6.72, p < 0.01$]. *Post-hoc* tests showed that for males the 1 and 2 mg kg⁻¹ AMP doses increased distance moved compared to saline controls ($p < 0.001$), while the 0.5, 1 and 2 mg kg⁻¹ doses significantly increased activity for females ($p < 0.05, p < 0.001$).

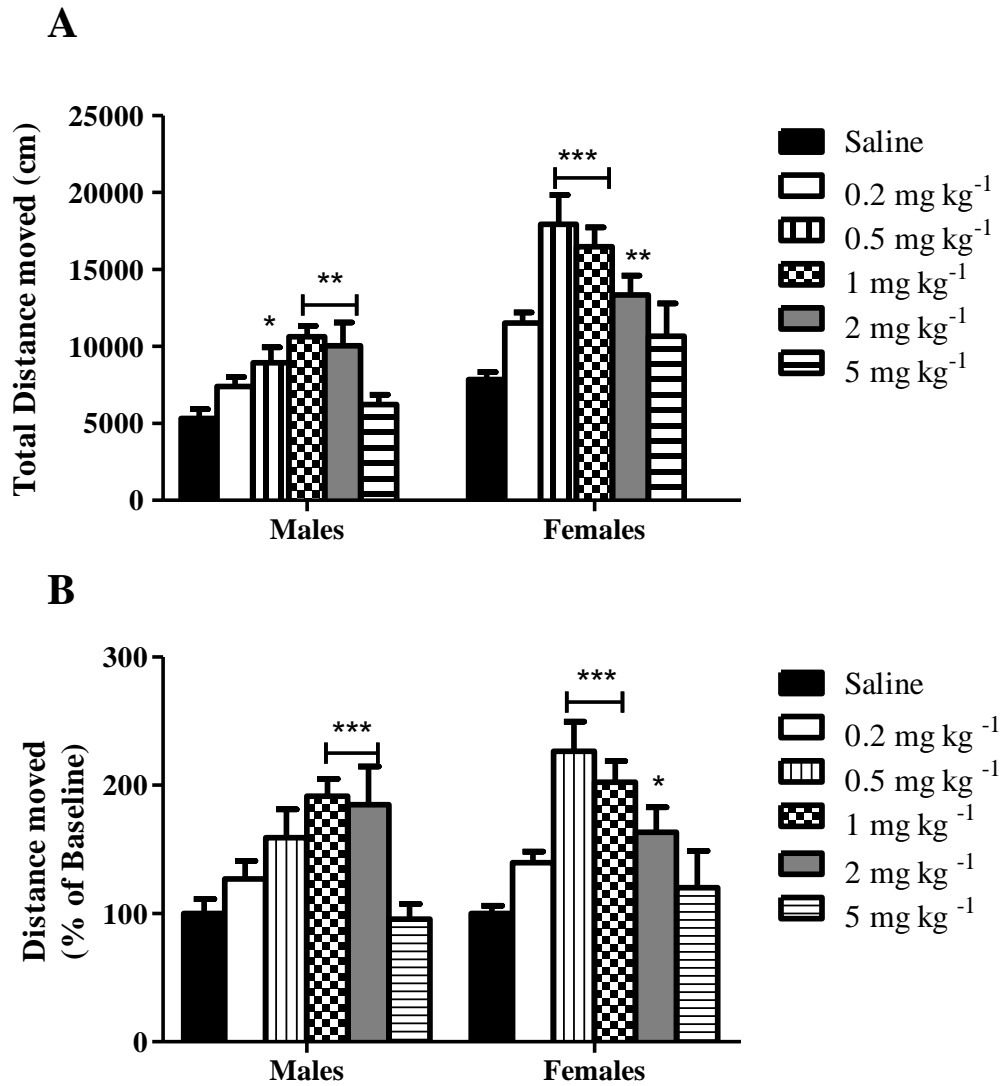


Figure 6.8: Amphetamine and sex effects on distance moved in the homecage. The effects of AMP (0.2, 0.5, 1, 2, 5 mg kg⁻¹, s.c.) on total distance moved (A) and distance moved expressed as a percent of baseline activity (B), in the homecage over 1 hour by male and female rats. Data expressed as mean + SEM ($n = 4$ per group), ** $p < 0.01$, * $p < 0.05$, *** $p < 0.001$ vs. same-sex saline control.

The distance moved (relative to baseline activity) by male and female rats in the homecage was analysed over 10-minute timebins. Male rats showed significant time [$F(5, 90) = 36.03$, $p < 0.001$], drug [$F(5, 18) = 5.57$, $p < 0.01$] and drug x time interaction effects [$F(25, 90) = 2.21$, $p < 0.001$]. One-way ANOVAs (Fig. 6.9) showed that AMP effects emerged after the first 20 minutes, the 1, 2 and (toward the end of the 60 minutes) 0.5 mg kg⁻¹ AMP doses significantly increased male rats' activity.

Repeated measures analyses for female activity in response to AMP over time also yielded significant time [$F(5, 90) = 22.78, p < 0.001$], drug [$F(5, 18) = 7.04, p < 0.01$] and drug \times time interaction effects [$F(25, 90) = 2.31, p < 0.01$]. One-way ANOVAs revealed significant AMP effects 10 minutes after drug administration. The 0.5, 1 and (to a lesser extent) 2 mg kg⁻¹ doses significantly increased activity for females. The drug effects are still potent in both males and females at the end of the 60-minute monitoring period.

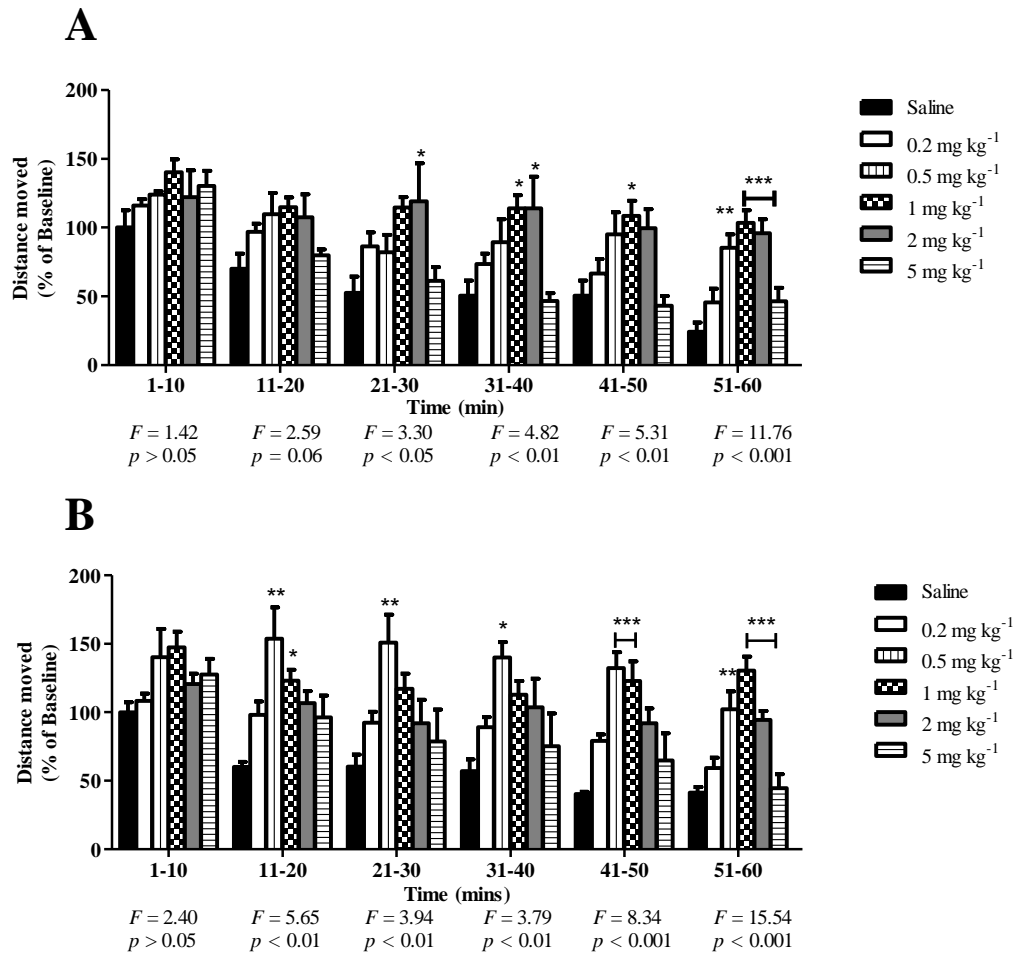


Figure 6.9: Amphetamine and sex effects on distance moved in the homecage over 10-minute timebins. The distance moved (as a percent of baseline activity) during 10-minute timebins by (A) males and (B) females in the homecage following saline or AMP (0.2, 0.5, 1, 2, 5 mg kg⁻¹). Data expressed as mean + SEM ($n = 4$ per group), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. saline controls, according to one-way ANOVA ($df = 5, 18$).

Sniffing behaviour was not significantly different between male and female saline controls [$t(6) = 2.15, p = 0.08$]. Stereotyped sniffing was manually recorded over one hour, there were significant drug [$F(5, 32) = 16.31, p < 0.001$], sex [$F(1, 32) = 15.11, p < 0.001$] and sex x drug interaction effects [$F(5, 32) = 3.56, p < 0.05$]. One-way ANOVAs revealed significant AMP effects for males [$F(5, 16) = 6.10, p < 0.01$], but not for females [$F(5, 16) = 2.88, p > 0.05$]. AMP (1, 2 and 5 mg kg⁻¹) significantly increased time spent engaged in sniffing behaviour for male rats compared to saline controls.

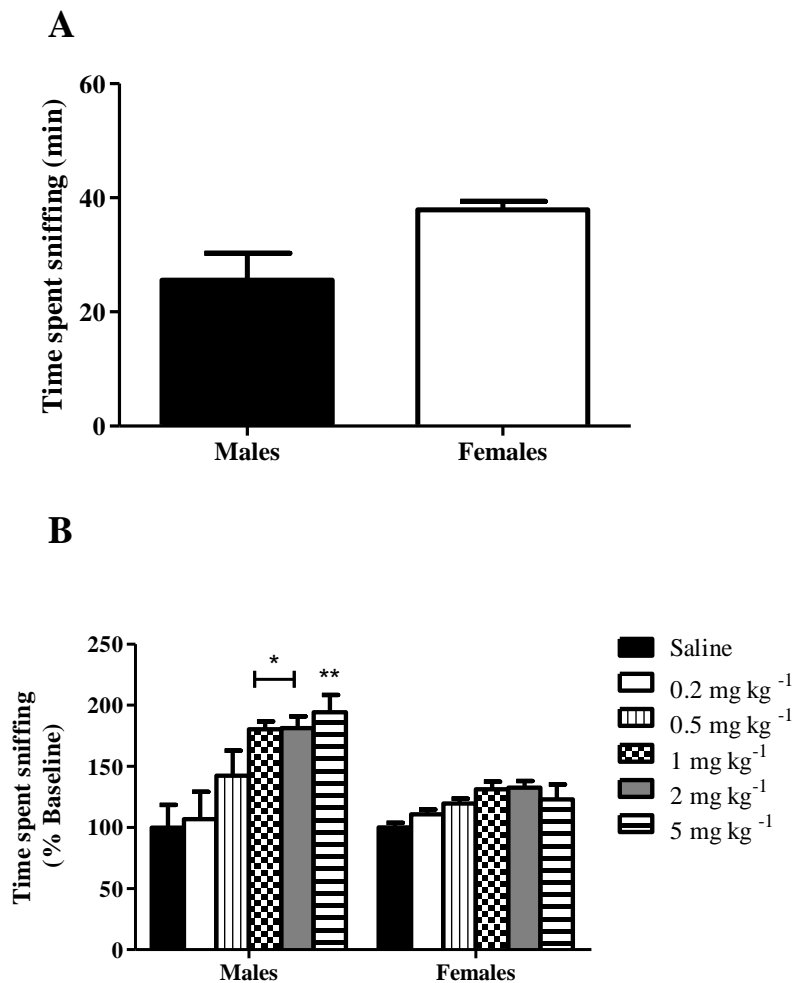


Figure 6.10: Amphetamine and sex effects on sniffing behaviour in the homecage. Baseline time spent sniffing (A) and AMP effects on time spent sniffing relative to baseline levels (B) in the homecage following saline or AMP (0.2, 0.5, 1, 2, 5 mg kg⁻¹). Data expressed as mean + SEM ($n = 4$ per group), * $p < 0.05$, ** $p < 0.01$ vs. male saline controls

Apomorphine effects on locomotor activity**Baseline activity (Figure 6.11)**

Females were significantly more active in the homecage with greater distance moved [$t(10) = 2.79, p < 0.05$] and greater velocity [$t(10) = 2.76, p < 0.05$] than male rats.

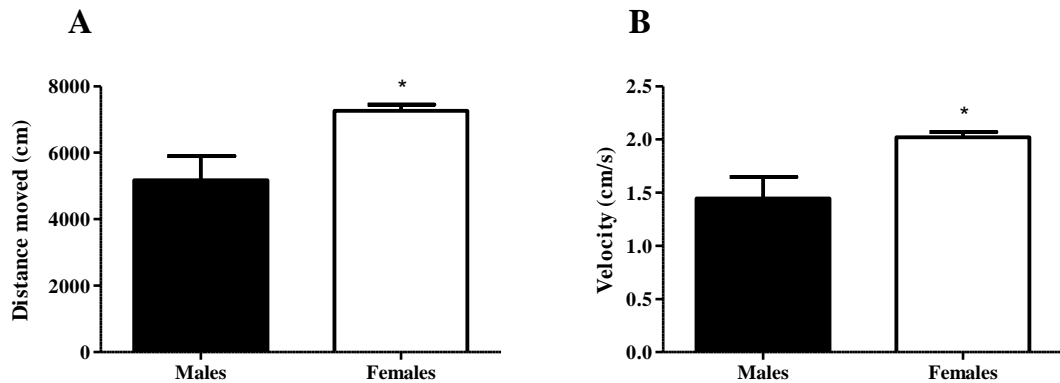


Figure 6.11: Sex effects on activity in the homecage. Distance moved (A) and velocity (B) by male and female rats in the homecage over 1-hour. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$ vs. males.

Apomorphine effects (Figures 6.12 and 6.13)

Analysis of overall distance moved across one hour in the homecage following APO or saline administration revealed significant sex [$F(1, 40) = 8.81, p < 0.01$] and drug [$F(3, 40) = 10.24, p < 0.001$], but no significant interaction [$F(3, 40) = 1.08, p > 0.05$] effects. One-way ANOVAs revealed significant dose effects for both male [$F(3, 20) = 4.55, p < 0.05$] and female rats [$F(3, 20) = 6.42, p < 0.01$]. *Post-hoc* tests showed 0.75 mg kg⁻¹ and 3 mg kg⁻¹ doses of APO significantly reduced distance moved compared to saline controls for males ($p < 0.05$). All three doses of APO employed significantly reduced females' activity compared to saline controls ($p < 0.01$).

To eliminate the sex difference in baseline activity the distance moved was represented as a percent of saline baseline controls. There was a significant drug effect [$F(3, 40) = 9.58, p < 0.001$], but no significant sex [$F(1, 40) = 1.70, p > 0.05$] or drug x sex interaction [$F(3, 40) = 0.71, p > 0.05$] effects. As with overall distance moved, there were significant dose effects for both male [$F(3, 20) = 4.38, p < 0.05$] and female rats [$F(3, 20) = 5.92, p < 0.01$]. *Post-hoc* tests showed 0.75 mg kg⁻¹ and

3 mg kg⁻¹ doses of APO significantly reduced distance moved compared to saline controls ($p < 0.05$) for male rats and all three doses of APO employed significantly reduced females' activity compared to saline controls ($p < 0.01$).

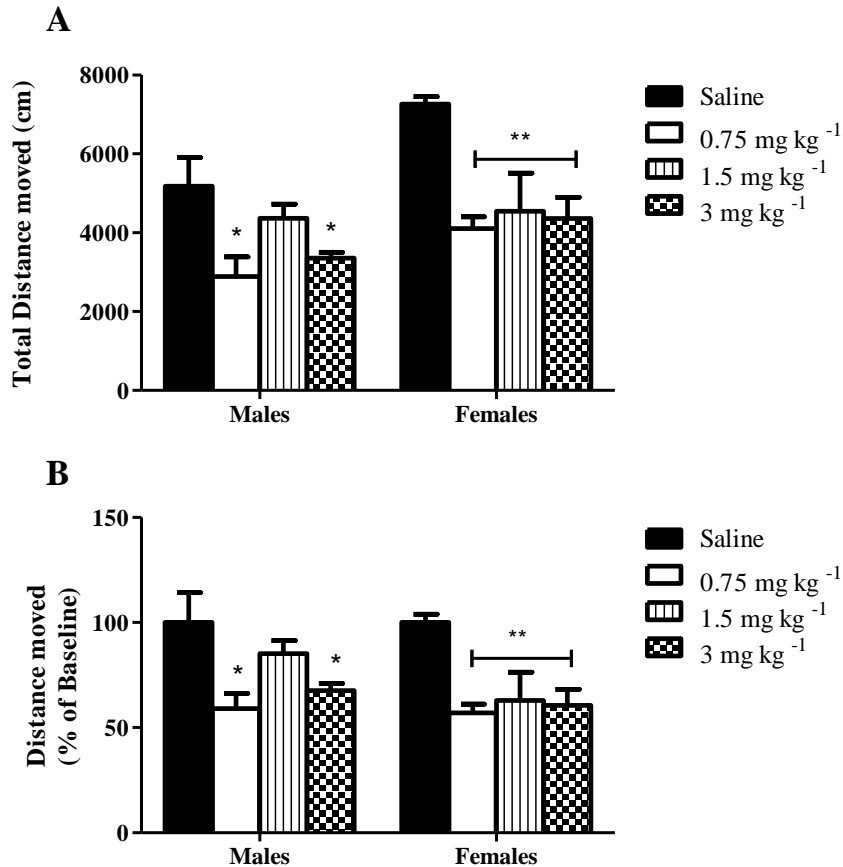


Figure 6.12: Apomorphine and sex effects on distance moved in the homecage. The effects of APO (0.75, 1.5, 3 mg kg⁻¹, s.c.) on overall distance moved (A) and distance moved relative to baseline controls (B) in the homecage over 1 hour by male and female rats. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$, ** $p < 0.01$ vs. same sex saline controls group.

When the distance moved (as percent baseline) was expressed over 10-minute timebins, there were significant main effects of time [$F(5, 100) = 2.56$, $p < 0.05$] and drug [$F(3, 100) = 4.38$, $p < 0.05$] and a significant interaction [$F(15, 100) = 3.19$, $p < 0.001$] for males. For females there were significant drug [$F(3, 100) = 5.92$, $p < 0.01$] and time x dose interaction effects [$F(15, 100) = 5.48$, $p < 0.001$], but no significant main effect of time [$F(5, 100) = 2.23$, $p > 0.05$]. As presented in figure 6.13, the 0.75 and 3 mg kg⁻¹ doses significantly reduced activity for male rats, while

the 0.75, 1.5 and 3 mg kg⁻¹ doses significantly reduced activity compared to saline controls for females. Drug effects on locomotor activity were most evident during the first 30 minutes of monitoring.

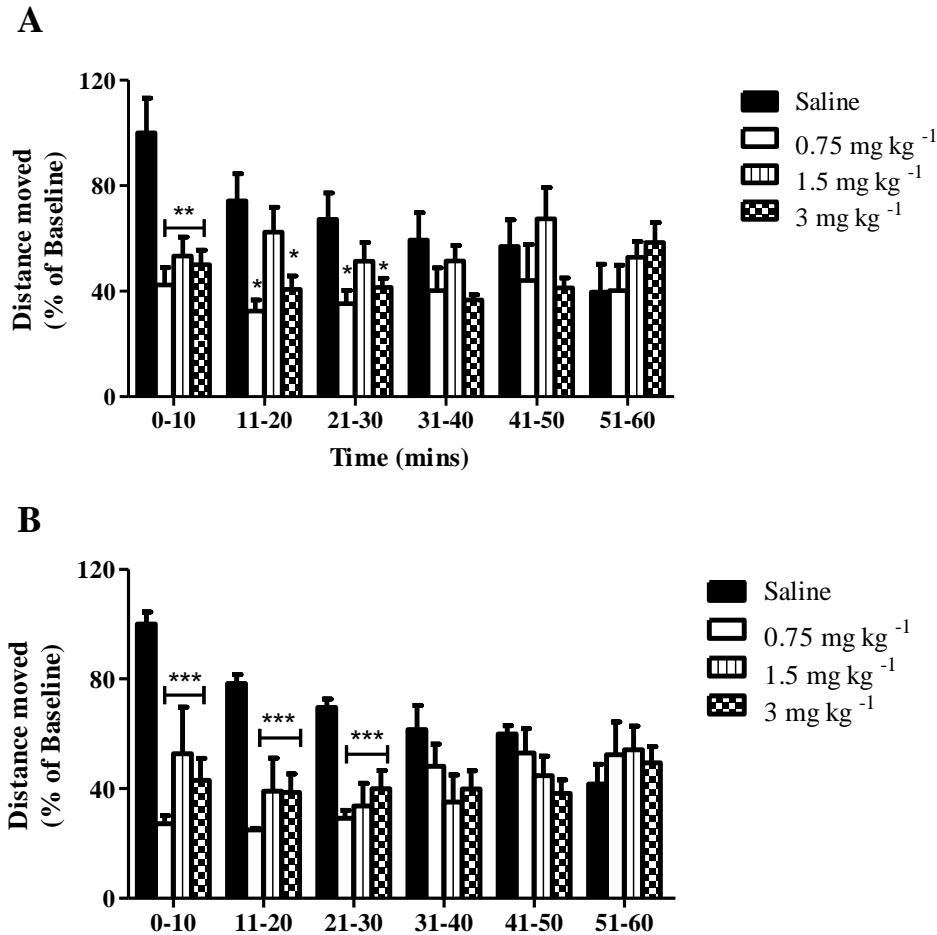


Figure 6.13: Apomorphine and sex effects on distance moved in the homecage over 10-minute timebins. The distance moved, relative baseline activity during 10-minute timebins by (A) male and (B) female rats in the homecage following saline or APO (0.75, 1.5, 3.0 mg kg⁻¹, s.c.). Data expressed as mean + SEM, $n = 6$ per group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. saline control.

6.5 Discussion

Overall the sex differences observed in the present study were primarily in baseline behaviour levels and these in turn influenced responses to psychotropic drugs. Saline-treated females were more active with greater distance moved and velocity in the open field test and in homecage activity tracking than their male counterparts. In addition, female rats displayed greater baseline velocity of movement and a higher number of open arm entries in the elevated plus maze and spent less time immobile in the forced swim test than males.

There was no effect of diazepam on distance moved in the open field test, demonstrating that the diazepam doses employed had no sedative effects on activity for males or females. In the elevated plus maze 1.25 mg kg^{-1} diazepam increased open arm entries and open arm time compared to saline controls but only for male rats. Diazepam had no effect on anxiolytic parameters for females; this may be attributed to the high baseline levels of open arm exploration displayed by females. Few studies have compared male and female responses to diazepam using the elevated plus maze. In a defensive burying test, where burying behaviour denotes anxiety levels and burying behaviour latency is inversely related to anxiety-like state, diazepam (1 mg kg^{-1} , i.p.) significantly reduced burying behaviour and increased burying latency in males and non-hormonal-cycling females compared to saline-treated controls but the drug had no significant effect on burying or burying latency in normally-cycling females (Fernandez-Guasti and Picazo, 1997). The anxiolytic effects of diazepam may depend on hormonal status. When the effects of diazepam on acquisition of conditioned avoidance responding were assessed it was found that the drug produced opposite effects on conditioned avoidance responding according to hormonal stage; 0.25 and 1 mg kg^{-1} diazepam (i.p., 30 minutes prior to testing) enhanced acquisition learning in rats at estrous and in ovariectomised rats treated with estradiol, however the 1 mg kg^{-1} dose impaired acquisition for males, females in diestrous, oil-treated ovariectomised females and ovariectomised females treated with progesterone. The authors suggest that lower conditioned avoidance responding and increased error rates seen in estrous and ovariectomised females treated with estradiol reflect high anxiety within these groups, and the improvements following diazepam may be due to its anxiolytic effects (Diaz-Veliz et al., 2000). Binding assays have shown that ovarian hormones can modulate gamma-aminobutyric acid

neurotransmission by increasing gamma-aminobutyric acid receptors (Maggi and Perez, 1984, Perez et al., 1986, Maggi and Perez, 1986), which may be responsible for the sexual dimorphisms in response to benzodiazepine drugs such as diazepam. Previous studies would suggest that females' response to the anxiolytic effects of diazepam may also be affected by the test employed. Sex differences in patterns of anxiety-like behaviours have been demonstrated as males display more fear-related behaviours such as freezing and locomotor inhibition while females display more active (Palanza, 2001) and defensive-like behaviours in response to a real threat stimulus such as exposure to a predator (Blanchard et al., 1990). The sex disparity in anxiety response has been analysed using factor analysis in the elevated plus maze. The contribution of each behavioural parameter to each factor is referred to as factor loading, the higher the loading, the greater the behaviour reflects a factor. The strongest factor for males was open-arm activity and this was interpreted to reflect anxiety, whereas the strongest factor for females in the elevated plus maze was activity (Fernandes et al., 1999). This suggests that male rats are driven by anxiety on the elevated plus maze but this test is more sensitive to activity levels than anxiety for females. The absence of diazepam effect in the present study may thus be reflective of the test used, rather than drug inefficacy. Activity in the elevated plus maze is also modified by the estrous cycle, proestrous and estrous females showed increased percent time in open arms compared to those in diestrous (who do not differ to males) (Mora et al., 1996, Marcondes et al., 2001). In the present study cycle phase was not verified, however females were randomly assigned to groups, one group were exposed to the elevated plus maze during the first week of testing, another group the following week and so on, with the aim of eliminating any cycle effects on behaviour.

In the forced swim test, females showed greater activity (swimming) at baseline and reduced immobility compared to males; this is in agreement with previous studies (Barros and Ferigolo, 1998, Brotto et al., 2000). In addition, previous research suggests that reduced immobility duration shown by females does not appear to vary across estrous cycle phases (Barros and Ferigolo, 1998). The low baseline immobility shown by females poses difficulties when trying to assess the anti-immobility effects of antidepressant drugs. Varying doses of the tricyclic antidepressant imipramine (5, 10 and 15 mg kg⁻¹) had no effect on immobility

duration for female Wistar rats but did reduce immobility frequency, and this was evident in each phase of the estrous cycle (Barros and Ferigolo, 1998). On the other hand, Marvan and colleagues (1996) found clomipramine to reduce females' immobility time in the diestrous phase only (Marvan et al., 1996). In addition, the serotonin reuptake inhibitor antidepressant drug fluoxetine (10 mg kg^{-1} for 7 days) decreased immobility and increased time spent swimming for male rats in the forced swim test but had no effect on females (Lifschytz et al., 2006). It has been proposed that female rats are less susceptible to learned helplessness models of depression than males, moreover, this disparity remains even in ovariectomised females (Dalla et al., 2008b). This is a relevant consideration when designing experiments for testing the efficacy of antidepressant compounds, and as women are twice as likely as men to experience depression (Nolen-Hoeksema, 2001) testing drug efficacy in both male and female preclinical models may be pertinent to potential therapeutic approaches.

There was a significant effect of amphetamine on distance moved for both males and females in the homecage monitoring apparatus. Females reached peak activity at 0.5 mg kg^{-1} whereas males required $1\text{-}2 \text{ mg kg}^{-1}$ amphetamine to reach maximum distance moved. The enhanced behavioural effects of low dose amphetamine in females compared to males was reported in early studies by Beatty and colleagues; they showed that females exhibited a more intense locomotor response to 1 mg kg^{-1} amphetamine (Savageau and Beatty, 1981) and 1.5 mg kg^{-1} (Beatty and Holzer, 1978) than males. Sex differences in response to psychostimulant and other drugs of abuse have been well documented in clinical (Lynch, 2006, Brady and Randall, 1999) and preclinical (Becker et al., 2001, Roth et al., 2004, Becker and Hu, 2008) research. Following both acute and chronic amphetamine treatment, female rats displayed greater activity (distance moved) (Bisagno et al., 2003) and greater drug-induced rotations than males (Becker et al., 1982). Females' enhanced rotational behaviour was present even when whole brain and striatal analyses demonstrated equivalent amphetamine concentration in males and females (Becker et al., 1982). The enhanced response shown by females is mediated by ovarian hormones, primarily estrogen. To assess the effects of sex hormones on amphetamine-induced behaviours male and female rats underwent castration or ovariectomy surgery. Ovariectomy attenuated amphetamine -induced

rotations compared to intact females, whereas castration had no effect on male rotations (Camp et al., 1986). In addition, estradiol treatment for gonadectomised rats increased females' amphetamine-induced rotations and head and forelimb movements compared to non-hormone treated females, while no effect was seen for males (Castner et al., 1993). Further evidence of ovarian hormone impact on amphetamine-induced activity is demonstrated during the estrous cycle; females in estrous showed greater amphetamine-mediated conditioned avoidance (Diaz-Veliz et al., 1994), locomotor activity (Becker et al., 2001) and rotational behaviour (Becker et al., 1982) than males and females in diestrous. Such studies indicate that ovarian hormones mediate amphetamine effects on behaviour, and it has been recognised that this is achieved by enhancing dopamine neurotransmission in the female striatum. The locomotor-reducing effects of the dopamine agonist apomorphine were moderately greater for female rats than males as measured by distance moved in the homecage. Previously, female rats have exhibited more stereotyped behaviours (including licking, sniffing and gnawing) than males in response to apomorphine ($0.1\text{--}1\text{ mg kg}^{-1}$). In the shuttlebox, apomorphine reduced locomotor activity for both males and female rats. Ovariectomy surgery enhanced drug effects on activity compared to castrated males (Savageau and Beatty, 1981). The present results are in some agreement with the aforementioned amphetamine studies wherein female rats have greater sensitivity to dopamine-stimulating drugs due to the effects of the ovarian hormones on dopaminergic neurotransmission.

Females' enhanced dopamine release in response to dopaminergic drugs compared to males is thought to be due to sexual dimorphisms in the organisation of the striatum, as amphetamine has sex- and region-specific effects on striatal neurons. (Castner and Becker, 1996). *In vivo* voltammetry results suggest that sex differences in striatal dopamine response to dopaminergic drugs are governed by more tightly regulated dopamine transporter or dopamine autoreceptor function in females than in males (Walker et al., 2006). Females also have a higher density of dopamine transporter mRNA in the striatum than males (Bosse et al., 1997). Jill Becker (1999) proposed that estrogen can act directly on striatal dopamine terminals to down-regulate autoreceptors and thus increase stimulated dopamine release. She also hypothesised that, via direct action on the striatum, estrogen inhibits calcium currents in gamma-aminobutyric acid spiny striatal neurons, thereby reducing

gamma-aminobutyric acid release and decreased response to gamma-aminobutyric acid at dopamine terminals, resulting in increased stimulated dopamine release (Becker, 1999). *In vivo* microdialysis showed sexual dimorphisms also exist in basal striatal dopamine concentration and that basal dopamine levels fluctuate during the estrous cycle; females in proestrous and estrous had significantly higher extracellular striatal dopamine compared to those in diestrous or after ovariectomy surgery, however castration of males had no effect on extracellular striatal dopamine levels (Xiao and Becker, 1994). Thus, ovarian hormones, but not testicular hormones, can directly modulate dopamine levels in the striatum of rats mediating the sexual dimorphisms in behaviour and neurochemistry seen following amphetamine administration. Finally, amphetamine is metabolised more quickly in male rats than in females (Yoshida et al., 1987, Milesi-Halle et al., 2005), thus, even when administered the same dose, amphetamine levels in the male brain are lower than in the female brain (Becker et al., 1982). This also contributes to the dose-response shift seen in the present study as females show a greater amphetamine-induced hyperactivity than males with the 1 mg kg^{-1} dose. The sex difference in sensitivity to psychomotor stimulants is thought to explain females' more rapid progression from initial drug use to dependence (Lynch et al., 2002, Frye, 2007). The mechanisms mediating amphetamine effects in rats therefore appear to be sexually dimorphic and evidence suggests that this is due to pharmacokinetic drug properties (Milesi-Halle et al., 2005) and the enhancing effects of ovarian hormones on striatal gamma-aminobutyric acid and dopamine neurons (Becker, 1999). Sex differences in drug metabolism in the human liver have been well documented in clinical studies (Waxman and Holloway, 2009), similarly, female rats display a slower rate of drug clearance than males and this is attributed to sexual dimorphic cytochrome p450 enzymes (Mugford and Kedderis, 1998). Female rats displayed reduced renal and non-renal clearance of methamphetamine and its metabolite amphetamine; female rats also had higher levels of excretion of unchanged methamphetamine and amphetamine at 1 mg kg^{-1} dose but lower levels at 3 mg kg^{-1} compared to males. Interestingly, the female rats' pharmacokinetic profile was more similar to that of humans compared to male rats, providing a potentially accurate model for studies of methamphetamine disposition in humans (Milesi-Halle et al., 2005).

To conclude, female rats display greater baseline activity and reduced responsiveness to the effects of the anxiolytic drug diazepam and the tricyclic antidepressant drug desipramine in the elevated plus maze and forced swim test respectively, compared to male rats. In contrast, females exhibited a greater sensitivity to the locomotor-stimulating effects of 1 mg kg^{-1} amphetamine than males. Sex differences in response to psychotropic drugs, particularly to stimulant drugs, are due to pharmacokinetic and pharmacodynamics factors, mediated by estrogen and (to a lesser extent) progesterone. Consideration should also be given to the behavioural tests used to assess drug efficacy, especially to their ability to assess behavioural indices of equivalent affective states in males and females.

Chapter 7

Examination of Strain Differences in Baseline and
Drug-Induced Behavioural Responses and Brain
Monoamine Concentrations in the Male Rat

Chapter 7

Results chapter 5

Examination of strain differences in baseline and drug-induced behavioural responses and brain monoamine concentrations in the male rat

7.1 Introduction

During the last century more than 500 rat strains have been developed for biomedical research and education (Voigt, 2010). The availability of numerous rat strains is advantageous for modelling various physical diseases such as cardiovascular disease, diabetes and tumours. Rat strains have also been developed to model symptoms of CNS conditions such as depression/anxiety, cognitive decline and drug abuse vulnerability. Strain differences have been identified and widely published; however, for the most part this research addresses differences between strains used to model particular diseases and their control strain. For example, the WKY rat strain display depressive-like symptoms including hypolocomotion, symptoms of behavioural despair and negative memory bias (Pare and Redei, 1993a, Pare, 1996, Rittenhouse et al., 2002a, O'Mahony et al., 2011) compared to the SD rat strain. The SHR is often used to model ADHD symptoms when compared to the normotensive WKY control strain. However, when compared to other strains such as Wistar and SD, SHR do not display hyperactivity (Ferguson and Cada, 2003, Hard et al., 1985). This illustrates the importance of employing a suitable rat strain as a comparable control. Furthermore, few studies have investigated strain differences between the strains such as Wistar and SD rats which are widely used as controls in behavioural research. Behavioural data are sensitive to strain differences and as varying baseline activities/behaviours will impact on results this will in turn affect behavioural responses to drugs in pharmacology studies.

In the OFT, SD and Wistar rats do not differ in activity (distance moved) (Rex et al., 2004, Tejani-Butt et al., 2003, Hlavacova et al., 2006) or time spent in the centre of the OF arena (Rex et al., 2004), suggesting that these two strains do not differ in anxiety-like behaviour in this test. However in an emergence test, where the rat's home cage is placed in the arena, thus making it an "unforced" OFT, SD rats

were more active in exploring the arena than were the Wistar strain (Pare et al., 2001), suggesting the SD were less fearful in the arena than the Wistar. In the EPM SD rats show less anxiety-like behaviours (greater open arm time and open arm entries) than Wistar rats (Rex et al., 2004). Wistar were more active with greater distance moved in the EPM than Fischer and WKY strains and less anxious (greater percent arm entries and percent arm time) than WKY (Shepard and Myers, 2008) and Fawn Hooded (Hall et al., 1998) rat strains. Lewis rats are an inbred strain characterised by hypoactive HPA axis reactivity (Gomez et al., 1996). The Lewis strain have shown greater OFT activity compared to Fischer, WKY and SD strains (van der Staay et al., 2009, Pardon et al., 2002, Rex et al., 1996) while no difference between Lewis and Wistar strains in OFT activity was found (Rex et al., 1996). In the EPM however Lewis rats had lower percent open arm time and open arm entries than the Fischer strain (van der Staay et al., 2009); while compared to the “anxious” WKY rats Lewis spent more time on the open arms of the EPM (Pardon et al., 2002). In the light/dark test of anxiety behaviour, Lewis rats showed greater anxiety-like behaviour, evidenced by less movement and less time spent in the bright section than the SHR strain (Ramos et al., 2002).

A recent study reiterated the issue of appropriate control, particularly when measuring and comparing monoamine levels in the brain. The “anxious” WKY strain displayed lower 5HT concentrations in the hypothalamus, substantia nigra, and nucleus accumbens compared to both SD and Wistar control groups. However, the control SD and Wistar strains differed in 5HIAA and 5HT turnover. SD rats had greater 5HIAA levels in the hippocampus, striatum and hypothalamus and greater 5HT turnover in the hypothalamus, raphe nucleus and locus coeruleus than Wistar rats. Striatal NA levels were significantly greater in the WKY strain compared to Wistar, but not compared to SD. Finally NA levels in the nucleus accumbens core were significantly greater in Wistar rats compared to SD, while in the nucleus accumbens shell SD rats had significantly greater NA concentrations than the Wistar strain. The authors suggest the decreased monoamine levels and alterations in transporter sites contribute to the predisposition of WKY rats towards depressive behaviour (Scholl et al., 2010). Though the relevance of the neurotransmitter differences between the Wistar and SD strains are not clear, the results suggest the need for using multiple control strains in such studies as results may be dependent

on the control employed (Scholl et al., 2010). Another study showed Wistar rats displayed greater anxiety behaviours in the EPM compared to SD and this was mirrored in increased hippocampal 5HT in Wistar rats compared to the SD strain, as measured by microdialysis on the EPM (Rex et al., 2004).

Given the strain differences in baseline behavioural measures, coupled with differing neurotransmitter responses to stress, it follows that various rat strains may respond differently to psychotropic drugs. The Lewis and Fischer F344 strains are widely used in models of drug abuse vulnerability as the Lewis strain have a greater propensity for self-administration and sensitivity to psychoactive drugs (Kosten and Ambrosio, 2002). The Lewis strain have displayed greater increases in AMP-induced locomotor activity and greater drug-primed reinstatement responding following extinction with cocaine and MA compared to the F344 strain (Gulley et al., 2007, Kruzich and Xi, 2006b, Kruzich and Xi, 2006a). Acute administration of 2 mg kg^{-1} AMP significantly increased locomotor activity for Wistar rats but not for the SD strain (McDermott and Kelly, 2008). The tricyclic antidepressant DMI has been shown by some to have no effect on (Jeannotte et al., 2009, Tejani-Butt et al., 2003), or reduce immobility time in the FST (McDermott and Kelly, 2008) compared to saline controls for the SD and Wistar strains (McDermott and Kelly, 2008). Finally the benzodiazepine DZP (2 mg kg^{-1}) increased percent open arm entries for SD rats but not for Wistar rats in the EPM (McDermott and Kelly, 2008). DZP (2 mg kg^{-1}) was found to have anxiolytic effects for the WKY strain in the emergence test, while SD rats showed no anxiolytic effects at this dose (Pare et al., 2001).

Taken together, the Lewis strain show high levels of activity and reduced anxiety in the OFT but avoid the bright section of the light/dark box and their profile of anxiety-like behaviours in the EPM depend on the strain of the rat to which they are compared. The SD and Wistar groups are somewhat intermediate in activity levels though the Wistar may display more anxious behaviours than the SD. While the WKY strain has a well characterised anxiety-like profile of avoidance of the EPM open arms and decreased 5HT (Pardon et al., 2002, O'Mahony et al., 2011), the differences in monoamine levels and behavioural correlates are not as well established in the “control” albino strains. The aim of the present study therefore was to investigate any strain differences in: activity in a novel environment using the OFT, baseline activity levels in the homecage, anxiety-like behaviour in the EPM

and time spent immobile in the FST. Furthermore, any strain differences in response to the anxiolytic DZP, the tricyclic antidepressant DMI and the psychomotor stimulant MA were assessed in the EPM, FST and homecage respectively. In addition, as few studies have looked at learning and memory in various strains, the MWM and NOR tests were carried out. Neurotransmitter levels in the hypothalamus, frontal cortex, striatum, hippocampus and cerebellum were analysed using HPLC.

7.2 Methods

7.2.1 Animals

Seventy-two male SD, Wistar and Lewis rats ($n = 24$ per group) weighing 100-150 g (approximately 6 weeks old on arrival) were singly-housed in standard plastic-bottomed cages (see section 2.2.2). All rats were handled daily for 5 days prior to behavioural testing. A counter-balanced design was used wherein animals were randomly assigned to one of six groups ($n = 4$ /strain in each group), each group was exposed to each behavioural test, but on different days, with the exception of the Morris maze due to the test duration. The aim of using this counter-balanced design was to avoid any confounding effects of test day on behavioural performance. The OFT, EPM and NOR tests were carried out between 08:00 and 12:00, while the FST pre-test and testing were carried out at 13:00-14:00 and the homecage monitoring and Morris maze tests were carried out from 08:00 to approximately 16:00, depending on the number of animals for testing on a given day. The order in which the animals were given trials in the Morris maze was alternated daily to avoid time of day effects on performance.

7.2.2 Drugs

See section 2.1 for details and drug sources.

DZP (0.625, 1.25 and 2.5 mg kg⁻¹, i.p.) was administered 30 minutes prior to EPM testing. DMI (2.5, 5 and 10 mg kg⁻¹, i.p.) was administered 24, 5 and 1 hour prior to the 5-minute FST. MA (0.25, 0.5, 1, 2 and 4 mg kg⁻¹, s.c.) was administered immediately prior to homecage monitoring.

7.2.3 Open Field Test

See section 2.3.2

7.2.4 Elevated plus maze

See section 2.3.3

7.2.5 Forced Swim Test

See section 2.3.4

7.2.6 Homecage Monitoring

Homecage activity was monitored over 2 hours. See section 2.3.5, homecage monitoring apparatus

7.2.7 Morris water maze

See section 2.3.6

7.2.8 Novel Object Recognition (NOR)

See section 2.3.7

7.2.9 Circadian Homecage Activity Monitoring

While two groups of animals were being tested in the MWM, the home cage tracking apparatus was used to continuously track locomotor activity of another two groups, as described in section 2.3.5.

7.2.10 Determination of monoamine levels using high performance liquid chromatography

Upon completion of the experiment rats were sacrificed by decapitation, the brain removed from the skull and frozen on dry ice before being stored at -80 °C. The hypothalamus, frontal cortex, striatum, hippocampus and cerebellum were later dissected as per section 2.5.1. HPLC analysis was carried out as described in section 2.6 to determine concentrations of NA, DA, 5HIAA and 5HT. Results are expressed as nanograms per gram fresh weight of tissue.

7.3 Statistical analysis

See section 2.8.

7.4 Results

7.4.1 Body Weight (Figure 7.1)

Lewis and Wistar rats were significantly lighter than SD rats during the body weight monitoring period; repeated measures ANOVA revealed significant week [$F(5, 345) = 3011, p < 0.001$], strain [$F(2, 69) = 63.24, p < 0.001$] and strain x week interaction [$F(10, 345) = 34.47, p < 0.001$] effects. Lewis rats were lighter than SD for the 6-week duration while Wistar rats were lighter than SD during weeks 2-6. Overall weight gain (i.e. body weight week 6 – body weight week 1) was significantly greater for SD compared to both Lewis and Wistar strains [$F(2, 68) = 43.04, p < 0.001$].

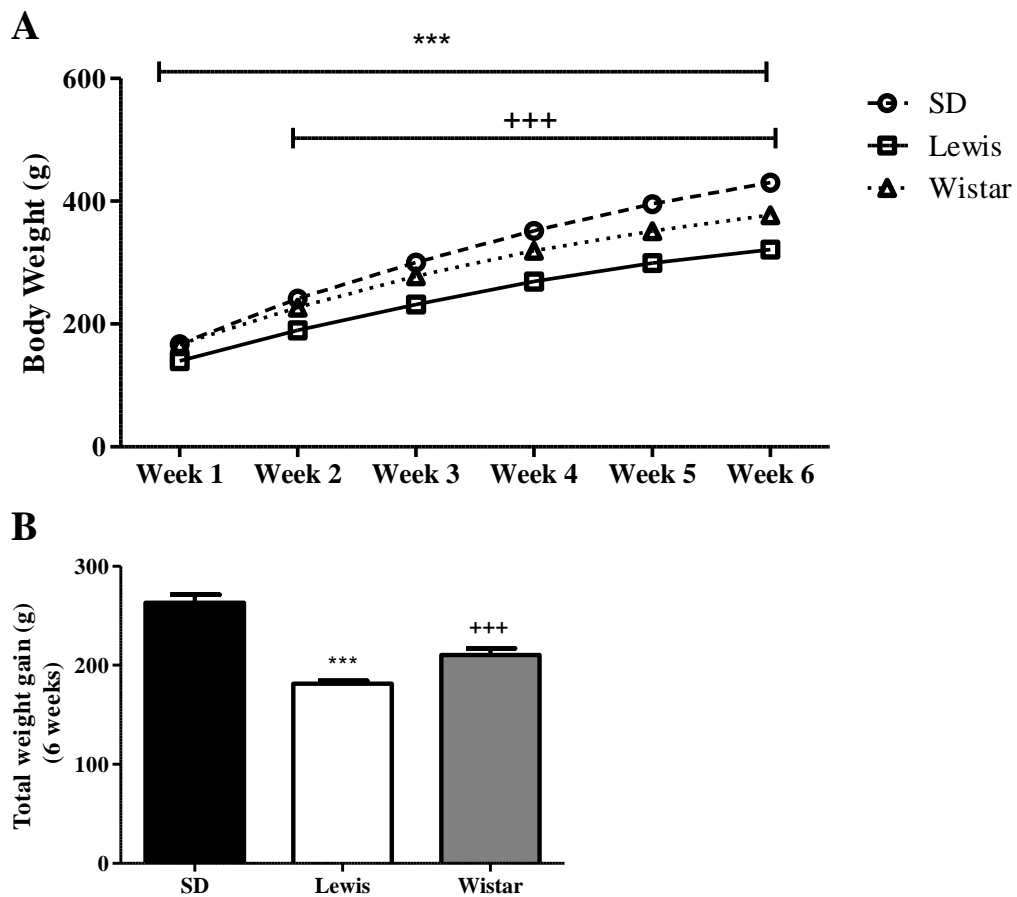


Figure 7.1: The effect of rat strain on body weight. Body weight (g) over the 6-week monitoring period (A) and overall weight gain over the 6 weeks (B) for Sprague-Dawley (SD), Lewis and Wistar rats. Data expressed as mean + SEM ($n = 24$ per group), *** $p < 0.001$ Lewis vs. Sprague Dawley, +++ $p < 0.001$ Wistar vs. Sprague Dawley.

7.4.2 Open Field Test (Figure 7.2)

There was no difference in total distance moved [$F(2, 21) = 1.80, p > 0.05$] or distance moved in the inner arena [$F(2, 21) = 0.56, p > 0.05$] between Lewis or Wistar and SD strains. Nor were there any strain effects on time spent in the inner arena [$F(2, 21) = 0.49, p > 0.05$] or frequency of entries to the inner arena [$F(2, 21) = 1.19, p > 0.05$].

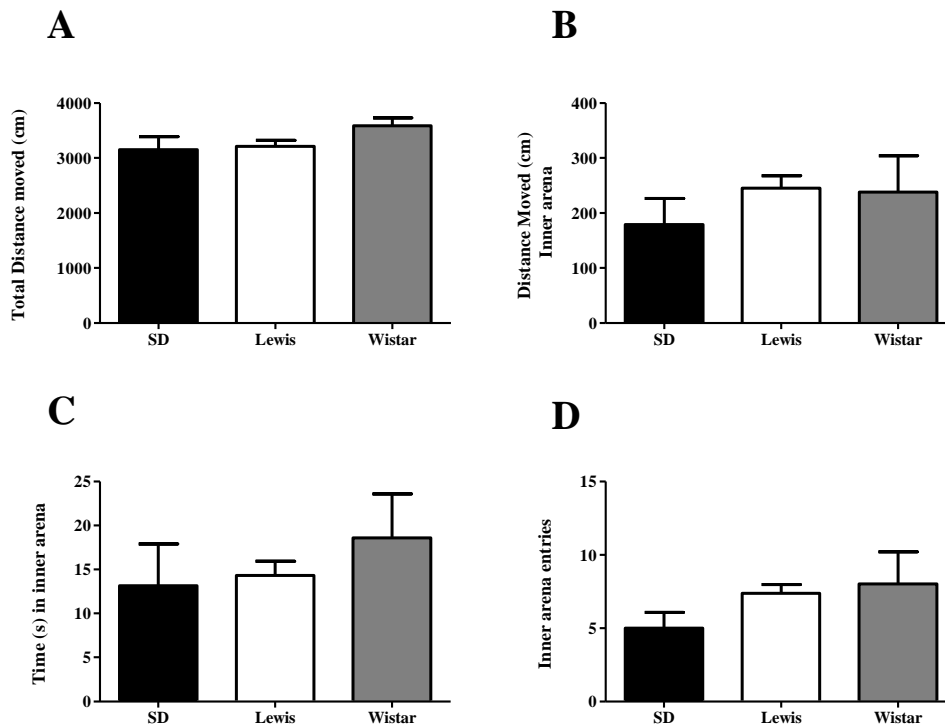


Figure 7.2: The effect of strain in the open field test. Total distance moved (A), distance moved in the inner arena (B), time spent in the inner arena (C) and inner arena entries (D) in the open field test by Sprague-Dawley (SD), Lewis and Wistar rats. Data expressed as mean + SEM ($n = 8$ per group).

7.4.3 Elevated Plus Maze

Baseline Behaviour (Figure 7.3)

There were no strain differences in distance moved [$F(2, 15) = 0.11, p > 0.05$], percent open arm entries [$F(2, 15) = 0.96, p > 0.05$] or percent time spent in the open arms [$F(2, 15) = 0.98, p > 0.05$] between the saline-treated controls in the EPM.

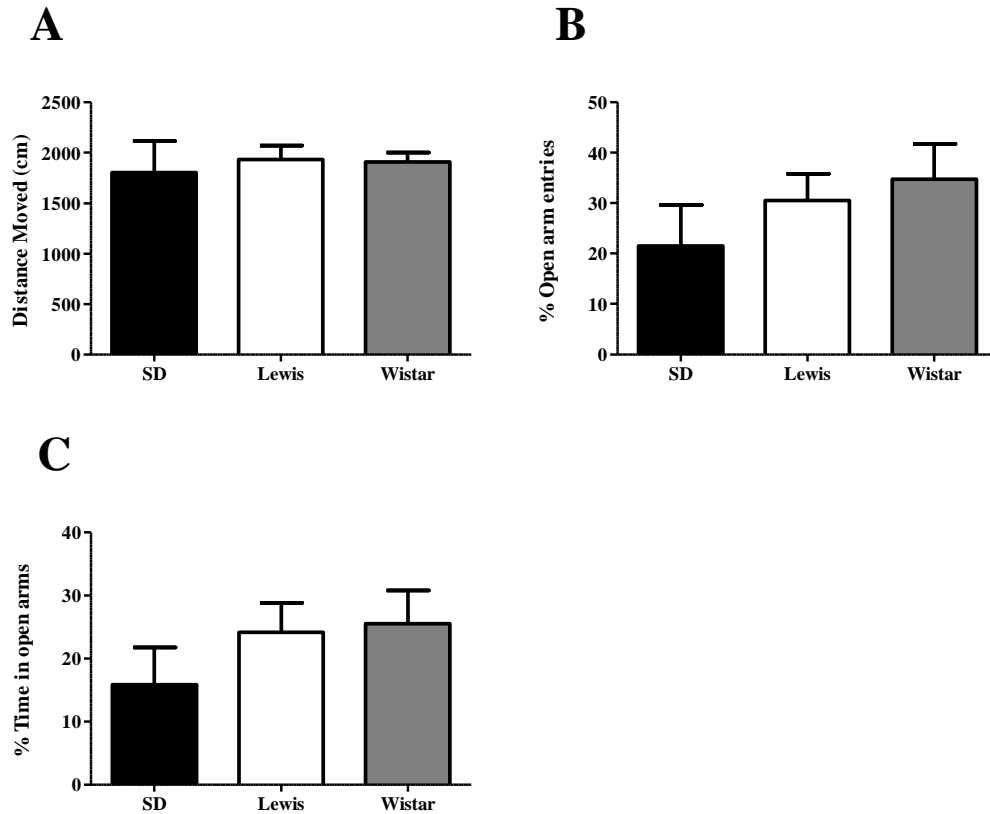


Figure 7.3: The effect of strain in the elevated plus maze. Distance moved (A), percent open arm entries (B) and percent open arm time (C) for Sprague-Dawley (SD), Lewis and Wistar rats in the EPM. Data expressed as mean + SEM ($n = 6$ per group).

Diazepam Effects (Figure 7.4)

In distance moved in the EPM there were no significant strain, [$F(2, 59) = 0.46, p > 0.05$] drug [$F(3, 59) = 1.56, p > 0.05$] or strain x drug interaction [$F(6, 59) = 0.49, p > 0.05$] effects on distance moved in the EPM. When percent open arm entries were analysed there was a significant effect of strain [$F(2, 59) = 7.28, p < 0.05$], but no significant drug [$F(3, 59) = 1.16, p > 0.05$] or interaction effect [$F(6, 59) = 0.20, p$

> 0.05]. Percent open arm time analyses revealed significant strain [$F(2, 59) = 5.67$, $p < 0.01$] and drug effects [$F(3, 59) = 3.17$, $p < 0.05$], but no significant interaction [$F(6, 59) = 0.81$, $p > 0.05$].

One-way ANOVAs showed significant DZP effects for the Lewis strain on percent open arm time [$F(3, 20) = 3.49$, $p < 0.05$] but not on distance moved [$F(3, 20) = 0.70$, $p > 0.05$] or percent open arm entries [$F(3, 20) = 0.63$, $p > 0.05$] in the EPM. *Post-hoc* tests showed that the 2.5 mg kg^{-1} dose DZP significantly increased percent open arm time for Lewis rats, compared to saline controls. No DZP effects were seen for SD rats (distance moved [$F(3, 20) = 0.30$, $p > 0.05$], percent open arm entries [$F(3, 20) = 0.45$, $p > 0.05$] or percent open arm time [$F(3, 20) = 1.78$, $p > 0.05$]). Nor were there any DZP effects for the Wistar strain in the EPM (distance moved [$F(3, 20) = 2.15$, $p > 0.05$], percent open arm entries [$F(3, 20) = 0.46$, $p > 0.05$] or percent open arm time [$F(3, 20) = 1.80$, $p > 0.05$]).

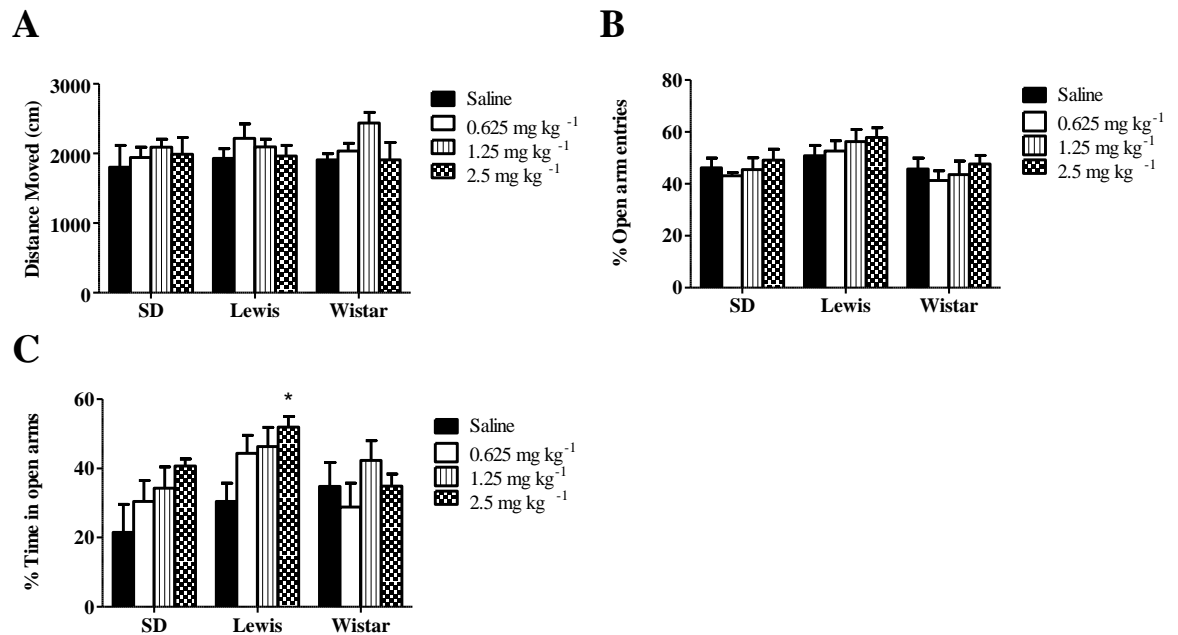


Figure 7.4: Strain and diazepam effects in the elevated plus maze. The effects of DZP (0.625, 1.25, 2.5 mg kg⁻¹, i.p.) on distance moved (A), percent open arm entries (B) and percent open arm time (C) of the elevated plus maze by Sprague-Dawley (SD), Lewis and Wistar rats. Data expressed as mean + SEM ($n = 6$ per group), * $p < 0.05$, vs. Lewis saline control.

7.4.4 Forced Swim Test

Baseline Behaviour (Figure 7.5)

There was no strain difference in time spent immobile in the FST [$F(2, 15) = 3.40$, $p = 0.06$], there was however a significant difference in time spent climbing [$F(2, 15) = 3.67$, $p < 0.05$]. *Post-hoc* tests revealed that saline control Wistar rats spent significantly less time climbing than SD rats in the FST.

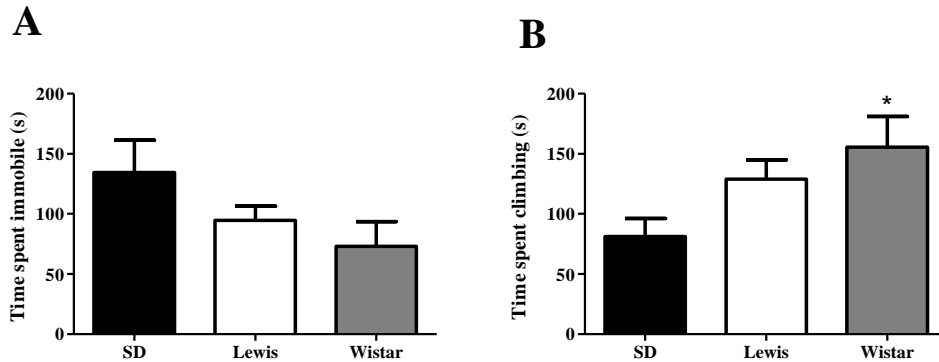


Figure 7.5: The effect of strain in the forced swim test. Time spent immobile (A) and climbing (B) by saline-treated Sprague-Dawley (SD), Lewis and Wistar rats in the FST. Data expressed as mean + SEM ($n = 5-6$ per group), * $p < 0.05$ vs. the SD strain.

Desipramine Effects (Figure 7.6)

Time spent immobile in the FST was analysed using a 2-way ANOVA; there was a significant strain effect [$F(2, 59) = 5.58$, $p < 0.01$] but no significant drug [$F(3, 59) = 0.79$, $p > 0.05$] or interaction [$F(6, 59) = 0.83$, $p > 0.05$] effects. One-way ANOVAs confirmed no significant DMI effect on immobility time for Lewis [$F(3, 20) = 0.16$, $p > 0.05$], SD [$F(3, 20) = 0.62$, $p > 0.05$] or Wistar [$F(3, 19) = 0.83$, $p > 0.05$] rats. Two-way ANOVAs for climbing showed a significant strain effect [$F(2, 59) = 4.21$, $p < 0.05$] but no significant drug [$F(3, 59) = 1.50$, $p > 0.05$] or interaction effects [$F(6, 59) = 0.75$, $p > 0.05$]. One-way ANOVAs also demonstrated there were no significant DMI effects on time spent climbing for Lewis [$F(3, 20) = 0.67$, $p > 0.05$], SD [$F(3, 20) = 2.20$, $p > 0.05$] or Wistar [$F(3, 19) = 0.69$, $p > 0.05$] rats.

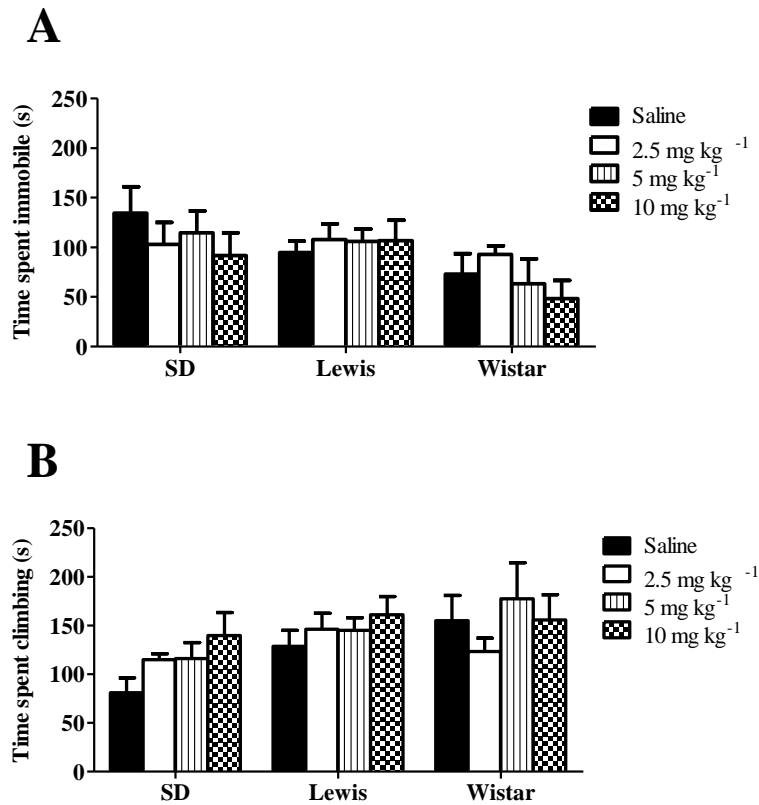


Figure 7.6: Strain and desipramine effects in the Forced Swim Test. The effects of desipramine (2.5, 5.0 and 10.0 mg kg⁻¹, i.p.) on time spent immobile (A) and climbing (B) in the forced swim test by Sprague-Dawley, Lewis and Wistar rat strains. Data expressed as mean + SEM ($n = 5-6$ per group).

7.4.5 Homecage Monitoring

Baseline Distance Moved (Figure 7.7)

There was no strain difference in distance moved in the homecage over the 2-hour monitoring period by saline controls [$F(2, 9) = 1.44, p > 0.05$].

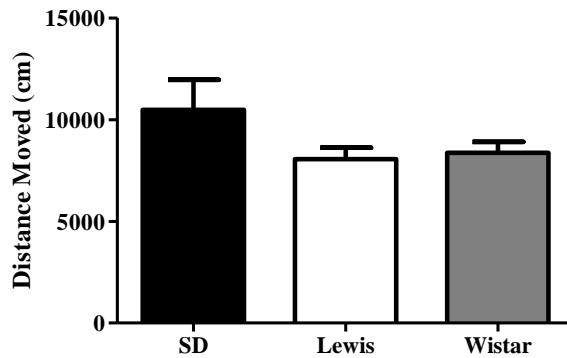


Figure 7.7: The effect of strain on distance moved in the homecage. Total distance moved by saline-treated Sprague-Dawley (SD), Lewis and Wistar rats in the homecage during a 2-hour monitoring period. Data expressed as mean + SEM ($n = 4$ per group).

Methamphetamine Effects (Figures 7.8 and 7.9)

Analysis of distance moved in the homecage following MA administration revealed significant main effects of strain [$F(5, 53) = 17.97, p < 0.01$] and drug [$F(2, 53) = 5.36, p < 0.001$] and a strain \times drug interaction [$F(10, 53) = 2.15, p < 0.05$]. One-way ANOVAs showed significant MA effects for Lewis [$F(5, 18) = 14.70, p < 0.001$] and SD strains [$F(5, 18) = 5.25, p < 0.01$]; and drug effects were just shy of significance for Wistar rats [$F(5, 17) = 2.70, p = 0.06$]. *Post-hoc* tests showed that 0.5, 1 and 2 mg kg⁻¹ MA significantly increased distance moved for SD and Lewis rats compared to saline controls, the Wistar strain however failed to show any significant response to the increasing MA doses.

When distance moved was expressed as a percent of the baseline (i.e. saline controls), there were overall significant strain [$F(2, 53) = 12.46, p < 0.001$], drug [$F(5, 53) = 18.57, p < 0.001$] and significant strain \times drug interaction effects [$F(10, 53) = 2.40, p < 0.05$]. One-way ANOVAs showed significant MA effects for Lewis [$F(5, 18) = 15.68, p < 0.001$], SD [$F(5, 18) = 5.45, p < 0.01$] and Wistar rats [$F(5, 17) = 2.98, p < 0.05$]. *Post-hoc* tests showed that the 0.5, 1 and 2 mg kg⁻¹ doses of MA increased activity significantly for Lewis and SD rats compared to saline controls;

while only the 0.5 mg kg⁻¹ MA dose significantly increased activity for the Wistar strain compared to saline controls.

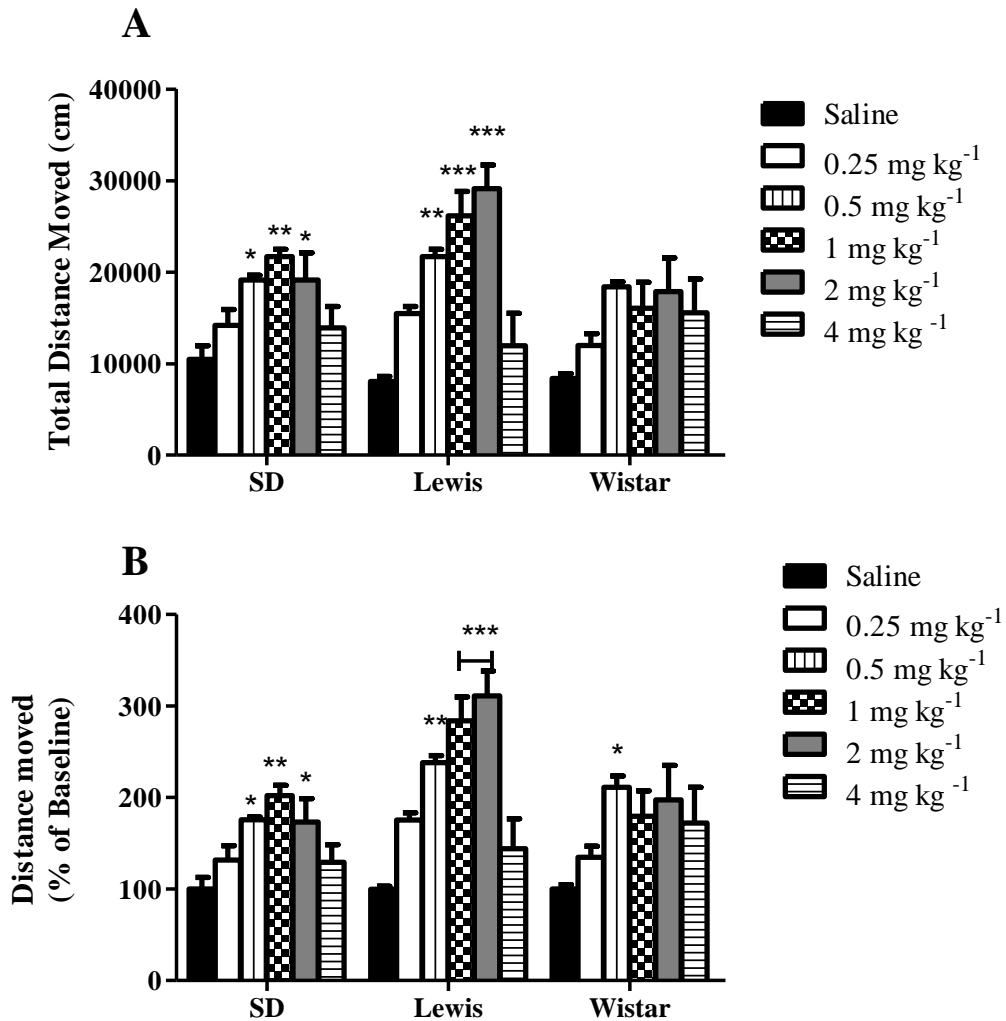


Figure 7.8: Methamphetamine and strain effects on distance moved in the home cage. The effects of MA administration (0, 0.25, 0.5, 1, 2 or 4 mg kg⁻¹, s.c.) on total distance moved (A) and distance moved as a percent of baseline levels (B) in the home cage over 2 hours by Sprague-Dawley (SD), Lewis and Wistar rat strains. Data expressed as mean + SEM ($n = 3-4$ per group), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. saline controls of the same strain.

The 2-hour activity monitoring period was analysed over six 20-minute timebins. Repeated measures analyses of distance moved (relative to baseline) showed significant drug and time effects for Lewis (time [$F(5, 90) = 49.93$, $p < 0.001$], drug [$F(5, 18) = 15.68$, $p < 0.001$]), SD (time [$F(5, 90) = 43.23$, $p < 0.001$], drug [$F(5, 18) = 5.45$, $p < 0.01$]) and Wistar (time [$F(5, 85) = 53.23$, $p < 0.001$], drug [$F(5, 17) = 2.98$, $p < 0.05$]) strains. There was a significant drug x interaction effect for Lewis rats [$F(25, 90) = 3.65$, $p < 0.001$] but not for the SD [$F(25, 90) = 1.45$, $p > 0.05$] or

Wistar [$F(25, 85) = 1.14, p > 0.05$] strains. One-way ANOVA analyses results for MA effects at each time point are presented in figure 7.9. The Lewis strain were most active following the 2 mg kg^{-1} MA dose, while the SD strain were more active in response to the 1 mg kg^{-1} dose. Significant dose effects were only seen during the first hour for Wistar rats, whereas drug effects persisted for the 2 hours in the Lewis and SD strains.

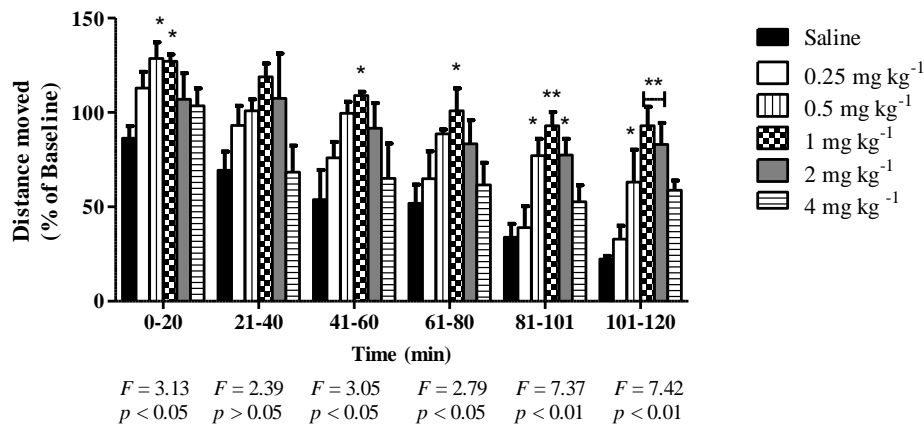
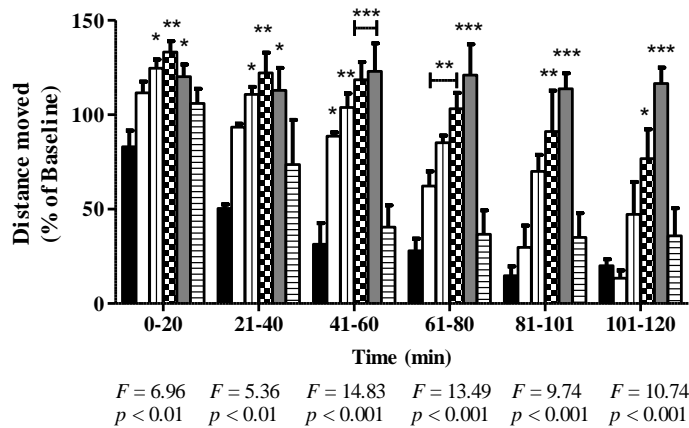
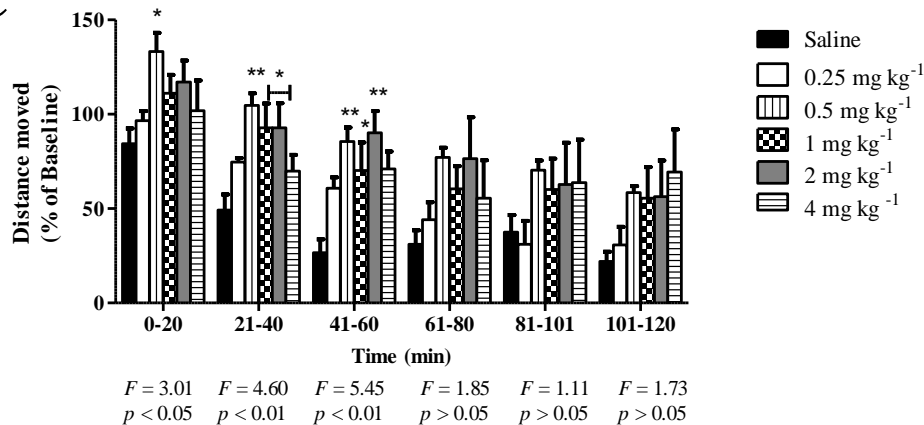
A**B****C**

Figure 7.9: Methamphetamine and strain effects on distance moved in the homepage over 20-minute timebins. The effects of MA (0, 0.25, 0.5, 1, 2 or 4 mg kg⁻¹, s.c.) administration on distance moved in the homepage, expressed as percent of baseline control, by Sprague-Dawley (A), Lewis (B) and Wistar (C) strains over 20-minute timebins. Data expressed as mean + SEM ($n = 3-4$ per group), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. saline controls of the same strain, according to one-way ANOVA ($df = 5, 18$).

7.4.6 Novel Object Recognition (Figure 7.10)

Rats of each strain spent significantly more time exploring the novel object than the familiar one. There was no strain difference in novel object discrimination ratio (i.e. time spent exploring novel object / time spent exploring the novel + familiar objects) [$F(2, 21) = 0.07, p > 0.05$].

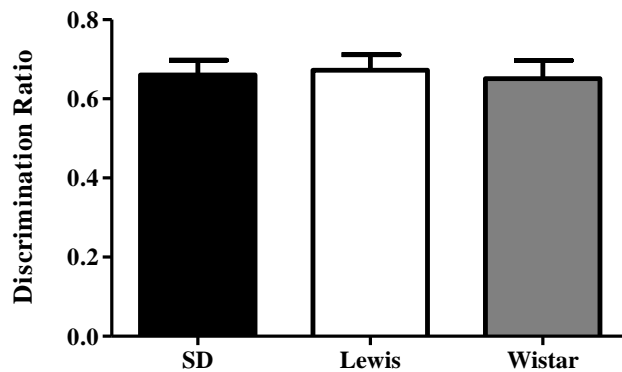


Figure 7.10: The effect of strain on discrimination ratio in the novel object test.

Discrimination ratio (i.e. time spent exploring the novel object / (time spent exploring the novel object + familiar object)) in the NOR test for Sprague-Dawley (SD), Lewis and Wistar rats. Data expressed as mean + SEM ($n = 8$ per group).

7.4.7 Morris Water Maze (Figures 7.11 and 7.12)

Repeated ANOVA for latencies to the platform in the acquisition phase of the MWM demonstrated a significant day effect [$F(3, 63) = 97.77, p < 0.001$] as rats learned the location of the platform over time; there was no significant effect of strain [$F(2, 21) = 0.13, p > 0.05$] but a significant strain x day interaction effect [$F(6, 63) = 2.84, p < 0.05$] on latencies. Swim speed was also significantly reduced over the four-day acquisition phase (time effect [$F(3, 63) = 8.88, p < 0.001$]), but there were no strain [$F(2, 21) = 0.24, p > 0.05$] or strain x time interaction effects [$F(6, 63) = 0.95, p > 0.05$] on swim speed.

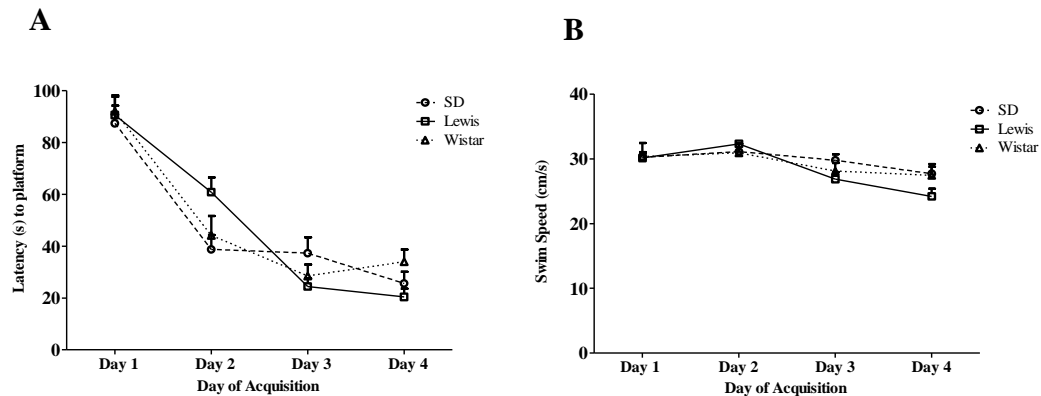


Figure 7.11: The effect of strain in the Morris water maze acquisition phase. Latency (s) to locate the platform (A) and swim speed (cm/s) (B) during the acquisition phase of the Morris water maze for Sprague-Dawley (SD), Lewis and Wistar rats. Data expressed as mean + SEM ($n = 8$ per group).

In the MWM probe trial there was a significant strain effect on path length [$F(2, 21) = 6.01, p < 0.01$] and on frequency of entries to the target (SW) quadrant [$F(2, 21) = 3.50, p < 0.05$]. *Post-hoc* tests revealed that Wistar rats swam for a shorter distance than both Lewis and SD strains ($p < 0.05$). Wistar also made fewer entries to the target quadrant than SD rats ($p < 0.05$).

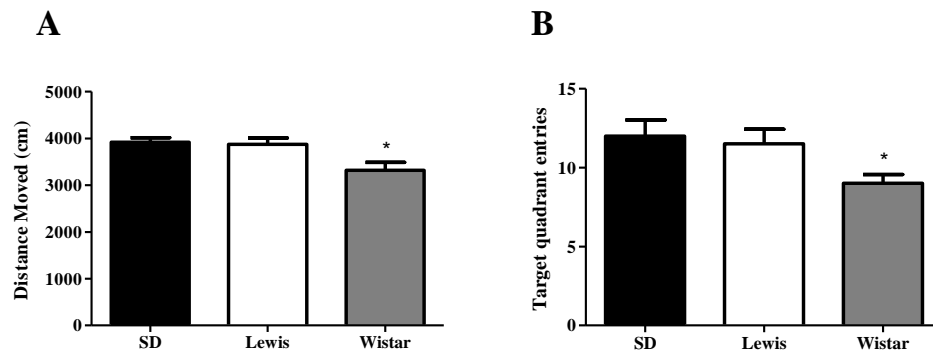
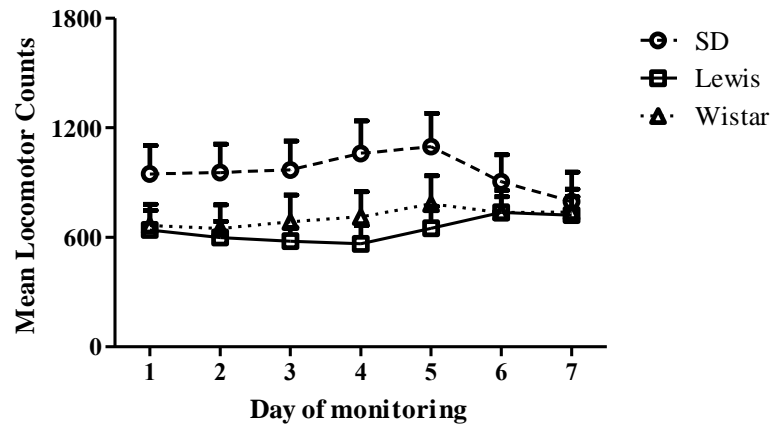


Figure 7.12: The effect of strain in the Morris water maze probe trial. Swim path length (A) and number of target quadrant entries (B) in the probe phase of the Morris water maze for Sprague-Dawley (SD), Lewis and Wistar rat strains. Data expressed as mean + SEM ($n = 8$ per group), * $p < 0.05$ vs. Sprague-Dawley strain.

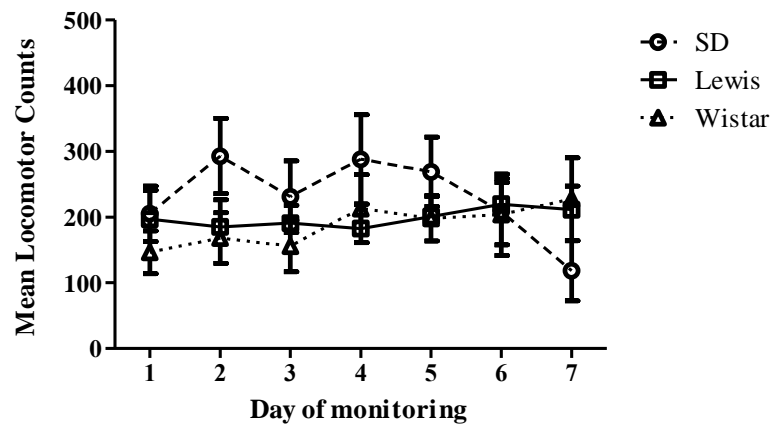
7.4.8 Circadian Homecage Activity Monitoring (Figure 7.13)

All animals were more active during the dark phase of the day (20:00 – 08:00 hours) than in light (08:00 – 20:00 hours). Homecage activity over 7 days was analysed; during the dark phase there were no significant day [$F(6, 126) = 0.42, p > 0.05$], strain [$F(2, 21) = 1.96, p > 0.05$] or strain x day effects [$F(12, 126) = 1.16, p > 0.05$]. Similarly, during the light phase there was no effect of strain [$F(2, 21) = 0.40, p > 0.05$] or day [$F(6, 126) = 0.96, p > 0.05$], but a significant interaction effect [$F(12, 126) = 1.86, p < 0.05$] on activity counts. When total daily activity counts (light + dark phase activity) were analysed there were no significant day [$F(6, 126) = 0.97, p > 0.05$], strain [$F(2, 21) = 1.63, p > 0.05$] or strain x day effects [$F(12, 126) = 1.79, p > 0.05$]. One-way ANOVAs revealed no significant strain effects on activity counts on any of the 7 days of tracking.

Dark Phase



Light Phase



Total Activity Counts

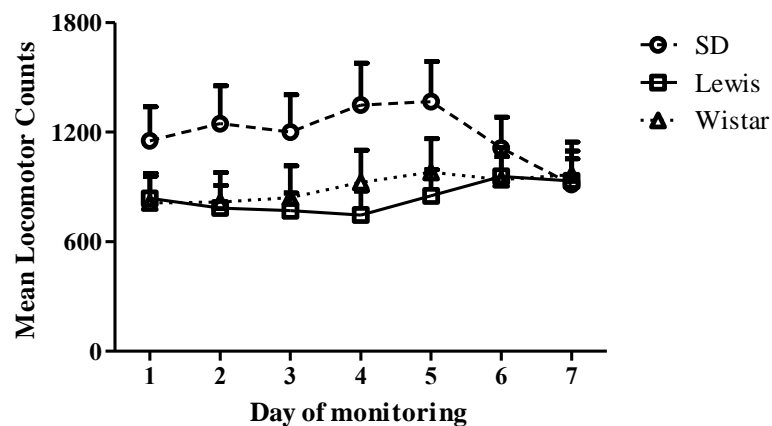


Figure 7.13: The effect of strain on locomotor counts in the homecage. Locomotor activity counts in the homecage for one week in the day-time, night-time and total counts over the 23-hour monitoring period for Sprague-Dawley (SD), Lewis and Wistar rat strains. Data expressed as mean + SEM ($n = 8$ per group).

7.4.9 Determination of monoamine levels using high performance liquid chromatography (Table 7.1)

There were significant strain differences in monoamine levels in the hippocampus and striatum (Table 7.1). In the hippocampus there was a strain effect on NA, 5HIAA and 5HT turnover (i.e. 5HIAA/5HT). There was also a significant difference in 5HT turnover in the striatum ($F(2, 21) = 5.20, p < 0.05$). *Post-hoc* tests revealed the Lewis strain has significantly greater hippocampal NA, 5HIAA and 5HT turnover compared to both SD and Wistar rats, which did not differ from one another. In the striatum the Lewis strain had significantly greater 5HT turnover than SD rats. No other significant differences were seen.

Table 7.1: Strain effects on monoamine concentration in the rat brain

	NA	DA	5HIAA	5HT	5HIAA/5HT
Frontal Cortex					
Lewis	214 ± 9	63 ± 4	443 ± 29	305 ± 20	1.47 ± 0.10
SD	220 ± 20	57 ± 12	388 ± 35	319 ± 41	1.26 ± 0.07
Wistar	228 ± 23	53 ± 8	354 ± 36	272 ± 25	1.26 ± 0.13
Strain	<i>F</i> 0.13	0.38	2.57	0.65	1.42
Effect	<i>p</i> > 0.05				
	<i>df</i> 2, 21				
Striatum					
Lewis	125 ± 14	2584 ± 420	354 ± 39	206 ± 28	1.78 ± 0.07*
SD	102 ± 19	2733 ± 372	262 ± 31	198 ± 40	1.28 ± 0.16
Wistar	163 ± 27	2986 ± 580	320 ± 35	225 ± 32	1.49 ± 0.11
Strain	<i>F</i> 2.20	0.19	1.75	0.72	5.20
Effect	<i>p</i> > 0.05				< 0.05
	<i>df</i> 2, 21				
Hippocampus					
Lewis	439 ± 19**+	67 ± 9	509 ± 41**+	279 ± 17	1.83 ± 0.11**+
SD	300 ± 39	39 ± 11	330 ± 24	282 ± 39	1.23 ± 0.08
Wistar	355 ± 14	41 ± 9	402 ± 28	250 ± 11	1.62 ± 0.12
Strain	<i>F</i> 7.94	0.76	7.94	0.49	8.02
Effect	<i>p</i> < 0.01	> 0.05	< 0.01	> 0.05	< 0.01
	<i>df</i> 2, 21				
Cerebellum					
Lewis	199 ± 9	ND	91 ± 10	32 ± 7	2.16 ± 0.22
SD	164 ± 9	ND	81 ± 5	19 ± 8	2.34 ± 0.14
Wistar	143 ± 25	ND	100 ± 8	31 ± 10	2.03 ± 0.26
Strain	<i>F</i> 3.07		1.45	0.73	0.42
Effect	<i>p</i> > 0.05		> 0.05	> 0.05	> 0.05
	<i>df</i> 2, 21				
Hypothalamus					
Lewis	1358 ± 146	247 ± 22	522 ± 55	270 ± 22	2.03 ± 0.29
SD	1109 ± 97	222 ± 14	495 ± 54	290 ± 25	1.69 ± 0.11
Wistar	1341 ± 131	251 ± 36	589 ± 65	294 ± 45	1.71 ± 0.15
Strain	<i>F</i> 1.22	0.39	0.70	0.16	0.87
Effect	<i>p</i> > 0.05				
	<i>df</i> 2, 21				

SD, Sprague Dawley; NA, noradrenaline; DA, dopamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HIAA / 5-HT, serotonin turnover; ND, not detectable. Data expressed as mean neurotransmitter concentration (ng / g tissue) ± SEM (*n* = 8 per group), * *p* < 0.05, ** *p* < 0.01 compared to SD strain; + *p* < 0.05 compared to Wistar strain.

7.5 Discussion

There were no significant differences between the Lewis, Sprague Dawley and Wistar rats in baseline anxiety-like behaviours in the open field test and elevated plus maze, nor were there any baseline differences in activity, measured by distance moved, in these tests or in either of the homecage tracking measures. There were no strain differences in immobility time in the forced swim test, though Wistar rats spent significantly more time climbing than Sprague Dawley saline control groups. There was no difference between the three strains in memory as assessed in the novel object recognition test, rats of all three strains recognised and showed preference for the novel over the familiar object. Though there was no difference between the strains in memory and latency to locate the platform in the Morris water maze, there were differences in the probe trial as Wistar rats swam for a shorter distance than the Sprague Dawley strain and made fewer entries to the target quadrant than Sprague Dawley and Lewis. Analysis of neurotransmitter levels showed Lewis rats had increased noradrenaline, 5-hydroxyindoleacetic acid and serotonin turnover in the hippocampus compared to both Sprague Dawley and Wistar strains, in addition, Lewis rats had greater serotonin turnover in the striatum compared to the Sprague Dawley strain.

The 2.5 mg kg⁻¹ diazepam dose exerted anxiolytic effects in the elevated plus maze by increasing percent open arm time for Lewis rats but this was not seen for the Wistar strain. There was no significant desipramine effect on time spent immobile in the forced swim test, though the 10 mg kg⁻¹ dose tended to increase climbing within the Sprague Dawley strain compared to saline controls. For the Lewis and Sprague Dawley strains, methamphetamine administration produced a significant dose-response effect as increasing methamphetamine promoted increased activity which declined at the highest dose (4 mg kg⁻¹) as stereotypical behaviours likely interfered with locomotion. However, this inverted-U dose-response relationship is absent for the Wistar strain who only displayed a significant increase in activity in response to the 0.5 mg kg⁻¹ dose. In addition, the Lewis strain appear to be more sensitive to the locomotor-stimulating effects of methamphetamine as the 1 and 2 mg kg⁻¹ doses increase activity by up to 200% of the baseline, while a 100% increase is seen for the Sprague Dawley rats at the 1 mg kg⁻¹ dose.

There were few strain differences seen in baseline behaviours in the present study. In the open field test all rats displayed comparable activity, this is in agreement with some previous studies that have shown no difference between Sprague Dawley and Wistar and between Wistar and Lewis rat strains in open field activity (Rex et al., 2004, Tejani-Butt et al., 2003, Hlavacova et al., 2006, Rex et al., 1996). The absence of differences in locomotor activity in the open field test corresponded with the elevated plus maze where no baseline difference in distance moved was seen between the three strains. Similarly, there were no differences between the saline controls in anxiety-like activity in the elevated plus maze. This contrasts with Rex and colleagues (Rex et al., 2004) who demonstrated Sprague Dawley rats were less anxious than the Wistar strain on the elevated plus maze. In their study, the open arms of the elevated plus maze were illuminated at lux 210, whereas in the present study the open arms were lit at lux 90. This is noteworthy as lighting levels influence behaviour in the elevated plus maze (Hogg, 1996) and varying the brightness may reveal strain differences in anxiety measures such as the elevated plus maze and social interaction tests (Hall et al., 1998). There were no significant differences in baseline activity in the homecage; this is consistent with Hlavacova and colleagues (2006) who showed no difference in ambulation counts between Sprague Dawley, Lewis and Wistar. Lewis rats displayed significantly more jumps and fewer rest (non-movement) periods than the Sprague Dawley and Wistar strains over the 45-minute observation period during the dark phase of the day (Hlavacova et al., 2006). Notably few studies monitor locomotor activity in the homecage, the use of the open field or novel environments may lead to erroneous conclusions as they do not necessarily reflect spontaneous activity levels.

In the present study, Wistar rats made fewer entries to the target quadrant in the Morris water maze which might suggest poorer spatial learning than the Sprague Dawley and Lewis strains. Little attention has been given to strain differences in the Morris water maze, with the exception of those exploring cognitive decline and aging, as often modelled by the Brown Norway and Fischer F344 strains (van der Staay et al., 2009, Weindruch and Masoro, 1991). When the Wistar strain were compared to the Brown Norway and Fischer progeny, the Wistar strain had greater latencies during the acquisition phase and reduced time in the target quadrant during the probe trial. As the authors suggest that poorer visual acuity of the albino strain

may have contributed to the result (van der stay and Blokland 1996), it may be unsuitable therefore to employ albino and pigmented rats in the Morris water maze as comparator groups (Prusky et al 2000). Similar to the present study, the albino spontaneously hypertensive rats, Wistar Kyoto and Sprague Dawley rat strains do not differ in latency to the escape platform (Ferguson and Cada, 2004).

In the forced swim test, the Sprague Dawley rat strain exhibited less climbing than the Wistar and Lewis strains; however there was no difference in time spent immobile between the saline control groups. The depressive-like profile of the Wistar Kyoto strain has been demonstrated in the forced swim test. Wistar Kyoto rats spend more time immobile than Wistar and Sprague Dawley strains, who do not differ from one another (Jeannotte et al., 2009, Tejani-Butt et al., 2003). Similarly, McDermott and Kelly (2008) saw no difference in immobility time between Sprague Dawley and Wistar rat (McDermott and Kelly, 2008). The absence of a significant effect of desipramine on immobility time in the present study was unexpected, as previously in this laboratory McDermott and Kelly demonstrated 10 mg kg^{-1} desipramine, administered using the same dose regime as the present study, attenuated immobility for both Sprague Dawley and Wistar rats (McDermott and Kelly, 2008).

In the elevated plus maze diazepam had no anxiolytic effect in the Wistar rats; this was also previously seen by McDermott and Kelly (McDermott and Kelly, 2008). This may be due to differences in diazepam metabolism; quicker metabolic elimination of diazepam in Wistar rats possibly reduces their sensitivity to the drug compared to Sprague Dawley rats (Saito et al., 2004). In addition, the Wistar strain displayed the least sensitivity to the locomotor-inducing effects of increasing methamphetamine dose. Previously in this laboratory, 2 mg kg^{-1} amphetamine increased Wistar activity compared to saline controls, while the increase seen for Sprague Dawley rats did not reach significance (McDermott and Kelly, 2008). However, the previous study only employed one drug dose and did not represent a dose-response investigation. The heightened response to methamphetamine shown by Lewis rats is in agreement with previous studies as Lewis rats showed greater behavioural activation to an acute injection of both methamphetamine and cocaine, with a left-ward shift in the dose-effect curves compared to Fischer rats (Camp et al., 1994). Few studies have compared methamphetamine sensitivity in Lewis and

Sprague Dawley or Wistar strains but strain differences in the mesolimbic dopamine system are likely to mediate differences in methamphetamine dose-response (Kosten and Ambrosio, 2002), however in the present study no strain differences were seen in dopamine concentration in the brain. Notably, strain differences in pharmacokinetics have been identified. As in humans, many factors can affect drug disposition, the most salient in strain studies is the expression of the liver cytochrome p450 enzymes. Differences between strains, and between same strains from different suppliers, in such enzymes can impact on metabolism, which may influence pharmacology research (Kacew and Festing, 1996). Previously, basal and pentobarbital-induced levels of microsomal cytochrome p450 1A and mRNA expression of cytochrome p450 1A, 1A2 and 1A3 were significantly greater in Wistar rats than in Sprague Dawley (Kishida et al., 2008). As the cytochrome p450 1, 2 and 3 families are involved in drug and steroid metabolism such differences can impact on detoxification (Rang, 2005). Furthermore, drug metabolites may differ according to strain, for example, the major metabolite of diazepam for Sprague Dawley rats is p-hydroxy-diazepam, whereas the major metabolite for Dark Agouti rats is 3-hydroxy-diazepam, which may contribute to pharmacodynamic differences (Saito et al., 2004). Moreover, in a study of diazepam metabolism the disappearance rate of diazepam from liver microsomes and overall hepatic clearance of the drug was significantly lower in Wistar rats than in Sprague Dawley (Saito et al., 2004). Differences in drug absorption, distribution, metabolism and/or elimination between rat strains are important to consider as they may contribute to different outcomes in studies employing various strains.

Neurotransmitter analyses showed Lewis rats had increased noradrenaline, 5-hydroxyindoleacetic acid and serotonin turnover in the hippocampus compared to both Sprague Dawley and Wistar strains, and Lewis had greater serotonin turnover in the striatum compared to Sprague Dawley rats. In previous studies the Lewis rat strain display reduced hypothalamic-pituitary-adrenal axis reactivity to a chronic mild stress model of depression compared to Fischer rats (Wu and Wang, 2010); conversely, Lewis rats are employed in anxiety studies with the spontaneously hypertensive rat strain due to their different anxiety-like profiles, with Lewis displaying greater anxiety behaviours than spontaneously hypertensive rat strain (Ramos et al., 2002). Elevated noradrenaline and serotonin in the brain are

associated with reduced anxiety and depressive symptoms (O'Mahony et al., 2011), however increased 5-hydroxyindoleacetic acid and serotonin turnover suggest greater serotonin metabolism, which has been associated with increased stress levels (Berton et al., 1998). In the present study, the neurochemical alterations seen in Lewis rats were not reflected in the elevated plus maze or forced swim test tests of anxiety and depressive-like behaviours as baseline activity was not different between strains in any of the tests carried out. As Lewis rats displayed a greater response to acute methamphetamine administration this may influence the altered monoamine levels observed in the present study as acute methamphetamine produces initial increases in noradrenaline, dopamine and serotonin in the brain (Rothman et al., 2001), which can be followed by decreased monoamine concentrations (Clemens et al., 2004). However, if it were the case that methamphetamine contributed to the hippocampal and striatal monoamine levels one would expect to have seen accompanying differences in serotonin and dopamine levels in the present study. Unlike in the present study, previously, Sprague Dawley and Wistar rats have differed in 5-hydroxyindoleacetic acid and serotonin turnover; Sprague Dawley rats showing greater levels of 5-hydroxyindoleacetic acid and serotonin turnover in the hippocampus, striatum and hypothalamus, and greater serotonin turnover in the hypothalamus, raphe nucleus and locus coeruleus compared to the Wistar strain (Scholl et al., 2010). The Lewis strain therefore exhibited noradrenergic and serotonergic alterations in the hippocampus and striatum, while no differences were seen between Wistar and Sprague Dawley strains. Neurochemical differences were not reflected in baseline behaviour levels. Previously immobility time in the forced swim test was negatively correlated with hippocampal noradrenaline and serotonin levels in the rat brain (Brenes et al., 2009); likewise serotonin depletion in the prefrontal cortex reduced open arm entries in the elevated plus maze compared to sham controls (Pum et al., 2009) These results support the association of monoamine and behavioural endpoints; however others suggest that neurotransmitter levels do not necessarily correlate with behavioural parameters. Serotonin turnover level in the hippocampus was positively correlated with horizontal activity in the open field test, but no serotonergic correlation was observed in activity in the elevated plus maze or light/dark box; similarly in two versions of the Morris Water maze serotonergic measures were not consistently associated with behavioural measures (van der Staay et al., 2009). Furthermore, baseline time spent immobile in the forced swim test was

not affected by serotonin (Page et al., 1999, Harkin et al., 2003) or noradrenergic lesions (Cryan et al., 2002) in the rat brain. Thus relationships between monoaminergic and behavioural measures are not necessarily linked, consistent with the results of the present study.

In conclusion, few baseline behavioural differences were found between the commonly-used Lewis, Sprague Dawley and Wistar albino strains in some commonly-employed behavioural tests carried out in this study. The absence of differences in baseline activity suggests that all three strains would be suitably comparable as control groups. On the other hand, strain differences were revealed in response to psychotropic drugs. In particular the Wistar strain demonstrated a blunted sensitivity to the locomotor-stimulating effects of methamphetamine and the anxiolytic effects of diazepam compared to both Sprague Dawley and Lewis rats. Conversely, the Lewis strain exhibit an exaggerated response to methamphetamine compared to the Sprague Dawley strain. In addition, the Lewis strain has significantly greater hippocampal noradrenaline, 5-hydroxyindoleacetic acid and serotonin turnover compared to both Sprague Dawley and Wistar rats and significantly greater serotonin turnover in the striatum than Sprague Dawley rats. The identification of strain differences can influence decisions on the employment of one strain over another and appropriate controls when designing experiments. Furthermore, it may be beneficial to utilise more than one strain when assessing animal models used in behavioural pharmacology (Koopmans et al., 2007).

Chapter 8

General Discussion

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As outlined in section 1.1.1, a challenge in the provision of environmental enrichment conditions for laboratory rats is its variability. In the present experiment the nature of environmental enrichment was unaltered over three studies, i.e. the cage size, the housing density and the types of enrichment objects employed were the same, however the age of the rats at enrichment onset was varied from early adulthood (Chapters 3 and 4) to immediately post-weaning (Chapter 5). In the early adulthood studies, differential housing conditions were maintained for 4 weeks prior to behavioural testing, whereas in the post-weaning study, rats were housed for 5 weeks to ensure rats were sexually mature by the time behavioural testing began.

Body weight, open field test and elevated plus maze analyses were carried out in all three housing-condition studies. Early adulthood environmental enrichment had no significant effect on body weight compared to standard group-housed controls for male rats, while singly-housed rats were significantly heavier than standard and enriched groups in weekly body weight analyses (Chapter 3) and in overall weight gain (Chapters 3 and 4). Female rats exposed to an enriched environment in early adulthood had significantly lower weekly body weights, but no significant difference in overall weight gain, compared to standard group controls. This incongruity is difficult to explain, however it may be due to at least four of the singly-housed female rats losing weight on the final week of weight monitoring. Post-weaning male rats housed in isolated conditions were also significantly heavier than those in standard conditions but there was no difference between the two groups in overall weight gain. There was no significant effect of environmental enrichment or isolated conditions during adulthood on open field test activity compared to standard conditions, however post-weaning environmental enrichment significantly reduced distance moved and inner arena entries and time compared to standard grouped controls. Baseline measures in the elevated plus maze were inconsistent as in the first study rats housed in isolation had significantly lower percent open arm time compared to those in standard social groups, on the other hand, housing conditions had no effect on elevated plus maze parameters in the second early adulthood or post-weaning studies. When the effects of the anxiolytic diazepam in

the elevated plus maze were assessed, adulthood environmental enrichment housing blunted drug effects compared to groups in standard social and isolated conditions. However, there were no significant diazepam-induced effects on open arm time or entries in the post-weaning study. Social isolation and environmental enrichment conditions had no effect on latencies to reach the platform or time spent in the target quadrant of the Morris water maze acquisition and probe phases, nor were there any effects of housing on baseline time spent immobile in the forced swim test or activity (distance moved) in the homecage, regardless of the age at which differential housing was introduced. Similarly, isolation housing enhanced the antidepressant effects of desipramine in the forced swim test and attenuated the locomotor-enhancing effects of amphetamine in the homecage compared to control group-housed rats in both early adulthood and post-weaning protocols. The absence of housing effects on baseline behavioural measures is somewhat in contrast to previous studies; particularly as rats in post-weaning social isolation did not manifest behavioural hyperactivity and heightened anxiety levels as previously shown (Bianchi et al., 2006, Kokare et al., 2010). In the present study however all rats were handled more than once weekly and thus may not represent a truly isolated condition. Holson and colleagues (1991) demonstrated that rats housed in hanging cages, without human contact for up to 8 weeks displayed high levels of freezing and defecation in the open field test and greater plasma corticosterone levels in a novel environment than socially-housed (3/cage) or singly-housed rats in plastic-bottomed cages and were handled weekly for cage bedding changing. The latter isolated group and the social group did not differ in open field activity or corticosterone levels, thus isolated rats do not necessarily always display characteristics associated with social isolation syndrome (Holson et al., 1991). Monamine levels in brain regions were analysed using high performance liquid chromatography. Significant effects of social isolation and environmental enrichment housing conditions were observed in young adult male Sprague Dawley rats in chapter 4, but not in chapter 5, when housing conditions were provided immediately post weaning. The reason for the difference between the two studies is most likely due to the methods of tissue dissection; in chapter 4 brain tissue was harvested using the micropunch technique, in contrast to gross dissection in chapter 5. It may be that the larger volume of tissue in the latter study impaired detection of subtle differences in neurotransmitter concentrations.

Sex differences in baseline activity in the open field test were seen only in the socially-housed (i.e. standard and environmental enrichment) conditions in Chapter 4, while in Chapter 6, sex differences were evident between male and female rats housed in isolated conditions. Similarly, there was a significant sex difference in the elevated plus maze in Chapter 6 as females had greater movement velocity and percent open arm entries compared to males, while there was no significant sex effect in the elevated plus maze between singly-housed rats in Chapter 4. As previous studies have shown female rats to have greater overall (Olivier et al., 2008) and open arm activity (Weintraub et al., 2010) than males in the elevated plus maze, it may be that the use of counterbalanced design in Chapter 6 facilitated the detection of significant sex differences. In Chapter 4 there was an absence of sex differences in the elevated plus maze, it is possible that the females were in diestrous or metestrous cycle phases and elevated plus maze activity is similar to that of male or ovariectomised female rats (Marcondes et al., 2001, Frye et al., 2000).

There were few differences in baseline activity among the three albino strains studied. Wistar rats spent significantly more time climbing than Sprague Dawley counterparts; however there was no difference between the two in time spent immobile. The Lewis strain displayed enhanced responses to the anxiolytic effects of diazepam and to the stimulant effects of methamphetamine, while the Wistar strain showed a weak response to methamphetamine effects, compared to the Sprague Dawley strain. Overall these results suggest there are few differences between the “control” Wistar and Sprague Dawley strains; however methamphetamine locomotor response is somewhat strain-dependent. Previously in this laboratory, it was shown that 2 mg kg⁻¹ amphetamine significantly increased homecage activity of Wistar, but not Sprague Dawley male rats, compared to saline controls (McDermott and Kelly, 2008). A single dose of amphetamine was used in that study, moreover, the Wistar rats tended to have lower baseline activity levels than Sprague Dawley rats in the earlier study, which was not seen in the present study.

The effects of diazepam and desipramine were not consistent across experiments, male Sprague Dawley rats housed in isolated conditions displayed significant anxiolytic responses to diazepam in Chapter 6 and Chapter 3, but not in Chapters 5 or 7. Similarly in the strain study (Chapter 7), there was no significant desipramine effect on immobility in the forced swim test for male Sprague Dawley

rats, but effects were seen using the same drug dose and dose regimes in the other experiments. The reason for the difference in diazepam efficacy is unclear. Sprague Dawley rats' immobility time was relatively low in the strain study (Chapter 7) which may account for the absence of drug effects. The development of dose-response relationships is central to behavioural pharmacology; dose response curves are used to identify the dose at which drug effects first appear, peak and decline. Variables such as environmental conditions, sex or strain can influence the expected dose-response relationship, thereby affecting data and research results. In the present study three bioassays were employed; the anxiolytic effects of diazepam in the elevated plus maze, the antidepressant effects of desipramine in the forced swim test and the locomotor effects of psychomotor stimulant drugs on homecage activity were assessed. Dose-response relationships were modelled in each of the three bioassays (Fig. 8.1). Male Sprague Dawley rats, housed in isolation, displayed robust desipramine dose-related reductions in immobility time in the forced swim test; this group were also sensitive to the effects of diazepam in the elevated plus maze however significant decreases in open arm time were less reliable than the drug effects observed in the forced swim test. On the other hand, housing with cage-mates and environmental enrichment, female sex and the Lewis rat strain facilitated pronounced dose-response effects to the stimulant drugs methamphetamine and amphetamine.

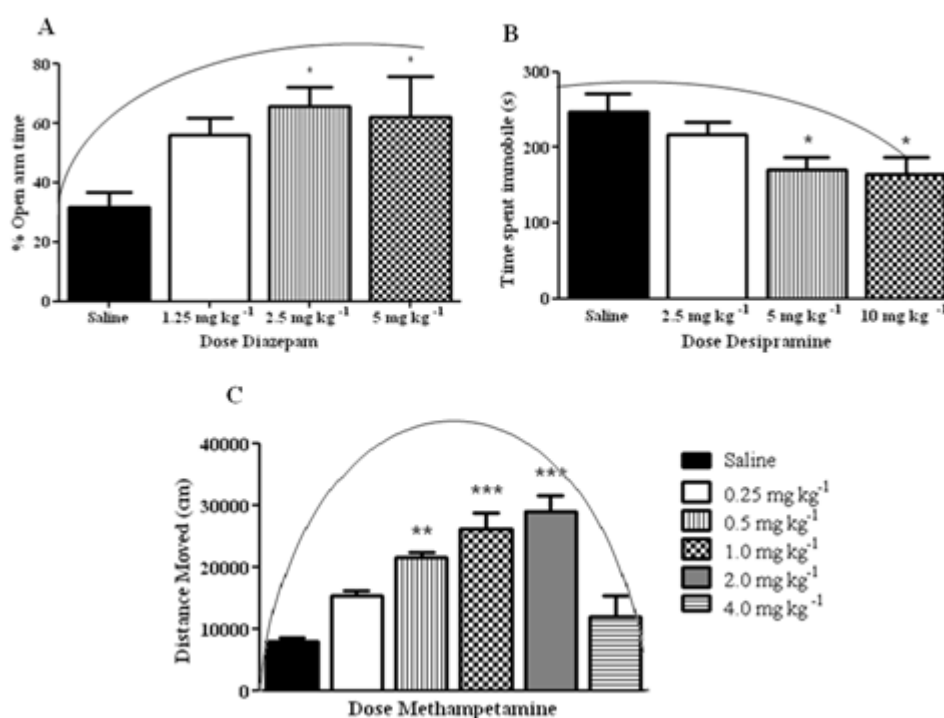


Figure 8.1: Examples of dose response relationships, diazepam effects on percent open arm time in the elevated plus maze (A), desipramine effects on immobility in the forced swim test (B) and methamphetamine effects on distance moved in the homecage (C).

Drug disposition also contributes to the development of dose-response relationships. As in humans, a number of factors can influence the rate and magnitude of absorption, distribution, metabolism and elimination of drugs in the rat. The intraperitoneal route of drug administration was used for the administration of diazepam and desipramine in these studies. This route of drug administration is beneficial as a large absorptive area is available and the drug enters systemic circulation relatively rapidly, however absorption may be comprised if care is not taken to inject to the intraperitoneal cavity and avoid any organ damage or injecting too close to the thigh muscle. The amount of drug reaching systemic circulation following subcutaneous bolus injection may be variable depending on local blood flow at the injection site. Drug distribution depends on the amount of free drug available, pH partitioning and drug lipid solubility. In rats, the metabolism and elimination of drugs can depend on factors such as age, sex and strain. Due to sex differences in the liver cytochrome P450 enzymes, female rats tend to eliminate drugs from the body at a slower rate than males (Mugford and Kedderis, 1998), in an

early study it was shown that male rats had over twice the rate of liver microsomal binding for hexobarbital and aminopyrine than females (Schenkman et al., 1967). In contrast, ethanol elimination from blood and the brain was faster in females than in males following intravenous administration; after intragastric delivery, blood ethanol levels peaked approximately 20 minutes faster in female rats than in males, this implies faster absorption or diffusion from the stomach in females. Likewise, microdialysis in the nucleus accumbens demonstrated ethanol concentrations peaked faster in females than males by almost 30 minutes, suggesting faster distribution to the brain (Robinson et al., 2002). Female Dark Agouti rats have deficient cytochrome P450 2D6 subtype enzymes compared to Sprague Dawley counterparts; as a result the Dark Agouti rat have higher serum and brain levels of drugs which are 2D6 substrates, such as the antidepressant drug citalopram, and slower drug clearance than Sprague Dawley rats (Kingback et al., 2011). Similarly, Wistar rats had a significantly lower plasma drug (naltrexone) concentration and greater clearance rates than an alcohol-preferring strain (Akala et al., 2008). Differences in pharmacokinetics such as these may contribute to differences in pharmacodynamics as the drug metabolites can have pharmacologic or toxicological effects in the rat.

In the present experiments, the importance of appropriate controls is apparent. For example, in the present research, male rats in environmental enrichment had significantly lower body weight than those in isolated conditions, but not compared to those in standard conditions; similarly enrichment promoted significantly greater open arm time in the elevated plus maze compared to singly-housed rats but not compared to standard grouped controls. Strain differences were demonstrated in drug responses and monoamine levels in the hippocampus and striatum. There was no difference between Sprague Dawley and Wistar rats, each of which are widely employed as control strains in behavioural pharmacology research, in baseline activity. However the blunted dose-response curves observed by the Wistar strain compared to Sprague Dawley and Lewis rats illustrate the importance of a comparator strain group in bioassays; diazepam and methamphetamine effects were attenuated in the Wistar rats but heightened in the Lewis strain and somewhat intermediate in the Sprague Dawley rats. This is relevant as results interpretation is dependent on the control group employed. In the present study, Lewis rats had significantly increased serotonin turnover in the hippocampus compared to Sprague

Dawley, but not compared to Wistar rats. Scholl and colleagues (2010) affirmed that strain effects on experimental manipulations (e.g. stress effects) may be identified when compared to one control strain but not when compared to another (Scholl et al., 2010). The identification of sex differences in baseline behaviour was not novel; however, the absence of dose-response effects in the elevated plus maze and forced swim test for female rats, validated tests in behavioural pharmacology, have not been previously characterised. Monitoring sex differences in stimulant dose-response effects on locomotor activity in the homecage is also novel. The diazepam/elevated plus maze and desipramine/forced swim test bioassays do not produce equivalent results for both males and females. Male rats were more sensitive to the development of dose-response curves than females due to their lower baseline open arm time in the elevated plus maze and higher immobility time in the forced swim test. Challenges exist therefore for identifying bioassays suitable for screening the efficacy of anxiolytic and antidepressant drugs in female rats.

Overall, the results of the studies described suggest minimal effects of social isolation or environmental enrichment housing conditions on baseline activity in the behavioural tests employed. Similarly there were few differences in baseline behaviours of Lewis and Wistar rats compared to the Sprague Dawley strain. Sex differences had the greatest effects on baseline activity. Psychotropic drug responses however were influenced by housing, sex and strain.

8.1 Future Work

Future work could be merited in all three areas addressed in this thesis. Regarding housing, sex and strain, one area of potential interest is the measurement of stress hormones. The absence of any baseline differences in behaviour in the elevated plus maze or open field test may be extended by analysis of plasma corticosterone and adrenocorticotrophic hormone levels. In addition, measurement at intervals throughout enriched and isolation housing periods could assess any temporal effects of housing conditions on stress markers. Sex differences in glucocorticoid levels have been established, however, to further investigate the sexual dimorphisms in response to the elevated plus maze and forced swim test, markers of the hypothalamic pituitary adrenal axis in response to these tests may be assayed in male and female rats. Similarly, any strain differences in stress reactivity may be noteworthy, in addition, an albino and hooded strain profile comparison may be

considered in future experiments as the Lister Hooded strain are often employed in this laboratory.

Future investigation of the effects of environmental enrichment may include an isolated condition with the provision of physical enrichment, as in some circumstances in this laboratory rats must be singly-housed. Any effect of the provision of enrichment objects in this condition will be pertinent as enrichment implementation is becoming mandatory while at the same time, researchers seek to maintain consistency with previously-conducted experiments without enrichment objects. Any effects of isolation and enriched housing on female rats' responses to drugs may also be a potential extension of the work carried out as part of this thesis in the future. The influence of post-weaning environmental enrichment or social isolation on neurogenesis in the rat hippocampus was proposed in the present study. Bromodeoxyuridine was administered to rats 24 hours prior to sacrifice, however, after several endeavours, immunohistochemical analyses were discontinued as it is believed that an insufficient amount of bromodeoxyuridine reached cerebral circulation due to an absence of widespread specific staining in the hippocampal brain sections; in future this experiment could be re-visited and refined.

Sex differences in male and female responses to desipramine and diazepam in the forced swim and elevated plus maze tests could be further addressed in future work, employing different tests. For example male and female serotonin knockout rats may be utilised to model equivalent immobility times between the sexes in the forced swim test (Olivier et al., 2008) and thus assess any sex differences in antidepressant efficacy. Alternatively a different behavioural model of depressive symptoms such as a chronic mild stress protocol may be used for screening antidepressant drug efficacy. Similarly, the potential confound of locomotion (which is affected by variables such as strain, sex, effects of circadian rhythms and sound) associated with anxiety tests such as the elevated plus maze suggest the use of other anxiety tests such as social stress or defensive burying tests to assess anxiolytic dose-response effects. On the other hand, it may be that it is not feasible to measure depression- and anxiety- like behaviours in male and female rats using the same tests and test parameters. Due to the effects of the estrous cycle on females' baseline behaviours (outlined above), a future experimental design of sex differences (and/or sex differences in response to differential housing conditions) will include

monitoring of the estrous cycle. Such studies would include investigation of whether cycle stage affects sensitivity to psychotropic drugs such as desipramine, diazepam and amphetamines, as this has not yet been extensively profiled.

In light of the results of the pharmacological studies in this thesis, it may be interesting in future studies to measure pharmacokinetic parameters in rat blood and brain. Differences in drug disposition based on sex and strain may account for the divergent responses observed in the present experiments. Plasma, serum, urine and post-mortem brain tissue levels of drugs and their metabolites may be assayed in this laboratory using specific high performance liquid chromatography. These measures may offer explanations for variations in psychotropic drug responses.

In summary, baseline behaviour was most affected by sex differences, while responses to psychotropic drugs were influenced by IC and EE housing, sex and strain. Monoamine levels differed according to sex, housing conditions and, to lesser extent, strain. The studies reveal that housing conditions, sex, strain and the employment of suitable control groups, in addition to methodological variables, can impact on behavioural and neurochemical endpoints. Future studies may extend this research in areas such as determination of stress hormone levels, estrous cycle effects, markers of hippocampal plasticity and drug disposition. The effects of housing, sex and strain on rats' responses to psychotropic drugs highlight the sensitivity of commonly-employed behavioural pharmacology tests to such variables. These findings stress the impact of appropriate subject selection and experimental design on behavioural and biochemical endpoints.

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Appendix (Published Works)

Simpson, J and Kelly, JP (2012). The effects of isolated and enriched housing conditions on baseline and drug-induced behavioural responses in the male rat. *Behavioural Brain Research*, **234**, 175– 183.
<http://dx.doi.org/10.1016/j.bbr.2012.06.015>

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Simpson, J, Ryan, C, Curley, A, Mulcaire, J and Kelly, JP (2012). Sex differences in baseline and drug-induced behavioural responses in the Sprague Dawley rat. *Prog Neuropsych Bio Psych*, **37** (2), 227–236.
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<http://dx.doi.org/10.1016/j.bbr.2011.12.036>

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