# THE EFFECTS OF *LAVANDULA ANGUSTIFOLIA* ON ANIMAL AND HUMAN LABORATORY MODELS OF ANXIETY.

**by**

**Belinda Fay Bradley**

A thesis submitted in partial fulfilment for the requirements for the degree of Doctor of Philosophy at the University of Central Lancashire

**November 2008**

# STUDENT DECLARATION

**Concurrent registration for two or more academic awards**

I declare that while registered for the research degree, I was, with the University's specific permission, an enrolled student for the Graduate Diploma in Psychology.

**Material submitted for another award**

I declare that no material contained in the thesis has been used in any other submission for an academic award and is solely my own work.

**Signature of Candidate**

_____

**Type of Award** _____

**School** _____

I also grant to grant powers of discretion to the depository library to allow this thesis to be copied in whole or in part without further reference to the author. This permission covers only single copies made for study purposes subject to normal conditions of acknowledgement.

# ABSTRACT

Lavender (*Lavandula angustifolia*) is a popular treatment for stress and mild anxiety. Currently, there are few reliable investigations of its efficacy because cognitive and associative effects of odours can confound pharmacological effects. Some of these problems can be overcome by testing the effects of odours in animals, and by using orally-administered lavender in sealed capsules in human participants. In addition, a criticism of current studies is that most employ short-term administration of lavender, even though humans most often use lavender over longer time-periods.

There are two parts to this thesis. The first part addressed two questions; whether lavender odour exhibits anxiolytic effects in animal models of anxiety, and whether chronically administered lavender is more effective than acutely administered lavender. The second part addressed the question of whether, in a randomised double-blind placebo-controlled trial, orally-administered lavender exhibits anxiolytic effects in humans.

This thesis makes three significant contributions:

First, these studies provided a validation of the gerbil elevated plus-maze model of anxiety in both male and female gerbils, a model that has only previously been validated in female gerbils (Varty et al., 2002).

Second, the studies on gerbils have shown that both lavender and rose essential oils have anxiolytic effects, which, rather than dissipating following acute odour administration (Cooke & Ernst, 2000), potentiate over time. Lavender's effects were particularly apparent in female gerbils on measures related to risk-assessment, a behaviour that has been related to the human anxiety trait of worry (Blanchard, Blanchard, Griebel, & Nutt, 2008).

Third, lavender had a clear dose response effect in reducing baseline anxiety in humans when tested acutely via oral administration, although there were no effects when more severe anxiety was induced. The route of administration and the fact that

lavender had dose response effects indicate that lavender's effects are not caused by psychological qualities of the odour, but are more likely to be due to direct pharmacological effects. Again, and comparable to results in gerbils, lavender's anxiolytic effects in human females were more noticeable, particularly during the anxiety task and in the recovery phase of the study.

In summary, prolonged exposure to lavender odour relieved anxiety in a validated animal model of anxiety, and orally-administered lavender alleviated mild anxiety in humans. In both cases, results were more prevalent in females.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ACKNOWLEDGEMENTS

Whom should I thank and acknowledge? The list is endless!

Firstly, I would like to thank my supervisors, Dr. Steve Brown, Dr. Bob Lea, Dr. Simon Chu, and Dr. Nicola Starkey, for their help, support, advice, encouragement and their endless patience. Thanks also to Paul Pollard (and his recent successor Professor Jane Ireland), Professor John Archer and Professor John Fisk, for their support of this work. Thanks too to Charlie Mullin, principal technician, and his successor, Kit Jordan, for encouraging me to climb this lofty mountain and for providing me with the support to complete it. Thanks to Nikola Bridges for assisting me in the validation of the elevated plus-maze and black white box. Likewise, thanks to Christine Woodcock and Rose Cley for taking good care of the gerbils and I really appreciated seeing another human face at those early hours on a Saturday and Sunday morning; to Adam Palmer for help editing the film-clips; Jim Donnelly and Richard McCabe for their invaluable support and advice with the GC/MS; Steve Atkins for arranging the phlebotomy course and Mark Roy for his useful advice, encouragement and for his patient supervision while I practiced my newly gained phlebotomy skills. Thanks also to Phil Roberts for reading the pharmacology part of my thesis and for his invaluable advice on this section; to my dear old friends Dr Mike and Doreen Willians, to Mike for statistical advice and to Doreen for her friendship and encouragement. Thanks also to Sarah Dennison for her cheerful encouragement during those months of writing up and also for testing some of my essential oil components in her lipid membrane system (hopefully we will be able to continue this work). I must also mention my Christian friends at home (St. Leonard's church) and at work (UCLAN) for their encouragement, prayer and initial proofreading (Brian Moore). Thanks also to William Sheridan, former Head of Technical Books at Oxford University Press, for his advice and for proofreading my thesis. Thanks also to Dr John Robertson, and Helen Farrell, Literary Fellow, UCLAN, for their invaluable advice. Finally, thanks to my family: Rob, Vicki, Mum, Dad, Auntie Pam, Grandma and my partner, Mark without whom I would not have got so far with this project and to whom I owe a great deal. Thanks, also to all the other people not mentioned here who I have met at conferences and in normal daily life and who have inspired me to continue with this work.

**Ladies fair, I bring to you**
**Lavender with spikes of blue;**
**Sweeter plant was never found**
**Growing on our English ground.**

**Caryl Battersby c. 1890**

# ABBREVIATIONS

5HT – 5 hydroxy-tryptamine (serotonin)

AC – adenylate cyclase

ACTH – adrenocorticotropic hormone

AMPA – alpha-amino-3- hydroxy - 5 - methyl 4-isoxazolepropionic acid

AOB – accessory olfactory bulb

APA – American psychiatric association

BDZ –benzodiazepine

BIS – behavioural inhibition system

BWB – black white box

CAM – complimentary and alternative therapy

cAMP- cyclic adenosine mono-phosphate

CCK – cholecystokinin

CNS – central nervous system

CNV – contingent negative variation

CRF corticotropin releasing factor

DBI – diazepam binding inhibitor

DR - dorsal raphé

DSM IV – The diagnostic and statistical manual of mental disorders 4$^{th}$ Edition

EEG – electro-encephalogram

EMG- electromyogram

EO – essential oil

EPM – elevated plus-maze

FRSA – free radical scavenging variation

GABA – gamma amino butyric acid

GAD – general anxiety disorder

GSL- galvanic skin level

GSR – galvanic skin response

HPA – hypothalamic pituitary axis

HR – heart-rate

HRV – heart-rate variation

i.p. - intraperitoneal

IBI – inter-beat-interval

ICD 10 – The international and statistical classification of diseases and related health problems 10th revision

ICU – intensive care unit

J-T – Jonckheere-Terpstra Test

LC – locus coerulueus

MAOI – monoamine oxidase inhibitor

mCPP – 1-(3-chlorophenyl) piperazine

MHPG – 3, methoxy, 4-hydroxyphenylglycol

MPEP – methyl (phenyl ethynyl) pyridine

MRN – median raphé nucleus

NA – noradrenaline

NICE - national institute of health and clinical excellence

NK1 – neurokinin receptor type one

NMDA – N-methyl-D-aspartic acid

NN 50% - the percentage value of consecutive R-R intervals that differ more than 50ms

NO – nitric oxide

NOS – nitric oxide synthase

NPY – neuropeptide Y

NT – neurotransmitter

OCD – obsessive compulsive disorder

PAG – periaqueductal grey

PANAS – positive negative affect schedule

PTSD – post-traumatic stress disorder

RCT – randomised double-blind placebo-controlled trial

RMSSD – root mean square value of consecutive R-R intervals

SAM – symapthoadrenomedullary system

SDRR – standard deviation of the inter-beat interval

SERT – serotonin re-uptake transporter site

SP – substance P

SSRI – selective serotonin re-uptake inhibitor

STAI-Y – Spielberger's state/trait anxiety inventory version Y

U –Mann Whitney's U

VNO – vomeronasal organ

# CHAPTER 1 INTRODUCTION AND BACKGROUND

## 1.1 OVERVIEW OF CHAPTER

This thesis is about an investigation into the ameliorative effects of lavender (*Lavandula angustifolia*) on mild anxiety. In order to describe this investigation and its implications it is important that the reader understands something about anxiety. The first section of the introduction is a description of anxiety, its possible biological causes and cures, and why sufferers of stress and mild anxiety might self-medicate by turning to complementary and alternative medicines, such as lavender essential oil. This section is followed by a discussion of the studies that have been conducted to examine lavender's effects on anxiety. The lavender section includes a discussion of possible psychological effects of lavender caused by its odour, and then a review of the animal literature and any relevant *in-vitro* effects of lavender, which might provide an explanation for its potential anxiolytic mechanisms. There is also a short section on rose oil, as there is a study included in the thesis which examines some of the effects of rose essential oil on anxiety. Since the mode of action of these essential oils on anxiety might be via the olfactory route, a brief description of the potential routes by which lavender and rose can exert their actions is provided. Finally, the aims and hypothesis for this thesis are presented.

## 1.2 ANXIETY

Anxiety disorders are the most prevalent form of diagnosed mental illness in Europe and the USA. In any 12-month period 15% of the population is estimated to suffer from anxiety, while over the average lifespan 11%-20% in Europe and 24.9% in the USA will be diagnosed with an anxiety disorder (Bruce et al., 2005; Andlin-Sobocki & Wittchen, 2005). These disorders often start early in adult life and last for many years. Having one anxiety disorder worsens the prognosis for other types of anxiety disorder and other mental health problems, such as major depressive disorder (Gale & Davidson, 2007). Anxiety disorders have been described as 'insidious', having a

chronic course, low rates of recovery, and a relatively high probability of recurrence (Bruce et al., 2005).

Thus, anxiety disorders have important implications in terms of medical costs and costs to employers because of time taken off work by employees. At least 35% of all sickness absences caused by mental disorders are reported to be due to anxiety disorders, for which sufferers are rarely given the correct diagnoses or support (Andlin-Sobocki & Wittchen, 2005). It has been reported that anxiety disorders represent the largest mental health problem in the USA, and affected men are four times more likely than non-sufferers to be chronically unemployed (Rachmann, 2002). In the USA, the total cost of anxiety disorders was estimated to be $40 billion (Andlin-Sobocki & Wittchen, 2005). In the UK, anxiety and depression were estimated to cost £12 billion per year in time off work (BBC, 2007).

### 1.2.1 Description of anxiety

Anxiety is an unpleasant, aversive, state characterised by high levels of negative affect and arousal, for example, feeling nervousness, apprehension, worry, a desire to escape from current circumstances, and hypervigilance. Somatic symptoms might include activation of the autonomic nervous system, characterised by racing heartbeat, sweaty palms, dry mouth, increased muscle tension, possibly increased breathing-rate (in the case of panic attacks) and increased secretions of hormones, such as adrenaline and cortisol.

Anxiety is unusual in that it represents both a normal state, allowing a person to adapt to his or her environment, and a psychopathology, leading to ill health. In normal state-anxiety, the systems required to deal with stressful situations are mobilised. For example, the fight, flight, or freeze (FFFS) response is mobilised to escape from, or deal with and lessen, the impact of an immediate threat to wellbeing (Gray & McNaughton, 2003). Alternatively, in situations where the threat is not immediate, approach-avoidance behaviour, such as risk-assessment, might be mobilised. A side-effect of this is an increase in levels of arousal; this sometimes aids performance in tasks such as public speaking (Argyropoulos, Sandford & Nutt, 2000). However, some people are more anxious than others, even in situations that are only mildly threatening. As a consequence of this, a distinction can be made between state and

trait anxiety. While state anxiety depends on circumstances at a point in time and mood variations, trait anxiety is linked to stable heritable factors and is a personality trait. Generally, people with high trait anxiety are more likely to develop pathological anxiety (Gray & McNaughton, 2003). Pathological anxiety is at the extreme end of the continuum and is a result of an interaction between state and high trait anxiety (Sandford, Argyropoulos & Nutt, 2000). It results in feelings of extreme, and often prolonged, anxiety without any obvious stimulus, which is very distressing for the sufferer, their friends and their relatives. These people are classed as suffering from an anxiety disorder (Chambers, Power & Durham, 2004; Carr, 2001).

Anxiety is not a unitary phenomenon and diagnosing anxiety disorders is not straightforward. Pathological anxiety encompasses a number of different conditions that present in different forms and respond to different treatments. Symptoms of anxiety might include the following: free-floating worry that is unlinked to anything in particular and can last for a number of years, panic-attacks that are short-lived and very intense, and phobias of specific objects. For this reason and because different types of anxiety respond better to different types of treatment, two systems of classification of anxiety disorders, based on symptom patterns, have been developed to assist physicians in making diagnoses. These are 'The International Statistical Classification of Diseases and Related Health Problems' (ICD10) and 'The Diagnostic and Statistical Manual of Mental Disorders Criteria', Version 4 (DSM IV) (American Psychiatric Association (APA) 2000). ICD10 is published by the World Health Organization (WHO) and classes anxiety disorders as neurotic, stress and somatoform disorders. In DSM IV, which is published by the American Psychiatric Association (APA), they are classed as anxiety disorders. Both manuals are virtually identical in their classification of anxiety disorders and both use the same diagnostic codes to identify the different types of anxiety. Table 1.1 (below) shows a summary of anxiety disorders as classed by DSM IV (APA, 2000; Antony, Orsillo & Roemer, 2001).

**Table 1.1: Summary Table of Anxiety Disorders According to DSM IV.**

| **Anxiety Disorders** | **Key Features** |
|---|---|
| **General anxiety Disorder** | Excessive anxiety and worry out of proportion to the event over at least six months. It is accompanied by fatigue, difficulty concentrating, irritability, muscle tension, and disturbed sleep. Could also include headaches, muscle twitching, sweating, nausea, and other hypervigilant reactions. |
| **Panic Disorder without Agoraphobia** | Recurrent, unexpected panic attacks, associated with persistent concern about the attacks. |
| **Panic Disorder with Agoraphobia** | Panic Disorder associated with agoraphobia (i.e. fear and avoidance of situations in which escape might be difficult or embarrassing, or in which help might not be available in the event of a panic attack or panic like sensations). |
| **Agoraphobia without history of Panic Disorder** | The presence of agoraphobia in an individual who has never met full criteria for panic disorder. |
| **Specific Phobia** | Clinically significant anxiety, fear and avoidance related to a specific object or situation. For example, heights, animals, blood, injections, flying, enclosed places. |
| **Social Phobia** | Clinically significant anxiety, fear and avoidance related to social and performance situations, associated with a fear of embarrassment or humiliation. |
| **Obsessive Compulsive Disorder** | The presence of obsessions (thoughts, urges, or images that are distressing and intrusive) and compulsions (repetitive behaviours meant to reduce anxiety or prevent perceived danger). |
| **Post Traumatic Stress Disorder and Acute stress Disorder** | Re-experiencing a traumatic event, accompanied by symptoms of increased arousal and avoidance of situations and thoughts that remind the individual of the event. |
| **Anxiety Disorder Caused bya General Medical Condition** | Anxiety symptoms that are the direct consequence of a general medical condition (e.g. panic attacks caused by hyperthyroidism). |
| **Substance Induced Anxiety Disorder** | Anxiety symptoms that are the direct consequence of a substance (E.g. cocaine). |
| **Anxiety Disorder not Otherwise Specified** | Disorder with prominent anxiety or phobic avoidance not meeting criteria for a specific anxiety disorder or for which there is inadequate or contradictory information. |

(APA, 2000; Antony et al., 2001).

Epidemiological studies reported by DSM IV show that the lifetime prevalence of anxiety disorders, such as panic disorder (PD) is between 1.5%- 3.5%, simple phobia between 10%-11.3%, social phobia between 3%-13%, obsessive-compulsive disorder (OCD) 2.5%, post-traumatic-stress disorder (PTSD) 1%-14%, and general anxiety disorder (GAD) 5% (Souetre et al., 1994; Andlin-Sobcoki & Wittchen, 2005). As mentioned previously, the chances of recurrence are high once diagnosed and often sufferers go on to develop other mental illnesses such as major depression. According to DSM IV about 55-65% of those presenting with the disorder are female and about two thirds of diagnoses are in females (APA, 2000; Kinrys & Wigant, 2005). This does not necessarily mean that fewer men than women suffer from anxiety. In many cases, anxiety goes undiagnosed and sufferers self medicate, for example with alcohol, which ultimately exacerbates the problem, or they are wrongly diagnosed by the GP and the somatic symptoms are treated rather than the psychological ones. For example, the patient might be sent to see a cardiologist for symptoms of raised blood pressure and heart-rate, rather than being treated for anxiety (Lydiard, 2000). In addition, patients often suffer from more than one of the disorders at once. Consequently, there is a lack of homogeneity in the types of disorders suffered. Added to this some sufferers do not have severe enough symptoms to be classified in one of the current categories, while clearly suffering from anxiety.

### 1.2.2 Current understanding of the aetiology of anxiety

Anxiety is not a clear phenomenon and there is confusion about whether it is a feeling, behaviour, or an expression of underlying processes. However, there is general agreement that it is expressed via somatic, cognitive, affective, and behavioural components (Lang 1968). Extremely complex in its aetiology, it is a transitory and unpleasant emotional state, involving both tension and apprehensive thought. It is also a relatively stable personality trait (Edelmann 1992; Rachman, 2004; Spielberger, 1984; Bolmont and Abraini, 2001).

Clinicians, psychologists, biologists and other workers have produced theoretical models as frameworks in which to understand and better treat anxiety. As a result there are many models of anxiety, some categorical (e.g. DSM IV and ICD 10), and some dimensional. The categorical models, such as DMS IV, are popular because

they enable clinicians to make simple yes/no diagnoses of anxiety in sufferers. However, these types of models of anxiety often are less than satisfactory, as they fail to distinguish between different types of illness. For example, GAD and major depression both contain insomnia, fatigue, irritability, restlessness, and difficulty concentrating as criteria for diagnosis (Brown, Chorpita, Barlow, 1998). Additionally, the categorical approach tends to assume that the disorders are as a result of abnormal psychopathology, rather than being at the far end of a continuum. This often results in failure to diagnose and treat someone with an anxiety disorder or leads to misdiagnosis. Dimensional theories overcome some of these problems and provide more information, making diagnosis more complex but better informing researchers about anxiety.

As already mentioned, at a very basic level anxiety can be described as a state or a trait condition. More often than not, it is brought on by an interaction of the two (Sandford, Argyropoulos and Nutt, 2000). Spielberger (1966) based his model of anxiety on these constructs and argued that the scales of state and trait anxiety that he created are bi-polar, each containing state or trait absent and state or trait present items, and that the two constructs are unidimensional (Spielberger, 1984; Endler & Kocovski, 2001). A person who is high in trait anxiety might be more likely to see a potentially threatening situation as more aversive than a person low in trait anxiety. More recently, it has been reported that Spielberger's model might also incorporate aspects of depression (Grös, Antony, Simms & McCabe, 2007). Nevertheless, Spielberger's model has been used extensively in anxiety research and the State/Trait Anxiety Inventory (STAI) provides a broad measure of changes in state anxiety in a research/clinical setting (Endler and Kocovski, 2001). Development of these models has led to the more recent suggestion that both trait and state anxiety are multidimensional constructs.

Eysenck's theories plot personality types on a grid consisting of two perpendicular axes: neuroticism and stability, and extraversion and introversion. These factors are orthogonal, that is, completely independent of one another. He argues that neurotic introverts are more likely to be open to conditioning than are extroverts. Consequently, those with neurotic introverted personality traits are more likely to develop anxiety disorders. From experiments with twins he also suggested that at least three quarters of the total variance for differences between individuals with

respect to neuroticism and extraversion is caused by heredity factors (Eysenck 1957). Eysenck also attributed the persistence of anxiety behaviours to the fact that, in order to avoid anxiety, people attempt to escape or avoid the stimulus thus relieving the symptoms of the anxiety and, consequently strengthening the behaviour pattern (Rachman 2004).

Watson and colleagues (1995) tripartite model is also multidimensional. This model provides a dimensional explanation for anxiety and depression. It is a psychological model based on affect or mood (Watson, Weber, Assenheimer, Clark, Strauss, & McCormick 1995). Moods are more permanent versions of emotions, which tend to be fleeting and short in time. This model has shown some use in separating constructs belonging to both anxiety and depression (Watson, et al., 1995). It also provides a way of distinguishing between anxiety and depression psychometrically. The model proposes two factors specific to anxiety and depression, and a general distress factor. It is based on Watson and Tellegan's (1985) work, dissecting mood into two components: negative affect and positive affect (Watson et al., 1995). Negative affect measures aversive and distressed mood states, such as those experienced in anxiety and depression, for example feeling upset, guilty, afraid, sad, scornful, and disgusted. Watson and Clark (1992) linked it to the neuroticism of Eysenck's theory; hence, anxiety would score highly on these factors. Positive affect consists of feeling active, delighted, interested, and enthusiastic, thus depression would be the opposite of these resulting in low positive affect. These two mood states are largely independent of one another, and show an interesting and differential pattern in response to depression and anxiety both centre on negative mood states and show high negative affect. By comparison, anxiety and depression show very different associations with positive affect; low positive affect is consistently associated with depression, whereas anxious mood is largely unrelated to positive affect (Watson, Weber, Assenheimer, Clark, Strauss, McCormick, 1984). The third dimension, which was later added to the model (Watson et al., 1995), somatic arousal, is characterised by tension, shortness of breath, dizziness, light-headedness, trembling and shaking. Hence, anxiety is characterised by high negative affect and high somatic arousal, and depression is characterised by low positive affect. This model has gained wide clinical acceptance in work with both adults and children (de Beurs, den Hollander-Gijsman, Helmich, Zitman, 2007).

In contrast with the dimensional theories, the cognitive theories place emphasis on faulty thought patterns and the subjective interpretation of events by the individual, resulting in a somatic response to the stimulus and an emotion, in the form of anxiety. Individuals interpret events differently depending on their mood, trait personality, circumstances and experiences. Also, thought processes differ from one individual to the next, consequently, some individuals will cope, while others will suffer from anxiety (Edelmann 1992). Hence, cognitive theories suggest that individuals who are more prone to anxiety add more negative valence to novel situations and interpret them as threatening. These individuals are also more selectively attentive to threat cues than other individuals and add greater weight to the likelihood of the occurrence of negative events (Mathews, Mackintosh, and Fulcher, 1997). These theories also make room for the acquisition of fears by the process of learning, including conditioning, but place emphasis on the affected person's interpretation of the events (Rachmann 2002). For example, one cognitive theory suggests that panic is the result of catastrophic misinterpretations of bodily sensations (Salkovskis, Clark, & Gelder 1996). A more recent cognitive model of anxiety is one that consists of three stages: registration of the threat, activation of the threat and evoked secondary elaborative checking (Beck & Clark 1997). This theory also states that the only way to overcome the anxiety is to deactivate the threat registration mode.

Gray's theories, which are the most comprehensive of all the models of anxiety, are based on dimensional theories, cognitive learning theories, and biological research. Gray has built on Eysenck's theory of anxiety, which is set in a learning framework. Gray's theory initially added to Eysenck's theory by adding another axis, this axis was at 30-degrees to the two axes in Eysenck's theory (Gray 1970; Matthews & Gilliland 1999). It was an axis of impulsivity and neuroticism. Within his theories, high trait anxiety is characterised by a combination of introversion and neuroticism. Gray's theory also takes into account both the biological and behavioural models of anxiety and so is mainly psycho-physiological (Gray & McNaughton, 2003). Gray has carried out a vast number of studies on learning in animals to provide evidence for his theory, which is about personality and places an emphasis on the interaction of emotions and physiology. He hypothesised that emotions, impulsivity, and neuroticism, which includes anxiety, are a result of three interacting systems; the behavioural inhibition system (BIS), the behavioural approach system (BAS), and the fight, flight and freezing system (FFFS). The BIS has been linked to the negative

affect in Watson's model (1985) and to neuroticism in Eysenck's model of anxiety (1957). Positive affect and extroversion have been associated with the BAS (Jorm, Christensen, Henderson, Jacomb, Korten, & Rodgers 1998). However, this model differs from Watson and Tellegan's (1988) model in which positive and negative affect are orthogonal (unrelated) constructs, in that the BIS, the BAS, and the FFFS are all assumed to interact with one another.

The BIS is involved in conflict resolution and all stimuli which activate the BIS should generate a conflict between competing goals. In fact, Gray argues that anxiety is a disorder based on conflicts between goals. For example, an approach-avoidance conflict might be observed when an organism wishes to approach an area previously occupied by a predator (Gray & McNaughton, 2003). The BIS enables screening of the situation for potential threat and then, depending on information gathered, either inhibits ongoing behaviour or increases arousal and vigilance ready for a FFFS response. Alternatively, if the situation is not assessed as threatening by the BIS, approach behaviour can continue. The BIS represents a neurophysiologic system, which is based in the hippocampus, the septum, parts of the limbic system and the frontal lobes of the cerebral cortex, in a pathway that Gray refers to as the septohippocampal loop, but which also involves the amygdalae, in forming a stop-look-listen, system. This system prepares the organism for action should it be necessary (Clark, Watson, Mineka, 1994; Gray and McNaughton, 2003). It is the BIS which Gray has proposed is involved in trait anxiety. The BAS is sensitive to stimuli associated with reward and comprises dopaminergic pathways.

The FFFS is hypothesised to mediate responses to all aversive stimuli, whether innate or conditioned. It is based in lower brain regions, such as the periaqueductal grey, the hypothalamus, and the amygdalae. Further additions to this hypothesis state, that these systems do not work alone, and that it is an interaction between the BIS, the BAS and the FFFS in an approach or avoidance conflict, which cause anxiety. Gray also takes the view, suggested by the Blanchards following extensive work on rodents, that fear is a response to actual threat and anxiety is a response to potential threat (Blanchard, Blanchard, Griebel & Nutt, 2008). In Gray's system, the FFFS mediates fear and the BIS mediates anxiety.

Gray's theory also goes some way to encompassing cognitive theories of anxiety. For example, when the BIS is screening for potential threat via parts of the brain that are involved in memory, such as the hippocampus and amygdala, it can recall and add negative valence to memories and provide more negative bias to them, resulting in an anxiety response (Gray & McNaughton, 2003).

However, anxiety in humans is complex and these theories, although going someway to aiding the understanding and therefore the treatment of anxiety, do not explain everything. Cognitive causes of anxiety might be more important than the biological basis suggested by Gray and Eysenck. A problem with the learning theory is that quite often individuals develop phobias to things that have never been presented to them as a threat. For example workers have shown that fear of monkeys, rats, spiders and snakes can be induced very quickly and can be triggered by observational learning, suggesting that certain phobias might be hardwired (Ohman & Mineka 2001). Further experiments have shown that the amygdala is the source of such fears. From an evolutionary survival point of view, having hard-wired fears to danger makes sense (Ohman & Mineka 2001). Cognitive theories tend to focus on phobias; they do not take into account general anxiety disorder, in which anxiety might not be linked to any particular event but is unspecific and future oriented (Edelmann 1992).

The biological models look to biological causes for the many different anxiety disorders, such as alterations in neuroanatomy, up- or down-regulation of receptors involved in the neurotransmitter systems that mediate anxiety, or alterations in the neurotransmitter systems/neurochemistry of sufferers. For example, some researchers attribute the cause of some types of anxiety to faulty GABA receptor regulation, whereas others look to the serotonergic, noradrenergic, and other chemical systems as the biological substrates of anxiety. This type of model looks for biological dysfunctions that are often treatable with drugs (Rachmann 2002). Eysenck and Gray's theories are unusual in that the psychology and biology overlap.

In reality all of these models are probably valid, in that anxiety works on a continuum and at a number of different levels and is the result of an interaction between environmental stressors, heritable traits, cognitions, behaviour, physiology, and each individual's neuroanatomy and neurochemistry. Normal anxiety is a stress response that allows the organism to adapt to its environment. Thus, in situations of normal

anxiety those who are affected often take actions to reduce the anxiety: this helps them to adapt more readily to the situation that they are experiencing. However, such action might include turning to alcohol or perhaps seeking help from alternative therapies, such as aromatherapy, herbalism or yoga. These therapies are popularly considered more holistic and healthier than prescribed medications. In contrast, at the extreme end of the continuum pathological anxiety occurs when there is a failure to adapt, either because of extremes of circumstance, or because of abnormal physiology or neurochemistry.

Anxiety is expressed via somatic, cognitive, affective and behavioural components (Lang, 1968). Whether the cognition first becomes aware of the stimulus, or whether the somatic symptoms make one aware of the stimulus, depends on the stimulus (Edelmann, 1992). Generally, in normal anxiety there is a real threat to wellbeing. Causes of normal anxiety can be things like having tests for a potentially life-threatening illness, serious illness in a close friend or relative, or the threat of losing one's house or job. In the case of pathological anxiety there might be an imagined stressor, a failure to recover from the original stressor, or the anxiety response is disproportionate to the threat. In all cases, recognition of the threat is followed by some form of appraisal and affective integration, which involves a neurological triggering mechanism involving the brain structures discussed in the next section. In addition, there is usually a physiological mediation of the stress response, such as increased heart-rate, sweating, increased circulation to the parts of the body that might be involved in the FFFS response e.g. leg muscles to enable flight.

## 1.2.3 The biological basis of anxiety

### 1.2.3.1 The neuroanatomy of anxiety

In order to understand the mechanisms involved in anxiety, and hence its treatment, the underlying neuronal circuitry must first be understood. Three important concepts underlie the functional anatomy of anxiety: a defence system, which makes immediate responses to a situation by using fight, flight or freezing behaviour; a behavioural inhibition system, which is involved in information gathering and suppressing behaviours that might endanger the individual; and, an avoidance system,

to escape or avoid situations that an individual recognises as dangerous prior to the event. These three systems work together as one, causing fight or flight, so preventing any behaviour that might lead to danger (Sandford et al. 2000). Evidence for these concepts is from years of animal and human studies (Gray & McNaughton, 2003; Blanchard, Blanchard, Griebel & Nutt, 2008).

The brain structures and pathways underlying these three systems are complex and involve many neural circuits at different levels depending on the type of anxiety and, in the case of pathological anxiety, the type of disorder (Kim & Gorman, 2005). What is known of the neuroanatomy of anxiety from animal, immnuocytochemistry and imaging studies can be simplified to three different levels (Carrasco & Van de Kar 2003). The brainstem is responsible for autonomic responses. The limbic system is responsible for the affective states of anxiety, emotions, mood changes and neuroendocrine changes in stress hormones, such as cortisol and other glucocorticoids. Finally, the higher limbic system and neocortex are responsible for conscious or cognitive responses to anxiogenic stimuli. There is bi-directional feedback between these pathways causing an enhanced response, leading to anxiety or a damping down of the response in normal individuals who do not suffer from anxiety (Carrasco & Van de Kar 2003). In sufferers of anxiety disorders, the control of these neural circuits might be impaired (Kim & Gorman, 2005). The next sections describe the involvement of these regions of the brain in the aetiology of anxiety in more detail.

Brainstem

In the brain stem, noradrenaline (NA) -releasing neurons in the locus coeruleus (LC) are involved in producing the immediate FFFS response to anxiogenic stimuli in co-ordination with the periaqueductal grey (PAG), the grey matter located around the cerebral aqueduct located in the midbrain (Singewald, Salchner & Sharp, 2003). In extreme danger when an organism is under immediate threat, the PAG is the region of the brain that is most likely to be activated, causing undirected escape, attack or catastrophic panic. In panic disorder, it is this region that is likely to be at fault (Grey & McNaughton, 2003; Blanchard, et al., 2008). The release of NA from the LC is responsible for arousal, drive and appetite (Smith & Nutt, 1996; Sullivan, Coplan, Kent & Gorman, 1999). Normally, during stress responses projections from the LC to

the amygdala, the hippocampus, the hypothalamus, the nucleus tractus solitarius, the periaqueductal grey, the prefrontal cortex and the thalamus are activated. The projections to these cortical and subcortical regions are responsible for mediating fear and anxiety. Most of these regions also innervate the LC. Thus, the NA neurons in the LC are in a position to integrate both external and internal data and activate structures that have roles in stress and anxiety responses (Kent, Mathew & Gorman, 2002). This neuronal activity focuses attention and promotes scanning behaviour (Jenck, Moreau & Martin, 1995; Singewald et al., 2003; Mansour et al., 2003). Additionally, noradrenergic projections from the LC activate the autonomic nervous system, including the cardiovascular and pulmonary systems, readying them for a FFFS response while suppressing systems which are not needed at this stage, such as the digestive and urinogenital systems.

Alongside the NA system, the raphé nuclei in the brain stem, which consist of many different subsets of serotonergic neurons including the median (MRN) and dorsal (DRN) raphé nuclei, are involved in the control of these anxiety responses (Abrams et al., 2005; Kim & Gorman, 2005). These neurons are thought to mediate a complex of differential responses to anxiogenesis, projecting to forebrain circuits involved in the regulation of anxiety responses and to the LC where they regulate NA release (Abrams et al., 2005). All of these regions communicate widely with the limbic system including the hypothalamus.

Additionally, the brainstem and limbic system send signals to the prefrontal lobes and paralimbic cortex, which include the orbitofrontal cortex, the bed nucleus of the stria-terminalis (BNST), the insula, the anterior-temporal and the anterior cingulate. This information is processed and a response co-ordinated (Liotti et al., 2000). Thus, all of these systems in the brain stem coordinate with other CNS structures and the external and internal environment to elicit a response.

Limbic system

The limbic system consists of many brain structures and pathways, the main ones being: the amygdala, the anterior thalamic nucleus, the fornix, the hippocampus, the septal nuclei, the hypothalamus, the mammillary bodies, the BNST, the medial forebrain bundle, and the prefrontal lobes. Phylogenetically the limbic system is the

oldest part of the brain and has a key role in the creation and processing of emotions (Chorpita & Barlow 1998; Phan, Wager, Taylor, & Liberzon, 2002).

The PAG is connected to the dorsal hypothalamus, which mediates the more sophisticated escape mechanisms that are likely to be employed when the threat is not as immediate and so panic does not ensue. In Gray and McNaughton's model (2003) the hypothalamus is responsible for simple active avoidance, for example, phobia. The hypothalamus also has a key role in coordinating neuroendocrine responses to anxiety via the Hypothalamic-Pituitary-Adrenal (HPA) axis, the slow stress response (see Figure 1.1), and the symapthoadrenomedullary system (SAM). The SAM is the immediate response to environmental stressors, mediated by cells in the adrenal medulla and different areas of the brain depending on the type of stress causing an increase in adrenaline and NA. Examples of the SAM include activity by nuclei in the brainstem in response to low oxygen, or the frontal lobes in response to a cognitive or psychological stressor (Mravec, 2005). The LC and the hypothalamus detect these hormone rises and activate the CNS (Mravec, 2005). The HPA axis causes the release of glucocorticoids, such as cortisol, which are involved in stress and the FFFS response (Carrasco & Van de Kar, 2003; Sandford et al., 2000). Interestingly, most of the hypothalamic nuclei are larger in males than females (Swaab, 1997). The hypothalamus is, in turn, connected to the amygdala.

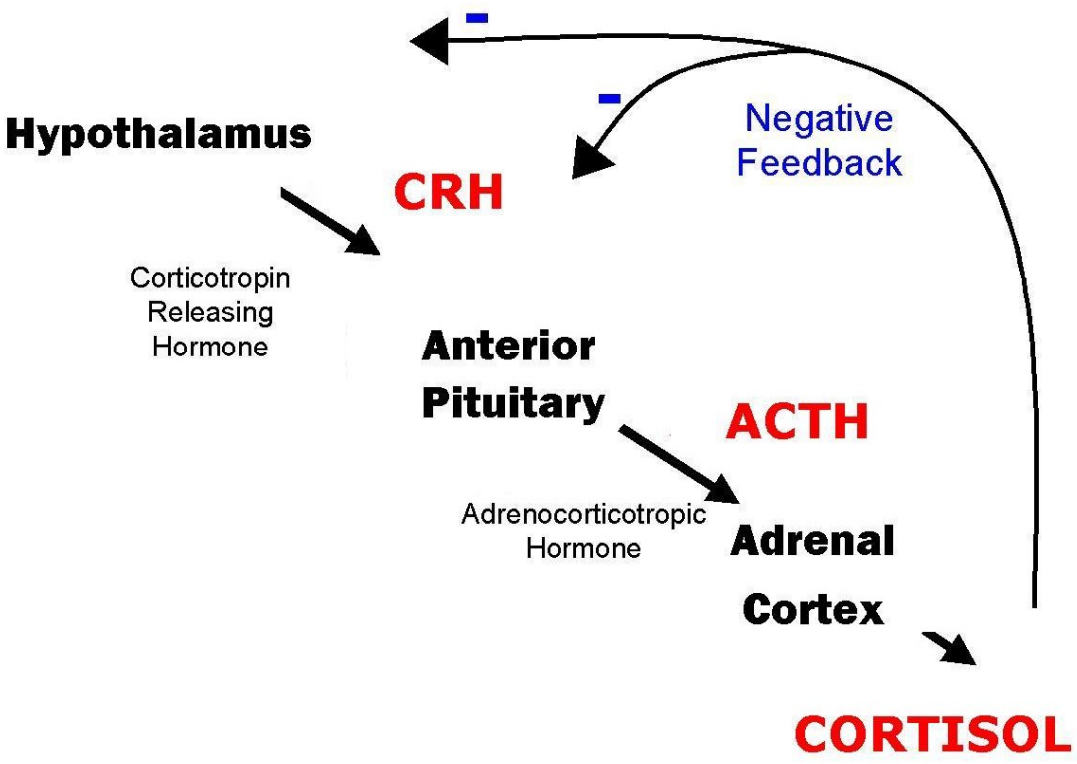



**Figure 1-1The HPA axis.**

The amygdala, is made up of many nuclei and is generally divided into the central, lateral and basolateral nuclei. The amygdala has been likened to a switchboard, controlling, co-ordinating and directing anxiety responses (Kim & Gorman, 2005). The central nuclei send projections to the hypothalamus, LC, and PAG, to activate the HPA axis and thus, cortisol secretion from the adrenals and NA release from the LC, to increase sympathetic arousal. Additionally, the lateral nucleus processes signals from the higher brain regions involved in the anxiety response, such as the prefrontal cortex, the cingulate gyrus, the hippocampus and the thalamus.

More specifically, studies have shown that the amygdalae co-ordinate simple avoidance and so co-ordinate with the hypothalamus in mediating avoidance during phobic states, as well as having a role in mediating the arousal components of GAD (Gray & McNaughton, 2003). Studies on animals and humans with damaged or removed amygdala, provide evidence for this role of the amygdala in the expression, memory conditioning of, and release, of fear and anxiety (Davies, 1992). Thus, the amygdalae are where memories of fearful events are created, which explains their

role in causing avoidance/aversion to situations (Singewald, Salchner, & Sharp 2003; Davis 1998).

There are hemispheric differences between the size and function of the amygdalae (Kim & Gorman, 2005). The size of the left amygdala is often diminished in anxiety sufferers and is thought to be linked to the control of anxiety responses. The right amygdala is thought to be more active in pathological states (Kim & Gorman, 2005).

In summary, the amygdala communicates with the neocortex where conscious thoughts are generated and processed. They also communicate with the thalamus, which co-ordinates sensory signals prior to sending them to the cortex (Davidson, 2002). The amygdala finally communicate back with the brain stem.

The bed nucleus of the stria-terminalis (BNST), a part of the extended amygdala, receives input from all three of the amygdaloid nuclei and has multiple connections to all of the limbic and cortical regions involved in the fear and anxiety response. It is involved in mediating anxiety responses, rather than the fear responses for which the amygdaloid nuclei are responsible, and for the longer-term regulation of the anxiety response (Bangasser, Santollo, & Shors, 2005). It is also thought to modulate the cortisol releasing factor (CRF)-cortisol pathway, rather than the immediate sympathetic pathways, which are co-ordinated by the hypothalamus (Carrasco & Van de Kar, 2003). Interestingly, the BNST is highly sexually dimorphic and densely expresses gonadal steroid receptors, and differs in size between males and females (Toufexis, Myers & Davis, 2006).

The septo-hippocampal formation, consisting of the hippocampus, the dentate gyrus, the entorhinal cortex, the subicular area, and the posterior cingulate cortex, acts as a comparator, comparing known information about a situation with the actual situation (Gray & McNaughton, 2003). One of its roles is in approach-avoidance conflicts (Degroot & Treit, 2003; Graeff, 1994). According to Gray and Mc Naughton (2003) the septo-hippocampal loop responds to threat by interrupting ongoing behaviour (behavioural inhibition) via the psychological BIS. This allows information gathering, known as risk-assessment behaviour (Blanchard et al., 2008). The BIS is active in situations where there is a conflict, for example, where information held is incongruous with information about the current situation, or proceeding with one goal

might involve entering a threatening situation thus creating an approach-avoidance conflict. The LC in the brain stem stimulates this hypervigilant, information-gathering, attentional output. Information gathering also involves the recall of memories stored elsewhere, for example in the temporal lobes (Rokers, Mercardo, Allen, Myers & Gluck, 2002; McNaughton & Corr, 2004). In recalling memories relevant to a potentially threatening situation, the septo-hippocampal system has the capacity to increase the valence of affectively negative stimuli. Gray and McNaughton (2003) suggest that this increasing of perceived threat and subsequent storage of these increasingly negative memories are what lead to the rumination and excessive anxiety seen in general anxiety disorder. Interestingly, lesions to the septo-hippocampal system closely resemble the effects of anxiolytic drugs (Gray & McNaughton, 2003); also, anxiolytic drugs and lesions to the hippocampus have very mild effects on mnemonic memory function (Gray & McNaughton, 2003). Finally, if the septohippocampal system determines that there is a threat, it sends information to the lateral nucleus of the amygdala, which mediates the appropriate avoidance reaction and the arousal components of GAD. Additionally, the BIS is able to interrupt ongoing motor behaviour and increase attention to the perceptual world (sensory vigilance) via connections from the subiculum, which is part of the septo-hippocampal loop system, to the nucleus accumbens (Gray & McNaughton, 2003). This is because the nucleus accumbens sends signals via the substantia nigra to the motor control areas of the basal ganglia and the entire thalamocortical sensory processing system. In extreme stress, there is often a degeneration of hippocampal volume, which might lead to extreme anxiety (Douglas Bremner et al., 1995).

Paralimbic system and prefrontal cortex

The orbitofrontal cortex, the insular, the anterior temporal and the anterior cingulate receive information from the limbic system and the brainstem (Liotti, Mayberg, Brannan, McGinnis, Jerabek, & Fox 2000). The cingulate cortex is a bridge in communication between the prefrontal lobes, the limbic system and the brainstem (Milad et al., 2007). Lesions to this region lead to a disinhibition of emotion and an inability to modify behaviour according to environmental circumstances (Milad et al., 2007). Damage to the orbitofrontal cortex and the cingulate gyrus cause decreased anxiety. Surgery in these regions has been used as a way of relieving suffering from extreme anxiety that is resistant to treatment by standard pharmacotherapy; often this

includes people who suffer from OCD (Ballantine, Bouckoms, Thomas, & Giriunas 1987). In PET studies, the orbitofrontal cortex is also implicated in worry (Wu, Buchsbaum, Hershey, Hazlett, Sicotte, & Chad Johnson 1991).

Anxiety can begin at any one of the levels discussed above, brainstem, limbic regions, paralimbic and prefrontal cortices (see Figure 1.2 below). These systems respond to stimuli and feed back to the other levels in order to co-ordinate a response, or create anxiety in the individual (Gray & McNaughton, 2003). The neurotransmitter systems involved in co-ordinating these responses at different structural levels are very complex. The following section continues the discussion looking at the neurotransmitters known to have a role in the aetiology of anxiety.

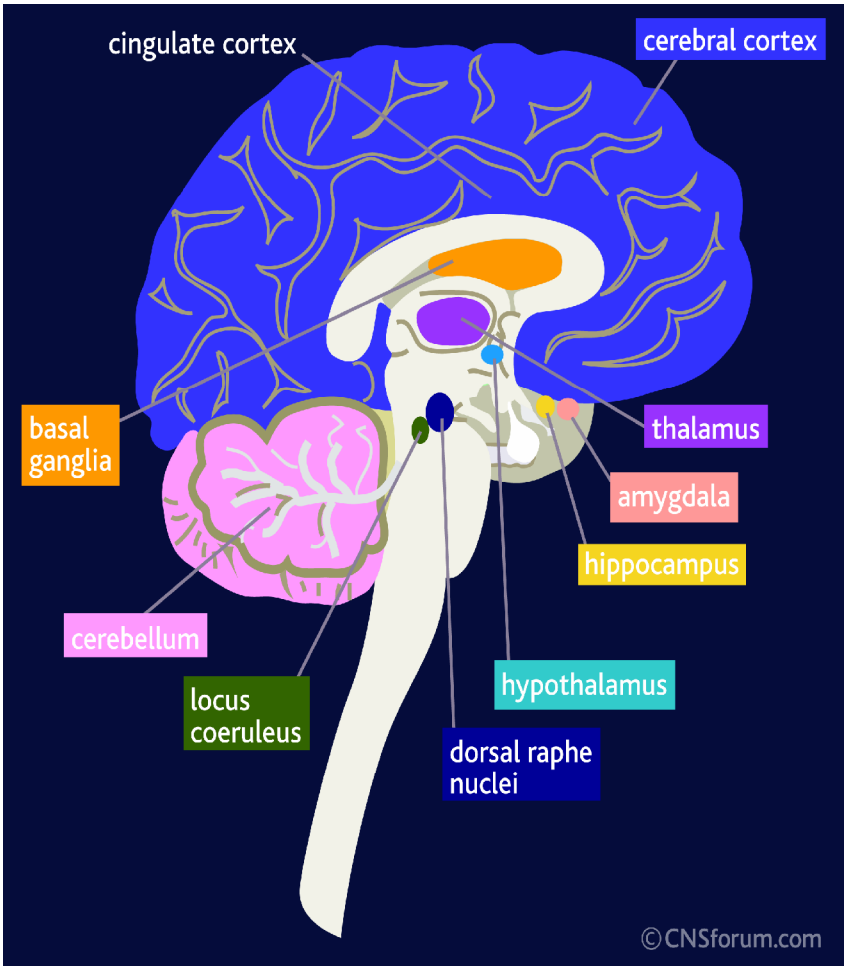



**Figure 1-2 Summary of the areas of the brain affected by anxiety (CNS Forum 2008).**

### 1.2.3.2 Neurotransmitter systems implicated in anxiety

In the CNS there are two opposing systems at work by which, all neurotransmitter systems are influenced: the excitatory and the inhibitory systems. These two systems influence and interact with all other neurotransmitter systems. Glutamate is the major neurotransmitter responsible for the excitatory system and Gamma Amino Butyric Acid (GABA) is responsible for the inhibitory system (Whiting, 2003). Both of these systems are found throughout every brain region and their receptors are evident in most neurons. They both occupy some 40% of all synapses and both are implicated in the pathophysiology of many diseases (Leonard, 1994).

#### Glutamate

Glutamate is the major excitatory amino acid in the CNS. There are many different types of glutamate receptor, and they have overlapping but different functions depending on their distribution (Pralong, Magistretti, & Stoop, 2002; Tapiero, Mathe, Couvreur, & Tew, 2002). The glutamatergic system has a role in synaptic plasticity associated with memory and learning (Cryan & Dev, 2008). Its fast actions are mediated by ionotropic receptors, N-methyl-D-aspartic acid (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate; these are mainly active in calcium and sodium ion transport (Cryan & Dev, 2008). While, its slower actions on neurotransmitter release and cell excitability are mediated by metabotropic G-coupled glutamate receptors (Cryan & Dev, 2008). There are three families of metabotropic receptor: Group 1 utilizes the phospho-lipase C and inositol-phosphate second messenger systems; whereas, groups 2 and 3 inhibit adenyl-cyclase, thereby decreasing cAMP production in the cell. Glutamate receptors have a role in memory and synaptic plasticity in the areas of the brain related to emotion, such as the hippocampus, amygdala and cortex, but some of these receptors, NMDA types, also require the binding of glycine to function correctly.

Experiments have shown a decrease in glutamatergic transmission when the HPA axis is deactivated (Makatsori et al., 2003; Moghaddam, 2002). In addition, activation

of the NMDA class of receptors is thought to lead to the release of CRF (Cryan & Dev, 2008). It has been demonstrated that serotonin reuptake inhibitors (SSRIs) have a significant impact on NMDA receptor function and therefore might exert their therapeutic effects via the glutamatergic system (Wegener, Volke, Harvey, & Rosenberg, 2003). In recent years, a growing number of studies have been conducted to investigate the anxiolytic effects of glutamate antagonists with promising results. Methyl, phenyl-ethynyl pyridine (MPEP) , a metabotropic glutamate receptor antagonist, has been shown to display anxiolytic effects in a number of unconditioned animal models of anxiety, with results similar to those exhibited by the drugs diazepam and buspirone (Brodkin, Busse, Sukoff, & Varney, 2002). It has also been shown to block startle responses in the potentiated fear model of anxiety (Kent et al. 2002). Additionally, although not directly proven to be an antagonist, a preclinical study looking at riluzole, which inhibits glutamate release from presynaptic terminals, has shown that it blunts the anxiogenic properties of the beta carboline, FG7142, in rats (Kent et al., 2002). Furthermore, it has shown some success in treating GAD and OCD patients (Cryan & Dev, 2008). Other investigations have shown glutamate antagonists to have some efficacy when used to treat PSTD patients (Hertzberg et al., 1999). More recently, drugs that activate the glycine site of the NMDA receptor, such as D-cycloserine, can promote the extinction of phobias in a clinical population and the extinction of learning in mice (Davis, Ressler, Rothbaum & Richardson, 2006; Davis, Myers, Chhatwal, & Ressler, 2006). Many drugs that work on both types of receptor have been tested in many animal models with mainly anxiolytic effects. Thus, the glutamatergic system is predicted to be one of the most promising areas for the development of new and novel anxiolytic drugs in the future (Cryan & Dev, 2008).

**GABA**

It is one of the most widely distributed neurotransmitters in the brain. The GABA system is thought to be integral in the pathophysiology of anxiety, mainly because of the effectiveness of benzodiazepines (BDZs), which act primarily on $GABA_A$ receptors, in managing anxiety. Many studies provide evidence of the mechanism of action of the $GABA_A$ receptor. These include neuroimaging studies of the receptors' locations in the CNS, and behavioural studies looking at their effects when specific agonists and antagonists are injected into specific brain regions (Nemeroff, 2003).

GABA is synthesised from glutamate by the enzyme L-glutamic acid decarboxylase. One explanation for the role of GABA in anxiety disorders has suggested that there is a role for the over or under expression of the enzyme that synthesizes GABA, L-glutamic acid decarboxylase, in the psychopathology of anxiety disorders, (Korpi, Grunder & Luddens, 2002). However, the main area of research involving GABA in anxiety treatments has focused at the level of the GABA receptor.

There are three types of GABA receptor, $GABA_A$, $GABA_B$ and $GABA_C$. The $GABA_B$ receptor is a G-coupled receptor and is involved in both post- and pre-synaptic feedback control of GABA release and synthesis. $GABA_C$ receptors are the most recently discovered of the GABA receptor family and although expressed throughout the mammalian CNS, they are predominantly expressed in the vertebrate retina (Pan, Khalili, Ripps, & Qian, 2005). They are ligand-gated ion-channels made up mainly of rho sub-units (Johnston, 1996). They might have a role to play in anxiety, but relatively little is known about them (Johnston, 1996). $GABA_A$ is a transmembrane, ligand-gated, chloride, ion-channel (see Figure 1.3). It is a heteropentamer, made up of ten subunits, from a pool of 19 subunits, divided into subfamilies according to amino acid sequence homology: Alpha (1-6) Beta (1-3) Gamma (1-3) Delta, Epsilon, Theta, Pi and Rho (1-3) (Herd, Belelli, & Lambert, 2007). This allows for a diversity of different $GABA_A$ receptors with differing properties and affinities for the receptor, which are distributed in varying brain regions including those implicated in anxiety (Whiting 2003; Nemeroff, 2003). Binding of GABA to the receptor opens the chloride channel, allowing chloride to flow into the neuron, thus depolarizing it and making it more difficult for other neurotransmitters to depolarize it; therefore it can be considered as inhibiting.

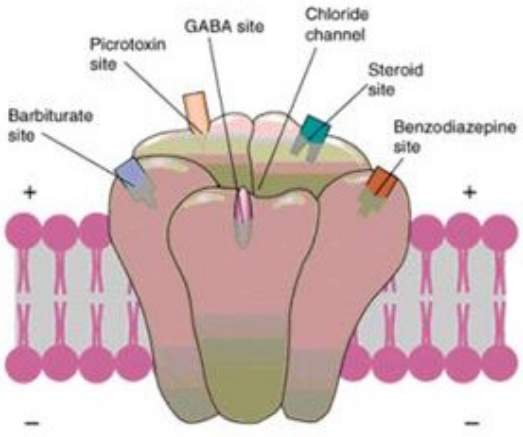



**Figure 1-3 GABAA receptor and its binding sites (Dubuc, 2002).**

The $GABA_A$ receptor is the one most implicated in the pathophysiology of anxiety and as the brain's main inhibitory receptor regulates the activity of the other neurotransmitter systems (Kalueff & Nutt, 2007; Nutt, 2006). The $GABA_A$ receptor is bi-directional in its activity, and has many different binding sites on its subunits for drugs that act as allosteric modulators of its activity (see Table1.2 below). For example, the $GABA_A$ receptor houses the BDZ binding site, and the allosteric binding of a BDZ to this site enhances the inhibitory actions of GABA by acting as a positive modulator of its action. $GABA_A$ receptors are also the binding site for many anxiogenic drugs, known as inverse agonists, which also allosterically modulate the action of the receptor to lessen the effects of GABA (See Table 1.2 for examples). Another group of drugs acts to prevent the action of agonists and inverse agonists at the $GABA_A$ receptor; these are known as antagonists (see Table 1.2 below) (Whiting, 2003; Kent, et al. 2002).

**Table 1.2 Examples of chemicals which bind at the GABA receptor and their binding sites.**

| Chemical/Drug | Type | Receptor binding site | Inc/Dec Anxiety |
|---|---|---|---|
| **GABA** | Neurotransmitter | GABA | Decrease |
| **BDZs** | Agonist, | BDZ | Decrease |
| **β-Carbolines e.g. RO 19 4603** | Inverse agonist, | BDZ | Increase |
| **Pentelynenetetrazol** | Inverse agonist | Picrotoxin | Increase |
| **Ethanol, Chloral hydrate, Chlormethiazole** | Agonist | Alcohol site | Decrease |
| **Neurosteroids** | Agonists | Neurosteroid site | Decrease |
| **Barbiturates** | Agonists | Barbiturate site | Decrease |
| **Bicuculline** | Antagonists | GABA site | Increase |
| **Ro 16-6028** | Partial agonist | BDZ | Decrease |
| **Ro 15-4513** | Partial Inverse agonist | BDZ | Increase |

(Leonard, 1994; Sanford et al., 2000).

The existence of inverse agonists and antagonists has led researchers to look for endogenous agonists and inverse agonists of the $GABA_A$ receptor. This in turn has led to the discovery of diazepam binding inhibitor (DBI), tribulin and neurosteroids. DBI inhibits the binding of diazepam and other agonists, inverse agonists, and antagonists, to the receptor (Sandford et al., 2000). Tribulin has been isolated from urine following stress in humans; it has a structure similar to the beta carbolines and has been shown to prevent BDZ binding, thus causing anxiety (Sandford et al., 2000). Neurosteroids have also been shown to act as modulators of the GABA receptor complex and gonadal steroids almost certainly regulate anxiety (Mitchell, Herd, Gunn, Lambert & Belelli, 2008). For example, pregnan-3a-ol-20-one and 5a-pregnan-3a, 21-diol-20-one, metabolites of progesterone, potently enhance the function of GABA (Herd et al., 2007).

Research suggests that the BDZ receptor structure might be altered in patients suffering from anxiety (Kosel et al., 2004). Further evidence is the fact that panic disorder patients exhibit abnormal GABA reactivity. Flumazenil, a BDZ antagonist, has little effect on normal controls, but causes panic in panic disorder patients. These patients are less responsive to BDZs and therefore need more potent ones to treat their condition (Sandford et al., 2000).

As $GABA_A$ receptors are widespread throughout the CNS they have a role in regulating other neurotransmitter systems, such as the serotonergic and noradrenergic systems, which are also involved in the psychopathology of anxiety (Korpi et al., 2002).

**Serotonin (5HT)**

The major 5HT systems in the brain are the raphé nuclei, situated in the brain stem, the two main groups of nuclei being the rostral and the caudal groups. The rostral group consists of 85% of all 5HT neurons in the brain and contains three sets of nuclei, two of which are implicated in the mediation and control of anxiety: the median raphé nuclei (MRN) and the dorsal raphé nuclei (DRN) (Hornung, 2003). The MRN is thought to modulate fear and anticipatory anxiety. Whereas, the DRN might modulate cognitive processes associated with anxiety (Graeff, Guimares, De Andrade, & Deakin, 1996) (Figure 1.4 below). Although these systems function in parallel, they are morphologically distinct, sending and receiving input from different regions of the brain (Sandford et al., 2000). Neurons from the MRN innervate the hypothalamus and neurons from both the DRN and MRN innervate the amygdala and other limbic forebrain regions (Carrasco & Van de Kar, 2003).

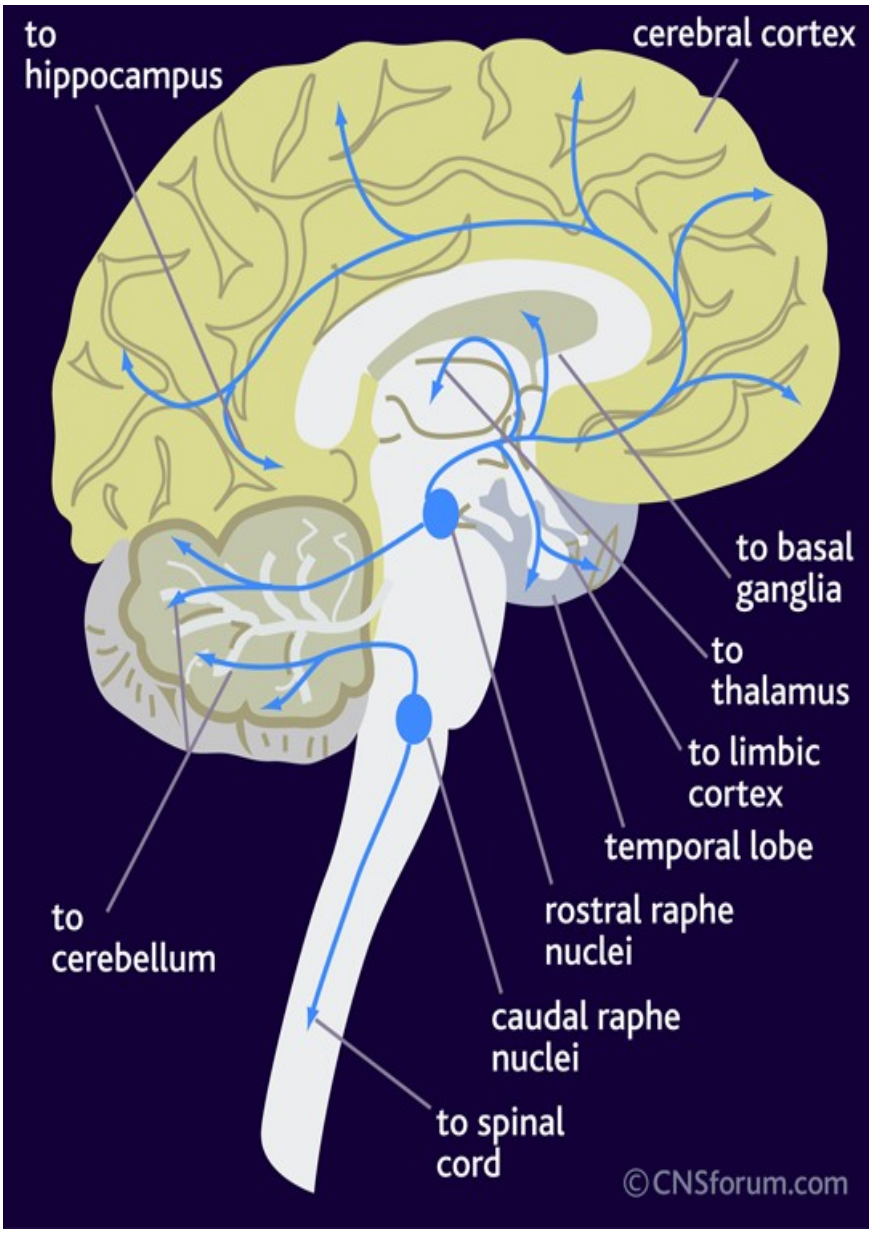



**Figure 1-4 Anatomical distribution of serotonergic pathways in the human brain (CNS Forum, 2008).**

Serotonin (5 HT), an indolamine, is widely implicated in the aetiology of anxiety and its involvement is complex (Carrasco & Van de Kar, 2003; Zhuang et al., 1999; Tye, Iversen & Green, 1979). Increases in 5HT in the amygdala are anxiogenic, whereas, in the PAG it inhibits panic (Graeff et al., 1996). It is thought to be the neurotransmitter that mediates the behavioural inhibition system (BIS) (Cools, Robinson & Sahakian, 2007). During the stress response, levels of 5HT decline. However, over time and possibly because of autoreceptor down-regulation, the levels of amines, such as 5HT and NA, increase (Tannenbaum & Anisman, 2003). It has been suggested that 5HT modulates homeostasis between other neurotransmitters, such as GABA, NA and dopamine, and this balance is upset in illnesses such as

anxiety (Vaswani, Linda & Ramesh, 2003). Dysfunction of 5HT is associated with anxiety and other mood disorders (Zhuang et al., 1999) and might be the result of deficient or excessive innervations to key structures, and/or cellular mechanisms resulting in aberrant neurotransmission (Kent, et al. 2002). 5HT was first implicated in anxiety when it was shown that inhibiting the release of 5HT had anxiolytic effects and that 5HT might mediate some of the BDZ anti-anxiety effects (Tye et al., 1979). Recent work has indicated that genetic abnormalities in one of the genes encoding tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5HT, might have a role to play in emotional regulation, leading to potentially abnormal anxiety responses (Gutknecht et al., 2007). Additionally, 5HT knockout mice, with the receptor 5HT-1A deletion, exhibit increased fear behaviours (Lesch, Zeng, Reif, & Gutknecht, 2003). Other studies demonstrated that a 5HT receptor agonist, mCPP, causes anxiety and panic in normal and psychiatric patients (Murphy, Mueller, Hill, Tolliver, & Jacobsen, 1989). Conversely, selective serotonin reuptake inhibitors (SSRIs) alleviate anxiety in some patients with anxiety disorders (Lucki, 1996). SSRIs enhance 5HT transmission by preventing its reuptake. Other evidence for 5HT's role in anxiety comes from the successful treatment of general anxiety disorder with buspirone, a 5HT-1A receptor partial agonist (Peroutka, 1985).

5HT works in conjunction with other neurotransmitter systems (e.g. NA GABA) and neuropeptide systems such as cholecystokinin and substance P (which are co-released with 5HT). Its neurochemistry is complicated by the fact that on the presynaptic membrane there are auto-receptors (5HT 1A/1B/1D) and serotonin reuptake transporter sites (SERTs) involved in release, regulation of synthesis, reuptake, and modulation of 5HT by other neurotransmitter systems. On the postsynaptic membrane there are at least fourteen different receptor subtypes, of which several are believed to be potentially important in mood and anxiety (5HT-1A, 5HT-2A, 5HT2C, and 5HT3) (Raymond et al., 2001). In addition, the presence of 5HT heteroreceptors on neurons of other neurochemical systems implies that 5HT also modulates/interacts with other non-serotonergic systems (Kent, et al. 2002). Clinical and preclinical studies have shown that abnormalities in 5HT receptors have a role in anxiety (Hoyer, Hannon, & Martin, 2002; Kroeze & Roth, 1998; Roth, Willins, Kristiansen, & Kroeze, 1998).

5HT presynaptic-autoreceptor-antagonists, and SERT-inhibitors prevent reuptake of 5HT, resulting in an increase in the intercellular 5HT concentration. This initially has an anxiogenic effect, in acute studies. However, when administered chronically they cause a down-regulation of the postsynaptic receptors responsible for provocation of the anxiety response via other brain structures and neurotransmitter systems, and have an anxiolytic effect (Bonasera & Tecott 2000). This can be seen in treatment with serotonin reuptake inhibitors (SSRIs), which have been designed to work specifically by blocking SERT. However, their resultant action on the up or down-regulation of the 5HT receptor varies according to which SSRI is used (Vaswani et al., 2003). Tricyclic antidepressants have also been used to treat anxiety; these inhibit reuptake of NA and 5HT (Leonard, 1994). Likewise, Venlafaxine, which is a NA and 5HT reuptake inhibitor, is very effective in the treatment of anxiety, especially GAD (Salinas & Hackett, 2001).

5HT-1A receptors are found on both pre- and post-synaptic membranes and are metabotropic adenyl-cyclase inhibitors. The presynaptic receptors are found mainly in the raphé nuclei and are responsible for feedback inhibition of 5HT synthesis and release. The postsynaptic receptors exert the stress-modulating effects of 5HT, mainly in the hippocampus, septum, amygdala and limbic system regions (Toth, 2003). Partial and full agonists of 5HT-1A have been shown to have anxiolytic effects in studies using animal models of anxiety (Griebel, Rodgers, Perrault, & Sanger, 1997; Bell & Hobson, 1994). Buspirone, a partial 5HT-1A agonist, is thought to exert its anxiolytic effects via these receptors (Yocca & Altar, 2006; Lim et al., 2008).

.

5HT-2A receptors have a low affinity for 5HT, they are found on the postsynaptic membranes. They are also metabotrophic receptors, but their second messenger system is the inositol phosphate/diacyl glycerol system. They are prevelent in high levels in the cerebral cortex and low levels in the basal ganglia and thalamus. Antagonists at these receptors reduce the stress response. 5HT-2A receptors activate the HPA by facilitating the release of stress hormones, such as adrenocorticotrophic hormone (ACTH), prolactin, oxytocin, and cortisol or corticosterone (Leonard, 2005). Antagonists at 5HT-2A receptors block their release, thus dampening the stress response (Bonasera & Tecott, 2000). Recently the personality dimension, neuroticism, has been found to have a high positive correlation with the occurrence of these receptors in the frontal limbic cortex (entorhinal cortex, superior frontal cortex,

posterior cingulate, inferior frontal cortex and the insula) (Frokjaer et al., 2008). Animal models have also added weight to the argument that the 5HT-2A receptors are involved in anxiety (Toth, Sibille, Gyulai, & Gal, 1996).

5HT-1B receptors are metabotropic adenylate-cyclase inhibitors. They are auto-receptors found on the presynaptic membranes. Genetic mutant knockout mice for 5HT-1B receptors have been shown to exhibit less anxiety than wild type mice, (Zhuang, et al. 1999).

Studies on 5HT-2C receptors, which are metabotropic, diacylglycerol/inositol phosphate-type receptors, have shown these receptors to be involved in weight-gain and the cognitive elements of anxiety, such as worry (Bonasera & Tecott, 2000). The anxiogenic effects of mCPP (Hoyer, Hannon & Martin, 2002) and the attenuation of SSRI induced anxiety with chronic treatment have been hypothesised to be mediated via this receptor (Bristow, O'Connor, Watts, Duxon, & Hutson, 2000). Additionally, recent research looking at neuro-active steroids and knockout mice has implicated antagonists at 5HT-3A receptor sites as potential treatments for anxiety (Kelley, Bratt, & Hodge, 2003; Rupprecht, 2003). Unlike the other receptors, these are ligand-gated cation-channels.

**Noradrenaline (NA)**

NA, a catecholamine synthesised from the amino acid tyrosine, is an excitatory neurotransmitter involved in modulating awareness of the outside world (Nutt, 2007). The NA system in the brain originates mainly from the LC, which is responsible for arousal, drive and appetite (Smith & Nutt, 1996; Sullivan et al., 1999). NA is the neurotransmitter that is most associated with the stress response, exerting its effects mainly via the sympathetic nervous system, increasing heart-rate and blood pressure, and causing the circulation to flow away from the digestive and urogenital systems to the areas where energy is likely to be needed rapidly in a fight or flight reaction (Kent et al., 2002). Furthermore, there are alterations in noradrenergic receptor activity in anxiety sufferers (Hollander et al., 1991; Sullivan et al., 1999). Enhanced turnover of NA has been reported in various animal models of anxiety, where the animals have been exposed to a stressor (Hayley, Borowski, Merali, & Anisman, 2001; Pardon, Ma, & Morilak, 2003). Like 5HT, NA also has receptor systems which might become

dysfunctional and cause anxiety. Studies have reported heightened NA levels and prolonged autonomic responding to stress in anxiety disorder patients (Kent, et al. 2002; Sullivan, et al.1999). It is known that the NA system works closely with the CRF system and has a role to play in the release of hormones involved in the stress response (Carrasco & van de Kerr, 2003). Pharmacological studies examining the effects of agents which act at adrenergic receptors have provided evidence for an altered NA activity in anxiety disorder patients. Studies have reported heightened NA levels and prolonged autonomic responding to stress in anxiety disorder patients (Kent, et al. 2002; Sullivan, et al.1999).

There are two main classes of NA receptor in the CNS, α and ß. Among the α receptors there are α1 and α2. The α1 receptors are excitatory, whereas the α2 are inhibitory in their action (Leonard, 1994). Among the β receptors there are β1, β2 and β3 (Carrasco & Van de Kar, 2003).

α Receptors

α Receptors have direct effects on the pathways immediately involved in the precipitation of anxiety. When NA binds to α1-receptors, glutamate is released. Antagonists such as urapidil and prazosin improve concentration and performance on cognitive tasks under stressful conditions (Birnbaum, Gobeske, Auerbach, Taylor, & Arnsten, 1999).

α2-Receptor antagonist, yohimbine, has been shown to cause anxiety in normal subjects (Bourin, 1998). It acts by blocking the presynaptic autoreceptors in the LC, preventing NA reuptake and increasing its availability. In panic disorder patients, yohimbine increased serum levels of the NA metabolite, 3-methoxy, 4-hydroxyphenylglycol, (MHPG) (Charney, Heninger, & Redmond, 1983). Also in depressed and anxious patients, yohimbine causes a blunted release of growth hormone, which would normally be released to allow for damaged cells to be repaired after the stressor has ceased (Charney et al., 1983).

Clonidine, an α2-receptor agonist, has anxiolytic properties (Sawynok & Reid, 1986). Panic disorder patients have been shown experimentally to have a hypersensitivity to

agonists and antagonists of the α2 receptors. This hypersensitivity might be either inherited or precipitated by a more stressful life in these patients, causing up-regulation of these receptors, (Marsland, Salmon, Terry, & Stanford, 1990). It should be noted that other α2 receptor antagonists, for example ethoxy-idazoxan, do not have the same effects as yohimbine except at high doses, indicating other possible routes of anxiogenic action (Sandford, et al. 2000).

### β Receptors

β Receptors, although involved in the mediation of the somatic effects of anxiety, have not been shown to directly affect the CNS pathways involved in anxiety. However they down regulate in patients who are undergoing chronic antidepressant treatment in a manner which corresponds to the time taken for the drug to have its therapeutic effect (Leonard, 1994). Propranolol, a β receptors antagonist, is used to treat Post Traumatic Stress Disorder (PTSD) patients, and has some success if used within a few hours of the traumatic event (Pitman et al., 2002). It is postulated that when it is administered soon after a traumatic event it might reduce the somatic symptoms associated with the stressful event, hence reducing the resultant fear conditioning which would ultimately cause the disorder. This lends weight to the learning theories of anxiety (Kent et al., 2002).

## Acetylcholine

The ascending cholinergic system has efferents to the limbic system in a similar way to the 5HT system, and both are involved in the control of hippocampal theta rhythms. Studies in rats have shown that the two systems are very closely linked and that the cholinergic system modulates the release of 5HT in the parts of the brain related to memory and anxiety such as the dorsal hippocampus (File, Kenny & Cheeta, 2000). The cholinergic system tends to be linked more with short-term memory and synaptic plasticity. There are two main classes of cholinergic receptor in the CNS, the muscarinic (metabotropic) and the nicotinic (ionotropic). The hippocampus in particular is extensively innervated with these types of neurons, which is not surprising given its role in memory formation. Likewise nicotine can modulate anxiety. Nicotinic receptor agonists can reduce anxiety when applied to the raphé and

have anxiogenic effects in the septum and hippocampus. In contrast, physostigmine, a general cholinergic agonist, also produced anxiolytic-like effects when applied to the hippocampus (Engin & Treit, 2008). Furthermore, recent studies have also suggested a role for interaction of the cholinergic system with the dopaminergic system in the modulation of approach and avoidance behaviours linked to anxiety (Hoebel, Avena, & Rada, 2007). Generally, though, anti-cholinergic drugs are used to treat Alzheimer's disease (Gray & McNaughton, 2003).

There is a growing body of experimental evidence that that other chemicals such as neuropeptides, co-released with neurotransmitters are also involved in the psychopathology of anxiety and might be useful targets in the treatment of anxiety disorders.

**Neuropeptides and other neurochemicals implicated in anxiety**

## Corticotropin Releasing Factor (CRF)

CRF, a 41-residue peptide is produced and secreted mainly by the paraventricular nucleus of the hypothalamus and is the main pituitary regulator of basal and stress induced release of ACTH, beta-endorphin and other pro-opiomalanocortin-derived peptides from the anterior pituitary gland (Carrasco, et al. 2003; Korte, 2001). It is responsible for eliciting stress effects such as activation of the autonomic nervous system, in order to facilitate the fight or flight response via the HPA axis (see Figure 1.1) and as a neurotransmitter (Carrasco & van de Karr, 2003). Its neurotransmitter effects are mediated via two transmembrane receptors, CRF R1 and CRF R2, which exert their second messenger effects via G-proteins (Takahashi, 2001). CRF R1 is most abundant in the neocortical, cerebellar and limbic structures. CRF R2 is usually found in subcortical structures such as the lateral septum and the hypothalamus. Stimulation of these receptors has been shown to increase anxiety-like behaviours in a variety of animal models (Griebel, 1999). CRF interacts with other neural substrates of anxiety for example NA, 5HT and neurosteroids; these interactions are thought to be responsible for some of its effects (Lukkes, Forster, Renner, & Summers, 2008; Millan 2003).

Cholecystokinin (CCK)

This is the most widely distributed neuropeptide in the brain. Additionally, it coexists with a wide variety of neurotransmitters such as dopamine, vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), GABA, substance P (SP), and 5HT (Griebel, 1999). There are multiple active forms of CCK of different length, some sulphonated and others not. The most predominant form in the CNS is the octapentapeptide (CCK8) which has a sulphonated tyrosine and the tetrapeptide (CCK4) which is found in smaller concentrations in the brain (Sandford et al., 2000). There are two receptor families for CCK. The CCKa receptors, which are the least numerous in the brain and show high affinity for CCK8, and the CCKb receptors, which are the main ones found in the brain and which show high affinity for both CCK4 and CCK8 (Sandford, et al. 2000). Interest in CCK-4 has arisen because it has been shown to induce panic attacks in healthy volunteers and, in lower doses, in panic patients when infused intravenously. Pre-treatment with BDZs or tricyclic-antidepressants, such as imipramine, can reduce this effect (Bradwejn & Koszycki, 1996).

The Peptide Tachykinins (Substance P and Neurokinin receptors, Neuropetide Y and Galanin).

These peptides are widely distributed throughout the CNS and peripheral nervous systems. Receptors for these peptides in the CNS are located in the limbic region and areas associated with mood and anxiety (Kent et al., 2002). In particular, Substance P (SP), which is held to be one of the most important neurotransmitters and neuromodulators present in the brain and which might have an important role to play in the modulation of anxiety, is released in response to aversive stimuli (Alvaro & Di Fabio, 2007; File, 1997). It is frequently co-localised with other neurotransmitters such as 5HT, GABA, glutamate, acetylcholine and dopamine, and influences their synaptic release. Depending on dose, its location within the brain, and the cause of the anxiety, SP can have anxiogenic or anxiolytic effects (Cheeta et al., 2001; De Ara-újo, Huston, & Brandão, 2001).

SP binds predominantly to neurokinin 1 (NK-1) receptors. Interestingly NK-1 antagonists have also been shown to be anxiolytic (Cheeta et al., 2001). Thus, there has been an intense search for antagonists to the NK1 receptor which can be converted to anxiolytic drugs (Alvaro & Di Fabio, 2007). This has led to studies in gerbil models of anxiety, since gerbils have a more similar NK-1 receptor to humans

than rats and mice (See Chapter 3; Varty, Morgan, Cohen-Williams, Coffin, & Carey, 2002; Varty et al., 2002). Further evidence for NK1 receptor ligands having a role in anxiety is provided by experiments using knockout mice, which have shown SP to modulate 5HT transmission and opioid transmission via glutamatergic neurons (Griebel, Mondroit, Aliaga, Simiand, & Soubrie, 2001; Lin, & Parson, 2002). Additionally, NK-2 receptor antagonists have also shown some anxiolytic activity but they are not sensitive to measures which traditionally detect BDZ activity (Luo & Wiesenfeld-Hallin, 1993). NK-3 agonists have been shown to have some anxiolytic effects (Haddjeri & Blier, 2001

Neuropetide Y (NPY) belongs to the pancreatic polypeptide family, but receptors are located in a number of brain regions, including the regions associated with fear and anxiety, such as the amygdala, hypothalamus, brainstem nuclei and cortex (Kent, et al., 2002). Studies injecting NPY into the para-ventricular regions of the brain have shown NPY to have anxiolytic effects, which can be reversed by alpha2-adrenergic receptor antagonists but not by GABA/BDZ receptor ligands (Kent et al., 2002). NPY is also known to be co-localised with NA in numerous brain regions (Kent, et al. 2002). Studies have shown that soldiers undergoing interrogation stress have increased levels of NPY. It has been suggested that this helps the soldiers cope with the stressor (MorganIII et al., 2000).

Galanin is co localised with NA in the LC, hippocampus and cortex (Kent, et al. 2002). It potentiates the local effect of alpha 2 adrenoceptors, where it acts to dampen firing rates (Khoshbouei, Cecchi, Dove, Javors, & Morilak, 2002). During stress, its levels decrease, returning to normal within a few days (Morilak, Cecchi, & Khoshbouei, 2003). However, if the stressor persists its level increases, which might explain the onset of depression with certain types of anxiety disorder (Yoshitake, Yoshitake, Yamaguchi, Ogren, & Kehr, 2003).

Neurosteroids

Anxiety is reportedly twice as prevalent in females as males and estrogens and progestrins have been linked to the increased vulnerability of women to anxiety (Toufexis, Myers & Davis, 2006). Neurosteroids can be synthesised denovo in the brain from cholesterol or by the adrenals and then cross the blood-brain barrier where they are modified once in the brain (Strous, Maayan & Weizman, 2006).

Neurosteroids have a role in non-genomic moderation of neuronal excitation, although progesterone might regulate gene expression by binding to receptors in the nuclei of neurons (Strous et al., 2006). It has already been mentioned that neurosteroids interact with the $GABA_A$ receptor and CRF; interestingly they might also interact with NMDA, AMPA, Kainate, 5HT and nicotinic acetylcholine receptors (Strous et al., 2006). Studies in rodents indicate that females are less anxious during periods of high oestrogen, such as proestrous, than when oestrogen concentrations are rising or falling, for example during oestrous and di-oestrous (Toufexis et al., 2006; Pandaranandaka, Poonyachoti, & Kalandakanond-Thongsong, *in press*). Likewise, testosterone injected into castrated male rats or female rats also caused decreased anxiety in the rats. Furthermore progesterone metabolite 3a-5a tetra hyroprogesterone (THP) obliterates anxiety caused by injections of CRF into the BNST, probably via its activity at the $GABA_A$ receptor (Toufexis, et al., 2006). It is interesting that neurosteroids have much more potent effects at the $GABA_A$ receptor than do the benzodiazepines or barbiturates (Strous, et al., 2006). Furthermore, SSRIs up regulate the enzyme responsible for the metabolism of dihydroprogesterone to THP (Texeira et al., 2003). Estrogens have been shown to cause an increase in synthesis of tryptophan hydroxylase, the rate-limiting enzyme in the formation of 5HT, in the mid brain of rodents during pro-oestrous, while not effecting SERT synthesis (Pandaranandaka, et al., *in press*).

Added to this complex neuroanatomy and neurochemistry of anxiety is the involvement of second messengers and cellular machinery such as the mitochondria, which are implicated in the pathophysiology of anxiety at a cellular level (Papadopoulos, Lecanu, Brown, Han, & Yao, 2006). This, as will be discussed in section 1.4.4, might also be a potential route for essential oils such as lavender to exert any potential anxiolytic effects.

Current Biological treatments of anxiety involve drugs that act specifically on some of the neurotransmitter systems described above and can have differing effects in reducing components of the anxiety symptoms without reducing all of them. For example, propranolol, a drug that acts on the beta-adrenergic receptors, reduces heart-rate but is not perceived to reduce anxiety in the sufferer because it has no effect on the cognitive symptoms of anxiety such as worry (Pitman et al., 2002; Tyrer & Lader, 1974). In contrast, drugs that act on serotonin receptor systems reduce the worry

component of anxiety but might not affect somatic symptoms (Leonard, 2003; Gray & McNaughton, 2003; Sinclair & Nutt, 2007).

## 1.2.4 Current treatments of anxiety

In diagnosing anxiety there are two lines of treatment, the psychological and the biological/pharmacological. The psychological usually involves some form of counselling and or behavioural therapy, such as cognitive behavioural therapy. The pharmacological usually involves treatment with serotonin reuptake inhibitors (SSRIs). According to the UK National Institute of Health and Clinical Excellence (NICE) guidelines, the recommended first line of treatment for anxiety (panic disorder, with or without agoraphobia, and generalized anxiety disorder) in adults is cognitive behavioural therapy, followed by pharmacological treatment with an SSRI (National Institute for Clinical Health and Excellence, 2007). CBT has the longest duration of effect, when compared with pharmacological treatments, but CBT is expensive and often anxiety disorders co-present with depression. Therefore, first line treatments are usually antidepressants, such as the SSRIs (Sinclair & Nutt, 2007). Examples of SSRIs are escitalopram, paroxetine, fluoxetine, citalopram and sertraline (Nutt, 2005). Since the subject of this thesis is to examine any pharmacological actions of lavender EO, the discussion here will focus on pharmacological treatments and minor mention of psychological treatments will be made.

### 1.2.4.1 Biological treatments

#### 1.2.4.1.1 The Serotonin reuptake inhibitors (SSRIs)

The SSRIs have been designed to work specifically by preventing 5HT reuptake, thus enhancing its transmission. However, their resultant action on the up- or down-regulation of the 5HT receptor varies according to which SSRI is used (Vaswani et al., 2003). Thus, initially, they prevent reuptake of 5HT, making it more available for postsynaptic receptors and presynaptic autoreceptors. This has the effect of down-regulating the number of receptors and thus increasing the amount of 5HT available to the postsynaptic receptors. Generally, SSRIs modify the 5HT/5HT-postsynaptic

receptor ratio, restoring and enhancing 5HT transmission; because of this they take a number of weeks to work. Thus, SSRIs often initially increase anxiety and take 4-6 weeks to become effective in providing relief (Sinclair & Nutt, 2007).

In addition to the SSRIs, there are the 5HT and NA reuptake inhibitors (SNRIs) such as Venlafaxine, which block both 5HT and NA reuptake; these are similar in efficacy and side-effects to the SSRIs.

Efficacy of SSRIs and SNRIs

The beneficial effects of both the SNRIs and SSRIs are unlikely to felt for up to four weeks following administration, and the symptoms of anxiety are often a lot worse in the first two weeks of administration (Sinclair & Nutt, 2007). Furthermore, in the longer-term, they can have inconvenient side-effects such as weight loss, movement disorders, insomnia, tachycardia, dry mouth, nausea, tremor, and sexual dysfunction (Mauri et al., 2002; Baldwin et al., 2005; Nutt, 1995; Vaswani et al., 2003; Linda & Ramesh 2003). Side-effects can sometimes be more serious, such as thoughts of suicide (Baldwin, et al, 2005). In addition, although not causing dependence and tolerance, SSRIs and SNRIs can cause unpleasant flu-like side-effects when stopped abruptly (Baldwin, et al. 2005). Also, even though SSRIs and SNRIs have very good short-term efficacy in the longer-term (18 months to 2 years), meta-analytical studies indicate that, at least 50% of patients seek further help; this is particularly the case in GAD sufferers (Westen & Morrison, 2001).

#### 1.2.4.1.2 Buspirone

In addition to the SSRIs, buspirone (commercial name Buspar) is often prescribed for GAD. Buspirone is a 5HT-1a partial agonist at postsynaptic 5HT-1A receptors in the limbic system and a full agonist at presynaptic 5HT-1A receptors in the raphé nuclei. The presynaptic receptors, found mainly in the raphé nucleus, are responsible for feedback inhibition of 5HT synthesis and release. The 5HT postsynaptic receptors mainly exert their stress-modulating effects in the hippocampus, septum, amygdala and limbic system regions (Toth, 2003). Buspirone reduces turnover of 5HT in the

hippocampus more than in any other brain region (Leonard, 2003). Like SSRIs this drug regulates the release of 5HT; initially, acute administration inhibits the release of 5HT. Buspirone also has effects at dopamine $D_2$ receptor sites, and buspirone's major metabolite, 1-pyrimidylpiperazine (1-PP), has a high affinity for alpha$_2$ adrenoceptors where it acts as an antagonist. Thus its activity might be an interaction between all three neurotransmitter systems (Leonard, 2003).

Like the SSRIs, buspirone's onset of anxiolytic action is slow, 4-6 weeks, and might not be effective in relieving anxiety if BDZs have previously been prescribed. However, buspirone is well tolerated and patients taking it do not develop dependence or withdrawal symptoms (Argyropoulos et al., 2000). Furthermore, buspirone does not interact with alcohol, and so is more patient friendly; it is safe and effective for long term use, as normally required in the treatment of GAD (Nutt, 2007). Buspirone can be used safely with many other drugs, and it exhibits none of the sexual dysfunction side-effects or the effects associated with abrupt cessation of administration of the drug, as exhibited with the SSRIs (Lydiard, 2000). However, its side-effects include appetite disturbances, dizziness and abdominal complaints (Lydiard, 2000; Struzik, Vermani, Coonerty-Femiano, & Katzman, 2004).

#### 1.2.4.1.3 Benzodiazepines (BDZs)

In spite of their many side-effects BDZs are still the treatment of choice, especially for short-term treatment of general anxiety disorder and during initial treatment with other drugs such as the SSRIs.

BDZs act allosterically on the $GABA_A$ receptor to enhance the action of GABA. Until the advent of SSRIs and novel anxiolytics such as buspirone, the BDZs were the drugs of choice for all of the anxiety disorders. They are still used as an adjunct treatment in the intervening time between start of treatment with SSRIs to prevent the increase in anxiety often associated with the first few weeks of administration, or when other types of treatment have failed to work (Nutt, 2007). They are very popular because of their rapid onset of action; they are well tolerated and thought to be of benefit in preventing recurrence of symptoms in long-term use (Lader, 1995; Nutt, 2005). The side-effects of the BDZs are many and vary according to each

individual. Some of the common side-effects include vertigo, weight gain, menstrual irregularities, drowsiness and psychomotor impairments (Vgontzas, Kales, & Bixler, 1995). A potentially serious consequence of BDZ treatment is their cognitive effects. They might cause forgetfulness and memory impairments, an increase in assertiveness and aggressive behaviour leading to violence and abusive behaviour in some patients, or shaking, worry, insomnia, depression and thoughts of suicide in others (Longo & Johnson, 2000; Saias & Gallarda, 2007).

To explain some of these unwanted behaviours it has been suggested that the anxiety, which is being treated by the BDZs, has masked other symptoms and once the anxiety is treated the underlying symptoms become apparent (Nutt, 1990). Hence, it is not the BDZs causing the negative behaviours. However, these side-effects can be very debilitating and even life threatening (Bramness, Skurtveit, & Morland, 2002). In addition to this, BDZs interact with alcohol and other CNS depressants, causing serious over sedation and lack of coordination (Longo & Johnson, 2000) and, depending on the type of BDZ used, dependence and tolerance effects can develop (Salzman, 1993). The Royal College of Psychiatrists UK (1997), recommend treating patients with BDZs for only 2-4 weeks to cover the worsening of symptoms caused by administration of antidepressants such as the SSRIs or as an occasional treatment for acute situations. They are not recommended for prolonged use unless the case has proved resistant to treatment with antidepressants.

Recently, in an attempt to find drugs that have all the benefits of the traditional BDZs, but none of the side-effects, there have been a number of new drug treatments (Millan, 2003). Some of these treatments work on the GABA-ergic system, in a similar manner to the SSRIs, by increasing the concentration of GABA and preventing its breakdown (Millan, 2003; Sinclair & Nutt, 2007). For example Vigabatrin, which inhibits the rate-limiting step in the breakdown pathway of GABA to glutamate, has efficacy in panic disorder (Millan, 2003; Ettinger & Argoff, 2007). Other drugs such as Tiagabine, which increase the concentration of GABA by inhibiting the uptake of GABA into neurons and glial cells, also decrease anxiety and might be of use in treatment of GAD (Pollack et al., 2005; Nemeroff, 2003). It is important to note though, that the GABA-ergic actions of these drugs might not be the only way in which the latter have an effect on anxiety. Drugs that increase the synthesis of GABA by inhibiting some enzymes and activating others, for example Valproate, also have

an anxiolytic effect and are being used clinically to treat anxiety (Ettinger & Argoff, 2006). GABA analogues, such as Pregabalin and Gabapentin do not bind to the $GABA_A$ receptor, but instead work by binding to calcium channels. Calcium channels play important roles in neurotransmitter release in the CNS and are also being used with some success to treat anxiety (Bateson, 2006). However, even these drugs are not without adverse side-effects. In a recent review of trials using Pregabalin to treat GAD more than 20% of patients withdrew from the trial because of adverse effects such as somnolence, dizziness, and headache (Tassone, Boyce, Guyer, & Nuzum, 2007).

#### 1.2.4.1.4 Tricyclics anti-depressants (TCAs)

TCAs, such as imipramine and chlormipramine, have traditionally been used to treat anxiety and might still be used as second line treatments when other forms of treatment fail. The onset of action of TCAs is not as rapid as that of the BDZs but they are more effective than BDZs when taken over a number of weeks (Struzik et al., 2004). However, while TCAs are very effective in the treatment of GAD and panic disorder, the SSRIs are at least as good and have fewer side-effects (Horst & Preskorn, 1998). The TCAs have multiple sites of action, including the 5HT reuptake transporter sites where they inhibit the reuptake of NA and 5HT. Although their actions at these transporters are responsible for their efficacy, these actions are also responsible for their toxicity. The use of TCAs is limited by the fact that they produce all of the side-effects that are associated with the SSRIs and also additional side-effects, such as such as postural hypotension, weight gain, drowsiness and constipation. Furthermore, the fact that TCA overdoses are associated with death caused by seizures, cardiac arrhythmias and metabolic acidosis has resulted in their replacement in most cases by the SSRIs which have a much safer side-effect profile (Nutt, 2003).

#### 1.2.4.1.5 Monoamine oxidase inhibitors (MAOIs)

MAOIs have been used less frequently since the advent of the SSRIs. MAOIs act by inhibiting the action of monoamine oxidase, an enzyme which is important in the deactivation of monoamines, catecholamines (including NA and dopamine), indolamines (including 5HT) and other trace amines (Leonard, 1994). MAOIs are not often used to treat GAD but they are used more frequently to treat panic disorder and social phobia, when other drugs have been tried and failed to work. Advantages to these drugs are that symptoms do not usually worsen at the start of administration, and they elicit less dependence (Nutt, 1990). They do have some undesirable side-effects; some, although not all, MAOIs are toxic when foods containing tyramine, such as cheese, are eaten. Eating these types of foods can lead to a hypertensive crisis (Sinclair & Nutt, 2007). Modern reversible MAOIs overcome this problem. However, they are slow to act compared with the BDZs (Nutt 1990) and have side-effects, which include dry mouth, blurred vision, undue sedation, postural hypotension and weight gain (Tyrer, 1989).

As discussed, none of these drug treatments is ideal, nor is any without side-effect. Additionally, the complicated aetiology of the disease and the fact that different individuals respond to, and tolerate, drugs differently mean that it can take time to find the right drug for each patient. Even the best treatments are only effective 50% of the time (Rachman, 2004; Westen et al., 2001). Further developments in understanding the neurochemical causes of anxiety might help to develop more effective remedies tailored to the needs of each individual sufferer, (Lydiard, 2000; Nash & Nutt, 2007).

### 1.2.4.2 Psychological treatments

The preferred treatment for many anxiety disorders, particularly the ones involving phobia and panic, is psychological intervention. There is widespread opinion among psychologists and other workers that cognition and faulty thinking is of primary importance in these disorders. It is thought that this faulty thinking leads to alterations in brain structure and neurotransmitter regulation in the aetiology of the disease and not the other way round. Faulty thought patterns are thought to predominate in both

trait anxiety, as well as state anxiety (Mathews, Mackintosh, & Fulcher, 1997; McLean, 2001; Nutt, 2005).

These psychological therapies include cognitive therapy, exposure therapy and cognitive behavioural therapy (CBT). Cognitive therapy is based on the cognitive theories of anxiety that place emphasis on the proposal that faulty thought patterns and the subjective interpretation of events by the individual result in a somatic response to the stimulus (see section 1.2.2). Cognitive therapy aims to help the patient understand his or her current thought patterns with the aim of modifying these unhelpful ways of thinking.

Exposure therapy is a behaviour therapy designed to help people overcome their fear of situations and objects, as it is this fear which leads to avoidance behaviours. Exposure therapy involves desensitization to the object or situation causing the anxiety by exposure to that object or situation. CBT is a combination of cognitive therpapy and a behaviour therapy such as exposure therapy. It is of interest that CBT has been shown to alter brain function in a similar way to that of drug treatment (Nutt, 2005).

All of these therapies include cognitive reframing, conscious alterations in behaviour patterns, and challenging negative thought patterns with a view to altering them (Rachman, 2004). Often, therapies are combined with relaxation therapies to help with the somatic symptoms of tension and arousal, which are associated with anxiety (Struzik et al., 2004).

However, similar to pharmacological therapies, these therapies are not without problems, for example good short-term results do not predict a good long-term outcome (Baldwin et al., 2005; Westen & Morrison, 2001). Meta-analyses have indicated that CBT over the long-term is effective only 50% of the time (Westen & Morrison, 2001; Struzik et al., 2004; Vaswani et al., 2003; Ressler & Mayberg, 2007). One reason for this is that the financial and temporal costs of CBT could prevent patients undergoing longer-term courses of therapy and the duration and number of therapy sessions that the NHS can provide, might not be sufficient to effect a cure. In addition, CBT has better long-term treatment results when patients are suffering from pure disorders rather than the more complex co-morbid disorders, such as GAD and major depressive disorder (Westen & Morrison, 2001).

More recently, combination therapy has achieved some promising results; cognitive enhancers, for example D-cycloserine, are administered to accelerate learning. New learning processes have been shown to be involved in the process of extinction, which is used to remove conditioned fears. In both rat and human trials, this process has worked effectively (Davis et al., 2006). It is thought that the effect of this drug is to enhance CBT in the unlearning of old negative memories and replace them with new positive memories (Davis et al., 2006). D-cycloserine is a partial NMDA agonist; NMDA receptors, as well as being a type of glutamate receptor, also, have a function in the action of SSRIs (Nutt, 2005).

Similarly, propranolol, a beta-adrenergic antagonist, has been used successfully to block PTSD by blocking the amygdaloid-cortical pathways involved in adding emotional salience to memories. This has been tested both immediately after the trauma, before the memories have been formed (Pitman et al., 2002), and later after the memories have been formed by using recall therapies and propranolol to prevent reconsolidation of recalled memories while, at the same time, enhancing the learning of new positive memories which have been produced with the help of CBT (Brunet et al., 2007).

Likewise, administering cortisol or glucocorticoids, such as hydrocortisone, after a stressful event might also reduce the likelihood of PTSD occurring. One reason for this is because sufferers of PTSD suffer from corticosteroid insufficiency during the time of, and immediately after, the trauma resulting in a decreased secretion of glucocorticoids. Administering glucocorticoids makes up for this deficiency (Schelling et al., 2006; Schelling, 2007).

### 1.2.5 Complementary therapies in the treatment of stress, anxiety and mental health disorders

Alongside these medically prescribed treatments sufferers of anxiety, who might be disillusioned with the failures of formal treatments or who do not wish to take prescribed medication, sometimes turn to alternatives in an attempt to relieve their

symptoms (Baldwin et al., 2005). Likewise, many undiagnosed anxiety sufferers or sufferers of other illnesses of which anxiety is a side-effect, such as cancer, AIDs, or anxiety resulting from major life changes, like divorce or bereavement, often turn to alternative therapies to relieve their anxiety-symptoms rather than taking medically prescribed drugs. This might be because they perceive alternative and complementary treatments to be more natural with fewer side-effects than prescribed drugs.

The use of complementary and alternative therapies (CAMs) to relieve conditions such as anxiety and stress is a very profitable in the developed world (Ernst & White, 2000), where CAM usage is increasing annually (Wootton & Sparber, 2003; Ernst, 2003; Xue, Zhang, Lin, Da Costa, & Story, 2007). It is widely reported that patients often use alternative therapies as palliatives because they are widely perceived to help them to better manage disease or treatment side-effects (Kessler et al., 2001 in Wootton & Spaber, 2003). This is a problem because some people, particularly those with chronic mental illnesses such as anxiety, self medicate with CAMs because they believe that these therapies are natural and therefore safe, but do not tell their medical practitioner that they are using CAMs. Studies have shown that people with chronic illnesses often try to avoid consulting with their current medical practitioner in favour of alternative therapists (Badger & Nolan, 2007; Badger, 2007).

A survey of complementary medicine providers found aromatherapy to be the most popular recommended treatment for stress and anxiety sufferers (Long, Huntley, & Ernst, 2001; Perry & Perry, 2006). Just some examples of where aromatherapy is popularly used to relieve anxiety are in child birth (Maddocks-Jennings & Wilkinson, 2004), as a palliative to relieve anxiety in cancer care (Graham, Browne, Cox, & Graham, 2003), in diagnosed (and undiagnosed) anxiety and depression (Wilkinson et al., 2007), to help anxious children to learn better in primary school (Kerl, 1997) and in the dentist's waiting room (Lehrner, Marwinski, Lehr, Johren, & Deecke, 2005). Use of aromatherapy has significantly increased in the last few years by both lay people and the medically qualified (Buckle, 2001; Emslie, Campbell, & Walker, 2002; Keegan, 2003; Buckle, 2001; Wilkinson, et al., 2007; van der Watt, Laugharne, & Janca, 2008). Also, it is being used increasingly by UK NHS nurses (Rawlings & Meerabeau, 2003) and nurses around the world to complement traditional medicine (Blunt, 2003; Perez, 2003).

In spite of its widespread use there is still little scientific evidence to substantiate the reported subjective effects of aromatherapy on anxiety (Cooke & Ernst, 2000; Wilkinson et al., 2007).

## 1.3 AROMATHERAPY

The broad definition of aromatherapy, as its name would suggest, is the treatment of illness using odours. In fact, aromatherapy is generally the use of essential oils (EOs) via inhalation and massage, some systems of aromatherapy even include ingestion and administration of EOs in pessaries, to treat a range of conditions and to improve health and wellbeing (Schnaubelt, 2005). It has been suggested that a better name would be 'EO therapy' or the use of plant extracts to treat ill health and improve wellbeing (Perry & Perry, 2006). It is essentially a branch of herbal medicine and is included in Western herbal medicine as well as Ayurvedic and Traditional Chinese Medicine.

### 1.3.1 Essential oils (EOs)

Essential oils, (EOs) are the volatile organic constituents of aromatic (fragrant) plants and volatiles are chemicals that easily evaporate at room temperature. EOs give plants their characteristic odours and flavours. They are especially used in perfumes and flavourings (Webster, 2003). However, EOs are not present in all plants (Tisserand & Balacs, 1995). Although they are called 'essential' oils, it is not because they are essential to the plant for its survival. The term 'essential oil' comes from the medieval-latin term '*quinta-essentia'* a name invented by Paracelsus, the founder of modern pharmacy, to describe those oils that he had distilled from medicinal plants believing the oils to be the quintessence of the plant (Worwood, 1997).

EOs are found in small, highly concentrated, scented droplets of liquid in the plant's flowers, leaves, roots, stem or bark (Dye, 1992; Crozier, Clifford & Ashihara, 2006). They are obtained for commercial use by one of three methods: steam distillation of various parts of the plant, extraction by squeezing the oil from the skin of citrus fruit,

or by dry distillation of woods, barks or roots (ISO 9235, 1997). The yield of oil obtained from plant material varies according to the plant species and although the yield of lavender EO from flowers depends on the variety of lavender and its environmental growth conditions, steam distillation of 250kg of flowers could produce approximately 500ml of lavender oil (Lis-Balchin & Head, in Lis-Balchin, 2004).

EOs have many roles in the plant, for example, their aroma and flavour attract insects to pollinate the plant (Dobson, Danielson, & Wesep, 1999; Quiroga, Sampietro, & Vattuone, 2001; Sampietro, & Vattuone 2001; Verpoorte & Memelink, 2002). In contrast, some EOs have potent insecticidal, anti-bacterial and anti-fungal properties and are thought to protect the plant from predators and therefore enhance survival: tannins and phenolic secondary metabolites, present in some EOs, taste bitter to predators (Baraza, Villalba, & Provenza, 2005; Barnea, Harborne, & Pannell 1993; Villalba, Provenza, 2005; Grassmann, Hippeli, & Elstner 2002). Furthermore, when EOs are present in extremely high concentrations they can have catastrophic effects on cells: their lipophilic properties enable them to pass through cell walls and cytoplasmic membranes and once EOs are in the cell they can disrupt the different cellular layers, leading to a cascade of events that eventually cause cell death (Bakkali, Averbeck, Averbeck, Idaomar, 2008; Basim & Basim, 2003; Jirovetz, Buchbauer, Ngassoum, Essia-Ngang, Tatsadjieu, & Adjoudji 2002; Kim, Roh, Kim, Lee, & Ahn, 2003; Opalchenova & Obreshkova, 2003; Quiroga et al., 2001; Tatsadjieu, Essia Ngang, Ngassoum, & Etoa, 2003; Sampietro, & Vattuone 2001).

The composition of any one EO is complex, sometimes consisting of hundreds of different chemicals, including terpenes, terpenoids, aliphatic and aromatic constituents (Humphrey & Beale, 2006 in Bakkali, Averbeck et al., 2008). The precise combination of these chemicals gives each type of oil its own unique and characteristic odour.

The terpene components of EOs are mainly synthesized in leucoplasts, a non-pigmented plastid, in the cells of the plant's oil glands (Clifford & Ashihara, 2006). The main terpenes are the 10 carbon compounds, the monoterpenes, made up of 5 carbon isoprene units. These are derived from the isomers isopentylpyrophosphate and dimethylallyl pyrophosphate. Terpenes are lipophilic and volatile. In addition,

there are C15 sesquiterpenes, in which the longer chain allows for more diversity of structure. These aromatic compounds are derived from phenylpropane and occur less frequently than the terpenes (Bakkali, et al, 2007). Within these groups there are alcohols, esters, aldehydes and ketones all of which contribute to their properties (Bruneton, 1999).

It is claimed by aromatherapists that it is the mixture of these components acting in synergy that cause the oils to have their therapeutic properties (Harris, 2002; Price & Price, 1999; Worwood, 1997). Alongside their anti-microbial properties, it is claimed that some EOs have mood enhancing properties and can alleviate the symptoms of anxiety and depression (Lawless, 1994; Price & Price, 1999; Tisserand, 1977). There are many EOs with reputations for relieving stress and anxiety (see appendix 1, Table 1.1 for the reputed properties of some EOs reported on internet web sites in 2007). Lavender, in particular *Lavendula angustifolia* 'Miller', has been reported in aromatherapy texts as being one of the most useful in this regard, and it is thought to have a wide range of therapeutic properties (Bowles, Cheras, Stevens, & Myers, 2005; Worwood, 1997; Lemon, 2004; Tisserand 1988). Bastard & Tiran (2000) cite lavender as being the most frequently used of all the essential oils for the relief of anxiety and depression. Indeed, lavender essential oil has been used anecdotally for many years to alleviate stress and anxiety and improve wellbeing, even though there is little scientific evidence to substantiate this.

## 1.4 LAVENDER (*LAVANDULA ANGUSTIFOLIA*) ESSENTIAL OIL

### 1.4.1 Lavender EOs anecdotal and traditional uses

Lavender has been used since ancient times as a sedative and tranquilliser (Buchbauer, Jäger, Jirovetz, Ilmberger, Dietrich, 1993). It was recommended by Dioscorides (c. 40-90 A.D.) for "griefs in the thorax" probably referring to the heart, which was thought to be responsible for maintaining mental-emotional equilibrium. Hildegard von Bingen in c. 1100 AD recommended it for relief from "malign spirits" (Throop,

1998); Culpepper recommended lavender for “the tremblings of the heart and the faintings and swoonings” (Culpepper, in (Castle & Lis-Balchin, 2002)). It is the EO most frequently recommended to relieve anxiety (Gattefossé, 1937; Price, 1998; Tisserand, 1977; Tisserand, 1988; Valnet, 1980; Worwood, 1997); one reason for this is that, as well as being used anecdotally for its calming propertieies for many centuries, it is one of the safest EOs and can be used with children, the elderly and the terminally ill alike (Wildwood, 2003; Tisserand et al., 1995).

#### 1.4.1.1 Species of lavender and properties of lavender oil

Lavenders (Lavandula spp.) belong to the family Labiatae (Lamiaceae). French botanist Joseph Pitton de Tourneforte (1656-1708) recognized the genus Lavandula, as two separate genera: Lavandula, consisting of *L. spica* and *L. multifidia*, and the genus Stoechas, consisting of *L. stoechas* and *L. pedunculata*. Linnaeus (1707-1758) united the two genera and provided binomial names for 5 of the species (Lis-Balchin, 2004). Also important, was Philip Miller (1758) who named *L. angustifolia*, as a consequence of which it is often referred to as *L. angustifolia* ‘Miller’ (Upson & Andrews, 2004). Although there are more than 32 species, lavenders fall into four main categories, listed in Table 1.3 (Epson, 2004 in Lis Balchin, 2004). All four of the groups have similar ethno-botanical properties, and major chemical constituents, but there are differences in the reported therapeutic uses for the different species

**Table 1.3 The four main categories of lavender**

| Category of lavender | Description |
|---|---|
| ***Lavandula latifolia,*** | a grass like lavender, grown mainly in the Mediterranean regions |
| ***Lavandula stoechas*** | has butterfly-like bracts on top of the flowers and is sometimes known as French lavender and was the species used medicinally by the ancient Greeks (Upson, Andrews, Harris, King, & Langhorne, 2004) |
| ***Lavandula x intermedia*** | a sterile cross between *L.latifolia* and *L.angustifolia* (Cavanagh & Wilkinson, 2002) |
| ***Lavandula angustifolia*** | a stocky plant and a fuller flower, commonly known as English lavender, formerly known as *L. vera* or *L. officinalis* |

### 1.4.1.2 The composition of lavender essential oil

Lavender essential oil is produced by steam distillation, either of the flower heads, or for commercial use, it is sometimes distilled from both the flower heads and the foliage. The chemical compositions of the two types of extract differ; hence, their odours differ. The oil of the flower is sweeter and more aromatic than that of the foliage and flower oil combined.

The composition of the EO is usually determined by gas chromatography with mass spectrometry; hence, the commercially used oil composition is well known and international standard specifications have been published for some of the species. For example, in France, the Appellation d'Origine Contrôlée (AOC) established in 1981

protects French lavender EOs against competition from lavender EOs from other countries and from adulteration, preventing synthetic components being added to oils to enhance their quality and hence their price (Upson & Andrews, Tisserand & Balacs, 2005).

The main constituents of lavender are linalool (25-38%), linalyl-acetate (25-45%) (see Figure 1.5 below), 1, 8 cineole (<1.8%), ß- ocimene, usually both *cis* and *trans* isomers (6-16% total for both isomers), terpin-en-4-ol (2-6%), and camphor (0.5%). Precise oil composition varies between lavender species. For example, oil derived from *L. stoechas* is higher in camphor levels than oil derived from *L. angustifolia* which is low in camphor (<2%). Low camphor varieties tend to have higher terpene content (e.g. ß- phellandrene) and sesquiterpenes (e.g. caryophyllene). Thus, *L. angustifolia* is used in the perfume industry; whereas, *L. stoechas* tends to be used as an insect repellant. Additionally, *L. latifolia* and *L. x intermedia* also have high camphor contents and because of this all three species are considered harmful as camphor can cause epileptiform convulsions. Therefore use of the high camphor content oils is commonly avoided in aromatherapy treatments; whereas, *L angustifolia* is considered, by aromatherapists, to be one of the safest and most useful EOs (Tisserand et al., 1995; Castle et al., 2002; Upson & Andrews, 2004). Because of its reputed safety lavender is widely used both at home, by aromatherapists, and by nurses trained in aromatherapy.

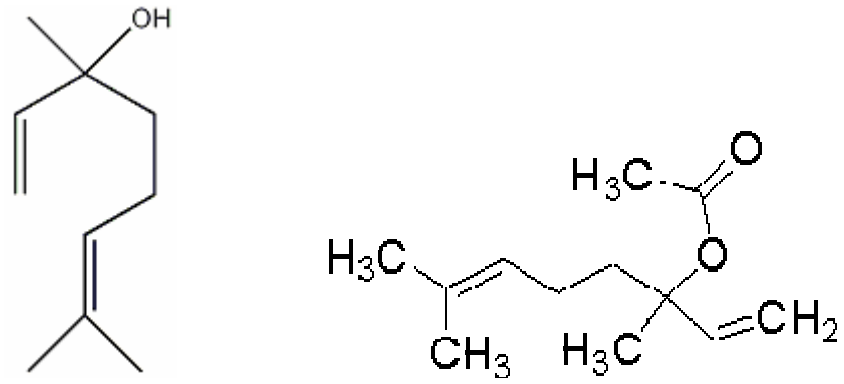



**Figure 1-5 The main components of lavender, left: Linalool (3, 7-dimethylocta-1, 6-dien-3-ol) and right: linalyl-acetate (3, 7-dimethyl-1, 6-octadien-3-yl acetate).**

## 1.4.2 Human research

### 1.4.2.1. Hospital and clinical studies

There is a great deal of interest in aromatherapy as part of nursing care when treating patients in hospital, in particular the use of lavender EO to improve mood and alleviate anxiety (for reviews see: Bowles, Griffiths, Quirk, Brownrigg, & Croot, 2002; Bowles, Cheras, Stevens, & Myers, 2005; Fowler, 2006; Graham, Browne, Cox, & Graham, 2003; Lee, 2003; Maddocks-Jennings & Wilkinson, 2004; Walsh & Wilson, 1999; Weier & Beal, 2004; Wilkinson et al., 2007). However, in a clinical setting the main aim is not one of research but, understandably, of doing everything possible to improve the patient experience. Hence, there are usually limitations to these studies (Wilkinson, et al., 2007). Limitations such as lack of standardized treatment (Dunn, Sleep, & Collett, 1995; Itai et al., 2000), lack of a control group (King, 1988), small sample size and lack of reported statistical data (King, 1988, Itai et al., 2000; Brownfield, 1998; Hardy, Kirk-Smith, & Stretch, 1995; Snow, Hovanec, & Brandt, 2004). Therefore, these types of studies will not be discussed further.

### 1.4.2.2 Lavender- pleasant odour effects or pharmacological effects on measures related to anxiety?

There are a number of unresolved issues when trying to ascertain the anxiolytic effects of a pleasant-smelling odour. First, the anecdotal effects of lavender odour on stress are widely known and thus, any stress/anxiety relief experienced could be caused by expectation effects. Second, its effects might be because of its pleasant odour, rather than to specific pharmacological properties of the whole oil and/or its component parts. Third, its effects might depend on the type of anxiety task used to elicit anxiety. As already discussed (see section 1.2), anxiety is not a homogeneous condition and different types of anxiety affect different parts of the brain; often each type responds differently to different forms of treatment. Fourthly, the route of administration might make a difference to its anxiolytic effects. Many different routes of administration have been used to deliver lavender and there is a lack of standardisation of delivery route. For example, lavender is often administered by inhalation (Ceballos, Matthews, Catledge, & Geisler, 2000) or dermal absorption (Burnett, Solterbeck & Strapp, 2004) and even within these two administration routes

there are further differences. In the odour studies lavender is often inhaled neat (Diego et al., 1998), in water (Saeki & Shiohara, 2001; Shiina et al., 2007) or via some other medium, for example propylene glycol (Atsumi & Tonosaki, 2007), or oil. As a result, participants are exposed to differing components of lavender depending on the diluent used. In the dermally administered studies diluted lavender might be massaged, or placed in a bath, to treat all or only part of the body such as the feet (Saeki, 2000), or a few drops might be placed on, for example, the wrist (Burnett et al., 2004).

The following sections attempt to address these points: the first section will examine whether lavender has pharmacologically anxiolytic properties or whether its effects are because of expectations; the second section asks whether lavender's supposed anxiolytic effects are due to non-specific odour properties; the third section will address whether the different type of anxiety-tests make a difference to the anxiolytic properties of lavender. Finally, since anxiety and stress are not likely to be experienced once only and are often prolonged conditions, there will be a short discussion on the acute versus chronic effects of lavender EO. The differing routes of administration, and whether lavender can exert its effects only by dermal administration or by volatilisation of the oil, will be covered in the section that follows, which considers the effects of lavender on anxiety in animal tests.

#### 1.4.2.2.1 Expectation effects versus pharmacological effects

To determine whether lavender has any anxiolytic properties that are specifically due to pharmacologcal properties, rather than to any non-specific odour-expectation effects, exposure to the odour needs to be controlled (effectively removing it from the study) so that participants are blind to whether they are receiving lavender treatment or not. There are difficulties in blinding when working with such a universally recognizable odour as lavender. In almost all studies reported, participants were not masked from the odour; as a consequence there is no blinding of participants or experimenter to which condition the participant belongs. This lack of blinding makes it difficult to determine if any anxiolytic effects are because of its pleasant odour, odour-expectations and associative effects, or indeed due to some pharmacological property of the oil, or whether all, or a combination of these factors are involved.

One solution to the problem of blinding would be to treat the nasal passages with zinc sulphate, to render participants anosmic, in order to measure any anxiolytic effects attributable to lavender besides its odour properties. However, this intervention is extreme and as well as causing possible permanent harm, might actually alter the behaviour of participants and so confound the study. Another approach might be to use anosmic patients. However, when anosmic participants inhaled 1,8 cineol (a component of some EOs) for twenty minutes the volatilised oil still acted directly on the brain in the same way as in normosmic controls (Nasel, Nasel, Samec, Schindler, & Buchbauer, 1994). In addition, results of MRI scans suggest that even at a concentration of volatalised odour lower than the detection threshold the brain is still activated, even though the odour is not consciously detected (Nasel et al. 1994; Sobel et al., 1999). Therefore, it would be difficult to interpret if any alteration in behaviour was due to a subliminal awareness of the odour or to direct pharmacological action.

In contrast, an alternative approach to blinding of participants to the odour, and thus the experimental condition to which they belong, is to block the capacity of participants to detect the odour. Heuberger, Redhammer and Buchbauer (2004) adopted this approach, by placing an oxygen mask over the nose and mouth of participants while concurrently administering linalool, a component of lavender essential oil, via massage. Linalool has been shown in animal research to have sedative properties reflecting those of the whole oil (Buchabauer, Jirovetz, Jager, Planck & Dietrich, 1991 & 1993). Furthermore, it has been shown that EO components can enter the circulatory system by using massage (Jager, Buchbauer, Jirovetz & Fritzer, 1992). The design of Heuberger et al.'s study was between subjects and laboratory based. Participants' abdomens (n=14 in each group) were massaged with 1ml of a 10% linalool solution in peanut oil. When compared with massage with a peanut oil control, linalool caused decreases in systolic blood pressure and skin temperature but had no effects on subjective wellbeing (Heuberger, Redhammer, & Buchbauer, 2004). This study indicates that in humans, linalool has an effect on physiological measures in an anxiolytic direction without the odour being present; indicating pharmacological rather than expectation effects. However, the fact that there was no effect on wellbeing might be because there was no odour present or because some other chemical (s) is (are) required from the oil to affect psychological measures.

#### 1.4.2.2.2 Odour Hedonic Effects versus pharmacological effects

Effects of EOs might be because of emotional effects of smell pleasantness rather than inherent pharmacological properties. Hedonic processing of odours has been shown to be an emotional rather than an analytical task (Vernet-Maury, Alaoui-Ismaili, Dittmar, Delhomme, & Chanel, 1999), which is affected by sex and the handedness of the individual, (Dijksterhuis, Moller, Bredie, Rasmussen, & Martens, 2002), the time of day and year, mental state (Goel & Grasso, 2004), ethnic origins (Schiffman, Suggs, & Sattely-Miller, 1995) and age (Fitzgerald et al., 2007). For example, in women and men at mid-life, pleasant odours (perfumes) significantly improved mood (Schiffman, Sattely-Miller, Suggs, & Graham, 1995). In general, pleasant odours have been shown to positively affect the emotions, and unpleasant odours negatively affect emotion (Knasko, 1992). For example, smelling lavender, which was viewed as a pleasant odour, increased happiness; while, camphor, which was neither pleasant nor unpleasant, produced less specific emotions, inducing both happiness, surprise or sadness; whereas, butyric acid, an unpleasant odour, elicited anger and disgust (Vernet-Maury, et al., 1999).

Even though the hedonic processing of odours is mainly emotional, the effects of odours are not limited only to emotion and odours can also play a large part in association and conditioned behaviours. Furthermore, odours can have direct effects on cognition and behaviour, even when not consciously perceived. For example, the smell of cleaning fluid caused participants to keep their direct environment cleaner than usual during an eating task (Holland, Hendriks, & Aarts, 2005).

In addition to the influence of odour hedonics on psychological and behavioural factors, odour pleasantness can influence physiological parameters too. Pleasant, versus unpleasant, odours have been shown to influence physiological measures in opposite directions. The inhalation of odours, which participants had identified as having an unpleasant smell, were found to increase heart-rate (Bensafi et al., 2002). However, smells that were pleasing to participants increased alpha EEG waves, which are related to relaxation (Yagyu, 1994).

Furthermore, although lavender is pleasant smelling at low concentrations it is often reported to be an unpleasant odour at higher concentrations. Low concentrations of

inhaled lavender (1/1000 in propylene glycol) decreased serum cortisol and increased FRSA, an index of stress that decreases in response to physical fatigue and increases when mood is positive (Atsumi & Tonosaki, 2007). However, very high concentrations of lavender, which participants found unpleasant, failed to have any effect.

Differences in the physiological response to odours are not limited to effects of pleasant versus unpleasant odours. Different pleasant smelling odours have been shown to elicit different effects on brain waves. Torii et al., (1988) found that lavender odour reduced a pattern of brain waves called contingent negative variation (CNV). Increased CNV is thought to be related to cortical arousal and increased stress (Nagai et al., 2004). In comparison, jasmine, regarded as a pleasant but stimulating odour, increased CNV in a similar manner to the stimulant caffeine in the same study. Congruent with these findings, electronically diffused lavender odour (for ten minutes in four sessions over two weeks) increased alpha and mean EEG activity (Ceballos, Matthews, Catledge, & Geisler, 2000). In another study, inhaled lavender (6 drops of lavender in 10ml of warm water for 10 minutes) decreased sympathetic arousal in young female adults (Saeki & Shiohara, 2001). Systolic blood pressure, galvanic skin conductance, and the low frequency component of heart-rate variation were reduced; while parasympathetic activity increased, as indicated by an increase in the high frequency component of heart-rate variation. In contrast, citronella and rosemary odours increased sympathetic nervous system activity. However, none of the participants or experimenters was blinded to the odours and these effects are just as likely to be due to odour hedonics as they are to pharmacological properties of the oils, and so they do not provide unequivocal evidence for specific pharmacologically anxiolytic effects caused by lavender EO.

In one study where odour pleasantness was taken into account, lavender was compared with rosemary (either oil was applied to the wrist, 3 drops in water, or a water only control) and participants were subjected to a timed crossword study (Burnett et al., 2004). When odour pleasantness was controlled, lavender and rosemary had differing effects and lavender did not affect physiological measures (heart-rate and body temperature). However, lavender did have positive effects on psychological self-report measures producing higher positive affect, increased vigour, activity and less fatigue and inertia, than the no-odour control. However, lack of

blinding to the odour in this study means that expectation effects were not controlled. This result is in contrast with Heuberger's study where the odour, albeit only linalool and not the whole oil, was removed and only physiological, and not psychological, measures were affected. There are other differences which make the two studies difficult to compare directly; in one study peanut oil was used as the carrier and in the other water, also the part of the body where the oil was administered differed. Thus, the bio-availability and absorption rates of the diluted oils might have differed.

Contrary to the Heuberger (2004) study, however, are the results of a study conducted in 5-day-old neonates of depressed mothers where there was found to be no difference between rosemary and lavender oil odour on measures related to wellbeing and negative affect. Both odours (3 drops of 10% in grape seed oil on a dental swab suspended above each baby's head) shifted brain activity (frontal EEG activity) to the left, particularly in babies who had predominant right frontal activity (Fernandez et al., 2004; Sanders, et al. 2002). Increased left frontal EEG activity is an indication of increased positive affect. It was not possible to ascertain whether the babies had any preference for either of the odours because they were too young to be asked! In addition, no information was given on whether the depressed mothers routinely used EOs to help lift their depressed mood state or which odour the mother preferred if any. Therefore, because there was no difference in response between the two odours, one could argue for non-specific effects rather than specific pharmacological effects.

#### 1.4.2.2.3 Does the type of anxiogenic stimuli have an effect on lavenders effects to relieve anxiety?

As previously discussed, a number of studies have examined lavender's effects in anxious or stressed participants. However, its ability to produce anxiolysis might be dependent on the type of anxiety that the test elicits. There is often no standardisation or validation of the conditions used to elicit anxiety. For example, lavender odour reduced self-reported mental stress and increased arousal when participants were left to wait in a small sound-proofed room (Motomura, Sakurai, & Yotsuya, 2001). However, being left in a peaceful and pleasant smelling situation for twenty minutes might be relaxing rather than stressful and there is no indication in this study of whether participants liked the odour or not. There have been a number of studies involving tasks in mathematics to elicit anxiety in participants. However, not all

participants find maths tasks anxiety-provoking and some, particularly from the student population, might even enjoy the challenge. Again, as with the studies mentioned earlier, none of these types of studies controlled for the odour. However, in determining whether lavender has specific pharmacological effects these studies do provide some evidence that either lavender odour or pleasant smells have the potential to reduce anxiety, but, they do not provide evidence for any direct pharmacological action caused by lavender EO, and so will be briefly mentioned.

In studies using mathematics tasks as the anxiogenic stimuli, exposure to lavender odour (3 drops of 10% on cotton wool inhaled for 3 minutes) (Diego et al., 1998) or lavender scented cleansing gel odour (Field et al., 2005) caused a decrease in anxiety and depression (STAI and profile of mood states (POMS)). They both also increased physiological relaxation (alpha and beta EEG increased) and shifted or maintained relative left frontal EEG activity (a re-analysis of the data in Sanders et al., 2002). Aromatherapists often claim that it is only the natural EO odours that have therapeutic properties (Tisserand, 1977). Whereas, lavender enabled participants to complete the maths task more accurately and more quickly than without any odour, rosemary odour increased alertness (Diego et al., 1998). Again, in both studies participants' odour preferences were not mentioned.

Another maths-based study employed the Uchida-Kraepelin test, a mathematical additions test, as the anxiogenic stimuli (Toda & Mormoto, 2008). Ten-minute lavender odour inhalation following the test decreased levels of chromogranin A. In contrast, there was no decrease in the control group who did not inhale lavender. Chromogranin A is an acidic glucoprotein, co-released with adrenaline and noradrenaline in response to anxiogenic stimuli (Kanno et al., 1999). However, since there was no comparison odour, and administration was not blind, it is difficult to attribute these effects specifically to lavender.

When a stronger anxiogenic stimulus, pain, was used lavender had no effect in improving mood, as measured by the positive and negative affect scales (PANAS) (Watson ,Clark and Tellegan, 1988), or anxiety (STAI), or on decreasing salivary cortisol elevations in response to the pain (Gedney, Glover, & Fillingim, 2004; Hoferl, Krist & Buchbauer, 2006). However, the authors did report that lavender odour had an effect on memory. Participant's self-reports after the study indicated that lavender

had different effects on pain perception depending on the sex of the participant. Males reported diminished pain sensitivity, whereas females reported diminished pain unpleasantness. However, when lavender was compared with rosemary, the findings with rosemary were similar, although to a lesser extent. The authors concluded that lavender or aromatherapy might have had memory effects, altering how participants viewed the experience retrospectively. Memory might have an important role to play in anxiety. This is because some aspects of memory, such as the laying down of new memories and memory recall, share common neurological substrates with anxious behaviour, for example the hippocampus and frontal cortex. Indeed, some types of anxiety do have a strong memory component to them, for example the flash backs associated with PTSD. Furthermore, there is some evidence that anxiolytic drugs might impair memory depending on the dose prescribed (Gray & McNaughton, 2003). There is other evidence for lavender odour giving rise to improved mood while impairing memory function. In a randomised between-subjects study, participants (n=142) were exposed to 4 drops of lavender EO on a diffuser pad, 4 drops of rosemary odour, or no odour (4 drops of water), and asked to complete a battery of cognitive tests (Moss et al., 2003). Lavender odour impaired working memory and reaction time. Additionally, it improved contented mood, but not calmness and led to a decrease in alertness, which continued beyond testing.

Pain has also been used as a stressor in five-day-old infants. The anxiolytic effects of linalyl-acetate odour were tested on endocrine responses to the heel-prick test for phenylketonuria (Kawakami et al., 1997). The stress hormone, cortisol, was used as the dependent measure. Infants (n=83) exposed to linalyl-acetate or baby milk produced less cortisol during the test than when compared with no odour. The odours were placed in a bottle with a smelling blotter which was placed 1.5 cm from the tip of the bottle and the bottle was held 2-3 cm from the infant's nose during the heel-prick. No mention is made of whether the mothers used lavender during pregnancy, or after the birth, and thus whether its odour might be associated with the reassuring presence of their mother, which might also account for the effects seen here.

Similarly, the effects of S-(+)– linalool odour exposure, R-(-)- linalool odour (the entantiomer prevalent in lavender) and a no-odour control were tested in a between-subjects design study on participants taking part in the Trier Social Stress Task (Kirschbaum, Pirke & Hellhammer, 1993; Höferl, Krist & Buchbauer, 2006). Both

physiological and endocrine measures were taken. Linalool –(R) – odour was reported as stress relieving: heart-rate decreased; whereas linalool – S-(+) had an activating effect: blood pressure (systolic and diastolic) and heart-rate were higher than both the control and the R-(-) - enantiomer. In both cases, cortisol was lower after twenty minutes than in the control group. This is reflective of a lower cortisol response during the resting phase in those exposed to either odour when compared with the control.

#### 1.4.2.2.4 Acute effects versus prolonged effects

Another failing in most studies looking at lavender odour's anxiolytic effects is that only acute effects are considered. Since sensory adaptation allows organisms to habituate to their surroundings, repeated or prolonged exposure to an odorant leads to decreases in stimulus-specific sensitivity (Dalton, 2000). Thus, when working with an odour, one might expect participants to habituate to that odour after a short time, for example after, or even before, the end of the experiment. Cooke and Ernst (2000) claim that aromatherapy has only short-lived and transient effects in alleviating anxiety; if this is the case, habituation to the odour would explain the observed effect. As most studies look at acute effects only, it is not clear whether any effects experienced are merely transient while exposure to the odour is taking place or are lasting and continue once exposure to the odour has ceased. There is little evidence of lavender's effects lasting for longer than the duration of the experiment. The effects of lavender, administered in a footbath, have been shown to last for ten minutes when compared with a control bath with no lavender in it (Saeki, 2000). In this study lavender increased blood flow and parasympathetic nervous system activity. The design of the experiment was within-subjects and participants were female trainee nurses. However, ten minutes is not long and the effect could be classed as a transient one.

#### 1.4.2.2.5 Summary

In summary, lavender's odour would appear to be important in eliciting any mood effects, but these effects might be only transitory. The studies described here indicate that lavender might possess some specific pharmacological properties. For instance,

Heuberger et al. (2004) indicated that linalool odour is not necessary for physiological deactivation; however, the odour might be necessary for perceived wellbeing. In addition there might be reasons other than simply a lack of perception of the odour to explain why linalool failed to reduce self-reported anxiety in Heuberger's study: other components of the oil could be required for this action. Studies on other oils have shown that the effects of EOs are complex and could act via a number of different physiological and psychological mechanisms (Hongratanaworakit, Heuberger, & Buchbauer, 2004).

It is not clear, then, whether any reported anxiolytic effects from the studies discussed here are caused by lavender's pleasant odour, or a combination between its odour and direct pharmacological effects. Thus with no standardisation of task and no blinding to the odour it is difficult to dissect exactly if lavender relieves anxiety. None of these studies provide evidence of pharmacological anxiolysis that can be attributed to lavender EO. At best, if lavender does relieve anxiety, then it looks as though its mechanism of action is not simple. Possible potential mechanisms of action for odours, such as lavender EO, have been summarised into the following four categories: quasi-pharmacological, in which smaller amounts than would normally be taken in by a drug are being absorbed to exert a mild pharmacological effect; semantic effects, in which memories formed from highly charged emotional situations, are associated with an odour; hedonic mechanisms, emotional responses, depending on their pleasantness or unpleasantness, evoked by odours; fourthly, placebo effects, arising as a result of suggestion or expectation (Jellinek, 1998). It has also been suggested that direct pharmacological effects reinforce each successive exposure to the EO odour (Broughan, 2001).

In conclusion, cognitive mechanisms of odour transduction often confound pharmacological effects in humans. Therefore, the next section will examine work conducted using animals naïve to the odour of lavender. This method potentially overcomes some of the difficulties presented in the human studies mentioned above. In particular, studying the effects of lavender on animals that are naïve to its odour and its reputed effects eliminates some of the cognitive difficulties encountered when testing lavender odour on human participants. It also allows for longer-term testing of the odour in a controlled environment, which would not be so easy to do when employing human participants.

## 1.4.3 Animal research

### 1.4.3.1 Studies examining lavender odour's effects on measures related to anxiety in animals

Animals that have been reared in a laboratory setting allow for a more controlled approach, as their complete past history of drug exposure and conditioning is known. *In-vivo* animal experiments examining lavender's anxiolytic effects have been mainly carried out in rodents. However, one study looking at dogs housed in a rescue shelter indicated that they spent more time resting and were less vocal following lavender inhalation for four hours per day, for five days (Graham, Wells, & Hepper, 2005). These effects were interpreted as increasing the wellbeing of the dogs. No details are given of how much lavender the dogs inhaled each day. Also, the reported effects could be because of sedation rather than anxiolysis, since lavender odour and its main components linalool and linalyl-acetate, when admisistered indivivually, were demonstrated to have powerful sedative properties and were able to reverse the effects of hyperagitation caused by caffeine injection in mice (Buchbauer, Jirovetz, Jager, Dietrich, & Plank, 1991). Injected caffeine increased locomotor activity to approximately 60% above normal activity. Lavender odour decreased this locomotor activity by about 90%, back to nearly normal activity. Its main constituents linalool and linalyl-acetate also reduced activity but to a lesser extent, 57% and 47% respectively, suggesting that the components work in synergy in the whole oil. The plasma content of the EOs was also measured one hour after inhalation to ascertain whether volatility (ability to evaporate) had anything to do with the measured effects. Results showed no correlation between volatility and locomotor effect (Buchbauer, Jirovetz, Jager, Plank, & Dietrich, 1993). However, a significant proportion of the linalool was conjugated to glucuronic acid when linalool was measured before and after incubation of the blood samples with ß-glucuronidase (Jirovetz, Buchbauer, Jager, Raverdino, & Nikiforov, 1990) et al., 1990). Buchbauer and his colleagues (1990) also noted that the more lipophilic esters were able to reduce the motor behaviour of the animals in relatively lower concentrations than their corresponding alcohols could achieve after inhalation, even though some of the alcohols were more volatile. Buchbauer's group attributed this sedative activity to their ability to cross

membranes such as the blood brain barrier more effectively rather than to their volatility (Buchbauer, Jirovetz, Jager et al., 1993). These results are interesting when compared with the effects of lavender odour in humans, where odour hedonics could also play a part in the behavioural effects. It is important to note that although many anxiolytics cause sedation, depending on dose, sedation does not necessarily equate with anxiolysis.

There are other rodent studies that indicate lavender's potential acute anxiolytic effects; lavender had anxiolytic effects similar to chlordiazepoxide injection after one hour's exposure in an open field rat model of anxiety (Shaw, Annett, Doherty, & Leslie, 2007). By contrast, inhalation of higher doses of lavender odour led to sedation. Chronic exposure to the odour was not tested.

#### 1.4.3.2 Studies examining orally-administered lavender's effects on measures related to anxiety in animals

Studies have also indicated that orally-administered EOs share similar effects to those of the odour (Kovar, Gropper, Friess, & Ammon, 1987). The effects of oral lavender in models of anxiety, such as the four plate test, the hole-board test, the elevated plus-maze and the potentiation of barbiturate sleeping-time have been examined (Guillemain, Rousseau, & Delaveau, 1989). Lavender, 1/60 dilution in olive oil at a dose of 0.5ml/20g, was administered acutely by oral gavauge to mice thirty minutes prior to testing. Results in the exploratory tests indicated that lavender caused sedation rather than anxiolysis, possibly because the dose was too high. A lower dose might have had an anxiolytic effect in the other tests rather than just a sedative one. In the barbiturate test, sleeping time significantly increased in response to lavender, indicating an interaction with the $GABA_A$ receptor complex (Guillemain et al., 1989). However, a further study revealed that oral lavender's effects on barbiturate sleeping time might be short-lived, as it was found to disappear after five days administration (Delaveau et al., 1989).

#### 1.4.3.3 Studies examining injected lavenders effects on measures related to anxiety in animals

Injected lavender EO provides evidence in favour of it having acute anxiolytic effects, aside from any effects attributable to odour hedonics. Twenty minutes after lavender essential oil injection (s.c) mice exhibited anti-conflict activity similar to that seen after diazepam administration (Umezu, 2000). The test used was the Geller-Seifter conflict test; it has been argued that this test is a test of phobia rather than general anxiety (Gray & McNaughton, 2003). Incidentally, the dose used was comparable with those that would be used in an aromatherapy massage session (i.e.1600 mg/kg). However, in another study lavender also had anti-conflict effects in Vogel's test at 800mg/kg, which is a lower dose than that estimated to be used in an aromatherapy session (Umezu et al., 2006). Umezu used injections as the administration route, rather than odour, as he hypothesized that odours are not potent enough to have anxiolytic effects. This route also controls any preferences due to odour, which might also affect animal behaviour. The results were comparable to those given by diazepam at doses ranging over 0.5-2mg/kg. Interestingly, and arguing for specific effects attributable to specific oils, Umezu has conducted a number of studies examining anti-conflict tests of essential oils and found anti-conflict activity for only two oils, rose EO (Umezu, 1999) and lavender EO (Umezu et al., 2006). Rose EO is not thought to act via the GABA binding site because flumazenil, the competitive $GABA_A$ receptor inhibitor, failed to antagonise rose oils anti-conflict effects; although, both the oils of rose and lavender exhibited similar overall effects when compared with those of diazepam.

In a further examination of lavender's anti-conflict effects and a search for the components of lavender responsible, linalool was identified, having an effect in both models, at 600mg/kg and 400mg/kg respectively (Umezu, et al., 2006). Other components that might also contribute to lavender's anti-conflict effects are: linalyl-acetate, which, at 1600 mg/kg significantly decreased the number of electric shocks received by the mice in Vogel's test; borneol, which increased the response rate in Geller's test, at 800 mg/kg, and significantly decreased it in Vogel's test; camphene, which increased responses in Geller's test, at 800 mg/kg, and had no effect in Vogel's test; and, cineol and terpin-en-4-ol, which produced significant responses in the safe period. These results indicate that although linalool appears to have the most potent

effects in both tests when compared with lavender's other components, the other components could also contribute to the effects of the whole oil lending evidence to the synergy effects described by aromatherapists (Price, 1998).

In contrast to these results, injected linalool (125 mg/kg, i.p) failed to have an anxiolytic effect in a study using rats in the elevated plus-maze (Cline et al., 2008). However, in the human study conducted by Heuberger and colleagues (2004), linalool also failed to have anxiolytic effects on self-report measures. The measurement of behaviours in this study (Cline et al., 2004) appears to be flawed because measures of fine and gross motor movement were taken instead of specific ethological measures, such as risk-assessment and exploratory measures (see Chapter 2 for an explanation of these behaviours). In addition, the linalool might have been present in too small a dose.

#### 1.4.3.4 Studies examining the effects of lavender and its components on pathways which might be implicated in the anxiety response.

There are only a few studies in animals which examine lavender's effects on anxiety. Therefore, it is important to look at the effects of lavender and its components on other systems; these could also share common pathways relevant to the mechanisms involved in anxiety responses.

More evidence in favour of lavender's pharmacological effects, and possible potential mechanism of anxiolytic action, are provided by studies on the effects of lavender on seizures induced by electric-shock or by convulsant drugs in mice (Atanassova–Shopova & Roussinov, 1970). Low doses of injected lavender (i.p.) exhibited depressive effects on the central nervous system, whereas high doses exhibited anticonvulsive effects. Lavender prevented convulsions induced by pentylenetetrazole, a non-competitive $GABA_A$ receptor inhibitor, which binds at the picrotoxin-sensitive site of the $GABA_A$ receptor but might also have activity on AMPA receptors. AMPA receptors are a class of glutamate receptor. However, lavender did not inhibit strychnine convulsions. Strychnine is a glycine receptor antagonist; glycine is one of the most widely distributed inhibitory neurotransmitters in the CNS. In addition, in this study, lavender potentiated the narcotic (anaesthetic) effects of chloral hydrate, alcohol and Evipan-sodium without having narcotic effects

when used alone (Atanassova – Shopova & Roussinov, 1970). It also checked the effects of amphetamine (Aktedron) and caffeine by inhibiting motor activity. Similar effects have also been found for linalool in mice (Elisabetsky, Marschner, & Souza, 1995).

Studies in rats have also indicated that dilute, but not neat, lavender odour (1 in 100 (vol/vol) in water) depresses sympathetic nerve activity in adipose tissue and increases parasympathetic (vagal nerve) activity (Shen et al., 2005). These effects lasted for 90 minutes following lavender odour exposure. Administration of a local anaesthetic or zinc sulphate, both of which inactivate olfactory receptors, completely abolished this activity, indicating possible odour mediated effects, rather than effects via the pulmonary route. Thioperamide, an H3 antagonist, also completely abolished these effects, providing evidence of an interaction of lavender and linalool with histaminergic pathways (Shen et al., 2005). In addition, later studies indicated that lavender decreased blood pressure in rats and implicated the hypothalamic suprachiasmic nucleus in the autonomic nervous system response (Tanida, Niijima, Shen, Nakamura, & Nagai, 2006; Shen et al., 2007). The suprachiasmic nucleus has a role to play in coordinating neuroendocrine circadian rhythms; these regulate a number of hormone systems including the stress hormone cortisol.

In accordance with this, vaporised lavender (*L. burnatti* super) odour also has been demonstrated to have effects on the endocrine system. Lavender oil and its major component linalool have been shown to alter plasma adrenocorticotropic hormone (ACTH), catecholamine and, gonadotropin levels in ovariectomised rats. These hormones have a role to play in the stress response and might explain any tension relieving properties of lavender (Yamada, Mimaki, & Sashida, 2005).

There is also evidence that orally or inhaled lavender has anti-nociceptive, pain-relieving effects following 60 minute lavender inhalation or oral ingestion of 100 mg/kg lavender (Barocelli et al., 2004). However, in this study linalool or linalyl-acetate did not have the same analgesic effects as whole lavender, possibly owing to too low a dose being used. The variety of lavender used here was not *L. angustifolia*, but *L. hybrida Reverchon Grosso* (a hybrid of *L. angustifolia* and *L. latifolia)*, which is hardier and yields higher amounts of the EO. In contrast to this study, in other studies linalool has been reported to have anti-nociceptive properties. Sub-cutaneous linalool injection had pain-relieving properties both in male mice and in rabbits in the

conjunctival reflex test (Peana et al., 2003). Linalool, in these studies, appeared to activate opiodic and cholinergic systems. Furthermore, in studies using Wistar rats, linalool was reported to have cholinergic local anaesthetic activity and the ability to block NMDA receptors. It also exerted some of its effects by: muscarinic (M2), opioid or dopamine (D2) receptor, potassium channels (Peana et al., 2004). Likewise, linalool significantly inhibited glutamate-induced nociception effects in mice in the induced paw-licking test; which is a test of how long an animal spends licking its injured paw when ionic but not metabotrophic agonists are present (see section 1.2.3.2 for a description of the different types of glutamate receptor) (Batista, et al., 2008).

The conclusions that can be drawn from these studies in animals are limited. Lavender and its main components appear to influence a variety of neurotransmitter systems. Whether this is a direct or indirect interaction is not clear. Although, the fact that lavender appears to have effects on GABA-ergic and glutamatergic systems as well as the HPA axis are all promising evidence for a role in anxiolysis. However, these studies in animals also indicate that there is no real consensus as to how lavender's components interact with neurotransmitter receptors, particularly those involved in anxiety.

## 1.4.4 *In vitro* research

### 1.4.4.1 Potential direct pharmacological effects of lavender and its components.

*In vitro* studies allow a closer examination of any potential pharmacological mechanisms of action of EOs and their constituents, aside from any psychological properties. There are, however, not very many of these types of studies, so again it is necessary to look more widely at studies that involve systems which could be implicated in anxiety-type behaviour.

Studies indicate that lavender's components interact with a wide variety of neurotransmitter systems, for example linalool's nociceptive mechanisms are probably mediated through a reduction in the production or release of nitric oxide (NO) in glutamatergic or cholinergic systems (Peana, Marzocco, Popolo, & Pinto, 2006). Inhibition of the enzyme that produces NO, neuronal nitric oxide synthase (nNOS), has been associated with anxiolysis (Spiacci, Kanamaru, Guimares, &

Oliveira, 2008). Linalool might also mediate some of its effects via the adenosine A1 and A2 receptors (Peana et al., 2004).

In experiments on muscle tissue, lavender EO and linalool at final concentrations of 2 x $10^{-5}$ and 2 x $10^{-4}$ g/ml were found to decrease tone in skeletal muscle and have a spasmolytic effect on smooth muscle (guinea pig ileum and rat uterus). The mechanism of action was thought to be postsynaptic and not atropine-like. Additionally, lavender caused a rise in intracellular cAMP, similar to that seen during 'stress' activation mediated by the sympathetic nervous system (Lis-Balchin & Hart, 1997, 1999). The increase in cAMP was suggested to be via a direct action on the adenyl-cyclase subunit rather than via receptor binding; thus, linalool might enter the cell to exert its effects.

Experiments examining the effects of lavender and its components on mouse cortical membranes have indicated that linalool exerts some of its effects by a direct interaction with the glutamate NMDA receptor complex (Brum, Elisabetsky, & Souza, 2001; Silva Brum, Emanuelli, Souza, & Elisabetsky, 2001). Additional evidence was provided by aqueous extracts of lavender flowers, which, although not the EO do contain similar components. The extract provided protection against glutamate toxicity in cerebellar granula cell tissue-culture (Buyukokuroglu, Gepdiremen, Hacimuftuoglu, & Oktay, 2003). Interestingly, in the Elisabetsky study it was noted that linalool did not interact directly with the $GABA_A$ receptor, although changes in GABA-mediated neuronal inhibition or effects on GABA release and uptake were not ruled out (Elizabetsky et al., 1995). In contrast to these results, cloning experiments on the $GABA_A$ receptor in *Xenopus oocytes* have provided further evidence for lavender exerting a direct pharmacological effect. The effect is similar to the effects seen with alcohol, tobacco, or BDZs when taken into an organism by the bloodstream, lungs, skin or intestine (Aoshima & Hamamoto, 1999).

More specifically, Re, et al. (2000) demonstrated that linalool inhibited acetylcholine release and decreased channel open time at the neuromuscular junction in the mouse left hemi-diaphragm. It was suggested that linalool's effects might be related to a reduction in calcium-ion influx in the presynaptic terminal channels. This might be caused by inhibition of the voltage-gated sodium and potassium channels or by an

interaction of linalool with the cell membranes (Re et al., 2000); thus linalool might also have indirect effects by interacting with cellular membranes.

#### 1.4.4.2 Potential indirect effects of lavender and its components.

EOs have been shown to react with cell membranes and to have cytotoxic effects at low concentrations (100nl/ml) (Teuscher, Melzig, & Villmann, 1989; Teuscher et al., 1990). Teuscher and his colleagues concluded that EOs which reach cells in high concentrations mediate unspecific effects such as irritation and cell membrane destruction. However, EOs usually reach cells in very low concentrations, where, depending on their physiochemical properties and their molecular shape, they can become integrated into the cell membranes. Here they can influence enzymes, ion-channels and receptors within their locality (Teuscher et al., 1989, 1990). Indeed, there is evidence for this in model membranes where terpenes easily incorporated themselves into monolayers of dipalmytoyl-phosphatidylcholine (Turina, Nolan, Zygadlo, & Perillo, 2006). The fact that terpenes are either amphypathic or hydrophobic, and therefore have a tendency to partition towards membranes, lends them the possibility of changing membrane structure and surface electrostatics. These properties would explain their concomitant effects on membrane permeability and on the activity of intrinsic proteins such as $GABA_A$ receptors, which are post-synaptic membrane proteins. Terpenes from EOs other than lavender have been shown to modulate the $GABA_A$ receptor and its allosteric binding sites in this way. In studies looking at the effects of terpenes on chlordiazepoxide behaviour in relation to an artificial membrane, the terpenes caused the chlordiazepoxide to remain in the membrane phase (Del Turina & Perillo, 2003). Terpenes have also been shown to enhance transdermal permeation of skin, transforming stratum corneum lipids by disrupting the interlamellar hydrogen-bonding network at the polar head group region. As a result, they increase hydration levels of lipid membranes, probably by forming new aqueous channels, altering the internal structure of membranes and making the latter more permeable to drugs (Narishetty & Panchagnula, 2005). It is interesting that linalool has been cited as being one of the sorbent promoters for dermal delivery of the psychotropic drug haloperidol (Aqil, Ahad, Sultana, & Ali, 2007).

EO terpenes have also been shown to act as pro-oxidants; they can depolarise inner cell membranes in organelles such as mitochondria, where they affect calcium-ion

cycling and reduce the pH gradient, thus affecting the proton pump and the ATP pool. Permeability of outer and inner mitochondrial membranes leads to cell death. However, EOs also contain molecules which protect the cell from such effects. This fact possibly provides evidence for their reported synergistic effects, in that trace components might moderate the activity of the major components. It has also been suggested that several components of an EO define its properties, such as fragrance, texture, colour, membrane permeability and, cell penetration. These properties influence its distribution within the cell and this ultimately determines its properties. For this reason it has been suggested that the study of EOs should involve the whole oil rather than the component parts (Bakkali, Averbeck, Averbeck, & Idaomar, 2008).

It appears that EOs can exert their effects *in-vitro* by both direct effects on systems such as GABA, glutamate, and acetylcholine and via indirect effects because of their solubility in cell and organelle membranes. Interestingly, in studies looking at the dermal toxicity of lavender and its components, linalyl-acetate and linalool were found to have cytotoxic effects, whereas the whole oil did not (Prashar, Locke, & Evans, 2004). Thus some of the components of the oil must have a protecting effect, perhaps lending weight to the use of the whole oil rather than its separate components in any form of treatment using lavender EO.

### 1.4.5 Summary of *in-vivo* and *in-vitro* effects of lavender EO and its components.

A number of possible routes both specific and non-specific are suggested by which lavender EO could cause anxiolysis. *In vivo* and *in vitro* studies provide strong evidence for the pharmacological properties of lavender both via the bloodstream and possibly also via an olfactory route (Shen et al., 2005; Heuberger et al., 2004; Buchbauer, Jirovetz, Jager et al., 1993; Kovar et al., 1987). Lavender might also work by a dual mechanism causing autonomic deactivation which is reinforced by direct CNS actions and in turn enhance perceived wellbeing (Heuberger et al., 2004).

By way of comparison, and arguing in favour of a specific effect attributed to lavender EO and not just EOs in general, rose oil will be discussed next. As mentioned above rose oil (Umezu, 1999) also has a long anecdotal history of being

anxiolytic, which is verified by some laboratory tests (Umezu, 1999), but its mechanism of action could be different to that of lavender.

## 1.5 THE EFFECTS OF ROSE, *ROSA DAMASCENA*, ESSENTIAL OIL ON ANXIETY

*Rosa damascena* Miller, EO, sometimes known as Rose Otto, also has a very long history of use for wellbeing and uplifting mood (Gurib-Fakim, 2006; Wells, 2005). There is only a small body of scientific evidence for rose oil having anxiolytic effects, for example, in the laboratory; volatalised rose oil odour caused a 40% overall reduction in sympathetic nerve activity, as measured by heart-rate variation, and a 30% reduction in plasma adrenaline concentration in humans (Haze, Sakai, & Gozu, 2002). In contrast to lavender oil, rose oil increased CNV indicating that it has a stimulating rather than a relaxing effect (Torii et al., 1988).

Animal studies also show support for its anxiolytic effects; in two conditioned models of anxiety (the Geller–Seifter and Vogel's tests), acute intraperitoneal administration of rose EO to mice had anti-conflict effects (Umezu, 1999). Rose oil's effects were not obliterated by the BDZ antagonist flumazenil (Umezu, 1999). This suggests that the anti-conflict action of rose oil is not mediated by the BDZ binding site on the $GABA_A$-receptor complex. However, like lavender, rose oil also potentiated barbiturate induced sleeping-time, indicating a possible interaction with the $GABA_A$ receptor complex (Rakhshandah & Hosseini, 2006). In contrast to lavender, studies in mice looking at the effects of rose oil and its components on locomotor activity did not confirm rose oil's anecdotally reported sedative effects. There was a very slight effect on sedation in untreated mice but after hyperagitation with caffeine, rose oil, unlike lavender, was not able to calm the mice (Buchbauer, Jirovetz, Jager et al., 1993). Additionally, acute (7 minutes), volatalised rose oil inhalation also showed direct behavioural effects by increasing the amount of time that rats tolerate an anxiety-provoking environment; the elevated plus-maze (de Almeida, Motta, Brito Faturi, Catallani, & Leite, 2004). Other studies have shown that reserpine antagonises the effects of rose oil in the forced swim test, suggesting that rose oil might act via a presynaptic mechanism (Khalaj & Farzin, 2006). Thus, the underlying mechanism of action of rose oil is, also as yet, unclear. However, the results of these few studies

would indicate that rose oil's anxiolytic effects differ from those of lavender in both their mechanism and mode of action.

Studies in animals would indicate that regardless of whether it is administered orally or via its odour, lavender's effects are similar. Studies in humans indicate that there might be a dissociation between lavender's effects depending on whether it is administered with the odour intact or with the odour removed, for example Heuberger's study (2004). In order to elicit direct anxiolytic actions, lavender odour first has to reach the brain. It can do this via a number of routes, three of which are mediated via the nose the olfactory, the vomeronasal and the trigeminal pathways (Savic, 2001). Alternatively, odorants can sometimes, depending on their properties, travel directly to the brain via the neuronal axon (for example cocaine) or in the bloodstream via the lungs and pulmonary circulation route.

The nasal pathways will be discussed more fully in the next section.

# 1.6 POTENTIAL ROUTES FOR ODORANTS TO REACH THE BRAIN AND AFFECT BEHAVIOUR

The olfactory and vomeronasal pathways share common CNS structures with those involved in the aetiology of anxiety, namely the limbic system, which was originally called the rhinencephalon or smell brain and later named the limbic lobe by Broca (Van Toller, 1988; Savic 2001; Spinella, 2002). The third pathway, the trigeminal pathway, might have a regulatory role to play in olfaction, but it is probably not involved in mediating any potential anxiolytic effects of EOs and so will be be only briefly discussed (Hummel, Futschik, Frasnelli, & Huttenbrink, 2003; Savic, 2002).

## 1.6.1 Olfaction

Olfactory pathways and the pathways involved in anxiety share some of the same brain structures (Song & Leonard, 2005). Aromatherapy's reputed effectiveness is that the olfactory system is closely linked to the limbic system, which as discussed earlier (section 1.1.3.1), is central to the biology of anxiety. The olfactory pathways are also the most common route of administration of EOs (Ehrlichmann & Bastone, 1992; Percival, 1995; Price & Price, 1999; Wormwood, 1997), see Figure 1.6 (below).

Potentially, olfactory pathways have the ability to produce a quicker effect on behaviour than the bloodstream route; this would be useful in the treatment of some anxiety disorders such as panic.

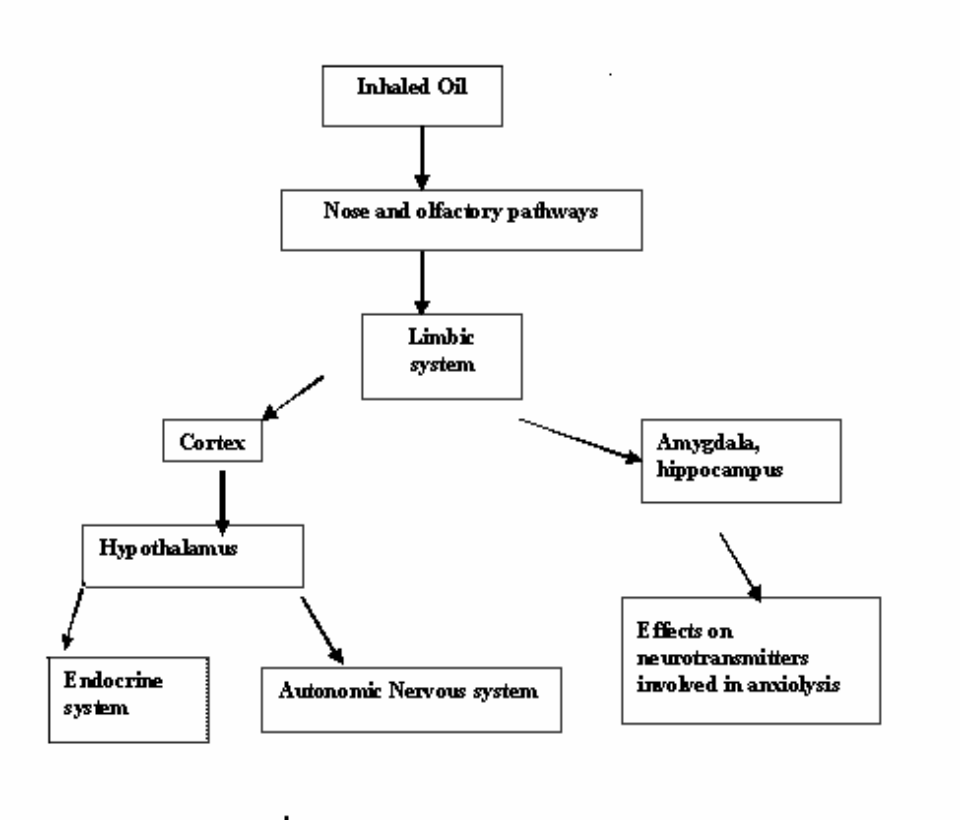



**Figure 1-6 The anecdotally suggested routes by which EOs might alleviate anxiety (adapted from Price & Price 1999).**

### 1.6.1.1 Neuroanatomy of olfaction and odour transduction

#### 1.6.1.1.1 Odorants

An odour is the sensory stimulation from a group of molecules that are airborne and travel to the olfactory epithelia, located in the roof of the nasal cavities in the nose, for detection by the nasal receptors. Not all molecules possess the properties to enable them to be odorants (Stoddart, 1976). In order to be an odorant a molecule or group of molecules has to be volatile, have a low molecular mass (below 350 Daltons) and have a high vapour pressure (Ohloff, 1986). They also have to have relatively low

polarity in order to travel through the lipid-rich nasal mucosa to the epithelium, where they can bind to nasal receptors (Ohloff, 1986). EO molecules are generally low molecular mass volatiles with these properties (Lamparsky & Müller, 1994; Kohlert et al., 2000). The olfactory sense is able to distinguish among a practically infinite number of chemical compounds at very low concentrations (Firestein, 1996; Mombaerts, 1999).

#### 1.6.1.1.2 Olfactory pathways

The nose is an external structure on the head of most mammals and the nasal cavity is usually bilaterally symmetrical, bisected by a septum. In mammals, this olfactory organ is incorporated into the respiratory system. Structurally, the nose is designed to inhale odours, although it is the olfactory nerve, cranial nerve 1, which is responsible for the sense of smell (Kandel, Schwartz, & Jessell, 2000). In order to facilitate odour-detection, sniffing takes place, drawing the volatilised odour molecules up the nose to the olfactory epithelium where the odour molecules can bind to the olfactory receptors of cranial nerve 1. This in turn facilitates detection and identification of the odour, although even without sniffing odorants can still be detected (Sobel et al., 1998).

The olfactory epithelium is situated at the top of the nasal cavity. In humans, this area is about $5cm^2$ whereas in rodents it is much larger (Dodd & Squirrell, 1980; Engen, 1982). The nasal epithelium contains the sensory cells also known as the olfactory receptor cells. It is bathed in mucus, which contains water, mucopolysaccharides, immunoglobins (IgA) and proteins (which include enzymes such as lysozyme, and other peptidases and xenophobic agents). Some processing of odour molecules takes place here by the enzyme molecules. The lipids in the mucus assist in the transport of the odorants to the olfactory receptor molecules; only molecules that are soluble in the mucus will reach the olfactory receptors (Dodd & Squirrell, 1980).

The sensory cells, or olfactory nerve cells (cranial nerve 1), are bipolar neurons that are unique in that they can regenerate (Graziadei, Levine, Monti Graziadei, 1978). Each one is likely to express only one type of receptor but one odour molecule could activate more than one kind of receptor. In humans there are approximately 10

million receptor molecules and in dogs there are approximately 200 million (Engen, 1982). In 1991, Axel and Buck discovered a family of roughly 1000 genes that encode the odour receptors (Axel, 1995). In humans and mice, this comprises 1-2% of the total number of genes, second only to the immune system. This family of olfactory receptor genes is part of an even larger group of proteins called G protein-coupled receptors. G proteins, so named because they derive energy from the hydrolysis of guanosine triphosphate (GTP), sit below certain types of receptors that cross the cell membrane (Gilman, 1987). When an odorant binds to a specific odorant receptor, it triggers the G (olf) protein to stimulate adenylate cyclase type III to synthesize cAMP. cAMP in turn opens a cyclic nucleotide gated cation channel, allowing an influx of $Ca^{2+}$ ions to open the $Ca^{2+}$ activated chloride channel. This leads to an influx of chloride ions and depolarization of the olfactory neuron (Bhandawat, Reisert, & Yau, 2005).

Each olfactory neuron cell has hundreds of olfactory receptors situated on non-motile cilia, which project into the mucus. The other end of the sensory cell, the axon, projects into the olfactory bulb at the front of the brain and terminates in structures called glomeruli, which are nerve junctions containing numerous synapses. A given receptor-type projects to one or at most a few glomeruli. The olfactory bulbs are elongated paired structures at the anterior (front) inferior (underneath) surface of the cerebral hemispheres. There are two olfactory bulbs lying on each side of the centre of the brain. The olfactory receptors in the nose project to the ipsilateral (same hemispheric side of the brain) mitral and tufted cells that form the glomeruli in the olfactory bulb. The axons of the mitral and tufted cells form the lateral olfactory tract; this projects to the olfactory cortex and via the anterior commissure to the other olfactory bulb. The mitral cell axons project ipsilaterally to the olfactory cortex, whereas the axons of the tufted cells are responsible for the connection between the two bulbs, allowing feedback control of the signal between the two olfactory bulbs (Engen, 1982; Savic & Gulyas, 2000; Savic, 2001).

The olfactory cortex consists of the anterior olfactory nucleus, the piriform cortex, the periamygdaloid and the transentorhinal cortex. The olfactory tract connects directly to the periamygdaloid cortex, while the olfactory tubercle connects to the mediodorsal thalamic nucleus. Additionally, the third neuron in the olfactory pathway projects from the olfactory cortex and the amygdala to the orbitofrontal cortex, the

subiculum, the thalamus, the hypothalamus, the brain stem, and, the caudate nucleus. All of these structures are involved in the processing of emotions and emotional information (Royet et al., 2001; Savic, 2001; Davis & Eichenbaum 1999; Zald & Pardo, 1997; Kalin, Shelton, & Davidson, 2007; Royet et al., 2001; Savic et al., 2000; Zald & Kim, 1996) (see section 1.1.3.1). Likewise, there are projections back to the olfactory bulbs from most of the main structures involved in the anxiety or defence response. Concurrent with this is the olfactory bulbectomy animal model of depression, in which treatment with antidepressants alleviates the behavioural symptoms that result from removal of the olfactory bulbs (Song & Leonard, 2005; McGrath & Norman, 1998).

Unlike rodents, humans are considered to be microsomatic, meaning that humans have relatively small olfactory bulbs. Rodents are classed as macrosomatic and thought to rely heavily on smell for their normal behaviour patterns. This idea has been challenged. Keverne, (1980) and Kohl, Atzmueller, Fink, and Grammer, (2001) argued that animals with more developed brains have the capacity to have a more sophisticated sense of smell (Keverne, 1980). In addition, in primates, olfactory receptors can respond to more than one odour type, and are thought to be detected via the patterns of signalling that the odours elicit, and the olfactory bulb is hypothesised to act more as a filter than a detection system.

Interestingly, a loss of olfaction can have a major impact on human wellbeing (Doty, 2001). This could be evidence for an influence of odorants, such as EOs, on health and wellbeing, Altered sense of smell has been reported after exposure to low levels of environmental chemicals; one example of this is cacosmia, which causes the sufferer to detect foul smells even when none is present (Bell, Schwartz, Amend, Peterson, & Stini, 1994). This illness is linked to multiple-chemical-sensitivity syndrome and can cause depression and anxiety in some individuals (Bell et al., 1994). Likewise, altered sense of smell has been reported in other psychological illnesses such as schizophrenia (Purdon & FlorHenry, 2000), hysteria (Weintraub, 1973), and alcoholic Korsakoff syndrome (Hulshoff Pol et al., 2002). Furthermore, in early Alzheimer's disease, olfaction is impaired and this might be an early marker of the disease (Kovacs, Cairns, & Lantos, 2001). Loss of olfaction has also been reported in HIV patients (Graham, Graham, Bartlett, Heald, & Schiffman, 1995) and in Parkinson's disease (Hawkes, Shephard, & Daniel, 1997).

It is also of interest that linalool interacts with adenyl-cyclases in *in-vitro* studies (Lis-Balchin & Hart, 1999 and see previous section), and cAMP is the most abundant second messenger in the olfactory neurons.

## 1.6.2 The Vomeronasal system

In many mammals the vomeronasal pathway mediates important behavioural and physiological effects in both sexes by detecting odours produced by pheromones (Wyatt, 2003). There is some evidence that components of EOs could act in a pheromone-like way in some species of insect (Wyatt, 2003).

### 1.6.2.1 Pheromones

Pheromones are airborne chemicals that are released by an individual into the environment affecting the physiology and behaviour of other members of the same species (Savic 2001). Pheromones are usually released as a warning of imminent danger, or as an advertisement of reproductive availability/status or position in the social structure of the group. In terms of communication, pheromones can be compared to hormones; they are slow-travelling compared to reflex reactions, can linger, and have an effect for a long time after the signal was sent (Bjering, Deinboll, & Maehlen, 2000; Savic, 2001; Tronson, 2001; Watson, 1999). Pheromones are thought to have evolved from hormones leaking from organisms to advertise to con-specifics, for example, events important to species' survival such as fertility and stage of oestrous (Wyatt, 2003).

It is interesting to note that in order to protect the survival of their species, plants evolved to produce odours that attract insects and animals, enabling their pollen to be passed on to other plants to enable cross-fertilisation (Wyatt, 2003; Reddy & Guerrero, 2004; Raguso, 2004). More evidence for pheromone-like effects of EOs is found in the orchid (Genus: *Ophrys*), in which EO components mimic the pheromones of the male *Aculeate hymenoptera:* wasps, bees and ants (Borg-Karlson & Groth, 1986). In particular, linalool, which is present in lavender EO, has pheromone like properties. These include enhancing the approach behaviour of

several male bee species, including *Colletes*, and *Andrena* (Borg-Karlson & Groth, 1986). EOs have been found to have pheromone-like activity in insects such as desert locusts (Assad et al., 1997) and, in common with other pheromone-like substances, EOs sometimes have hormone-like activity (Howes, Houghton, Barlow, Pocock, & Milligan, 2002; Wyatt, 2003). This includes lavender essential oil, which was found to increase breast size in prepubescent boys and interact with the oestrogen receptor in tissue-culture tests (Henley, Lipson, Korach, & Bloch, 2007).

#### 1.6.2.2 Vomeronasal pathways

In many, but not all mammals, pheromones are usually detected first by the vomeronasal organ (VNO), Jacobson's organ, located in the nasal passages (Watson, 1999).

In animals which possess an accessory olfactory bulb (AOB), such as the gerbil, it is positioned behind the main olfactory bulb and has a similar but more simplified structure (see Figure 1.7 below).

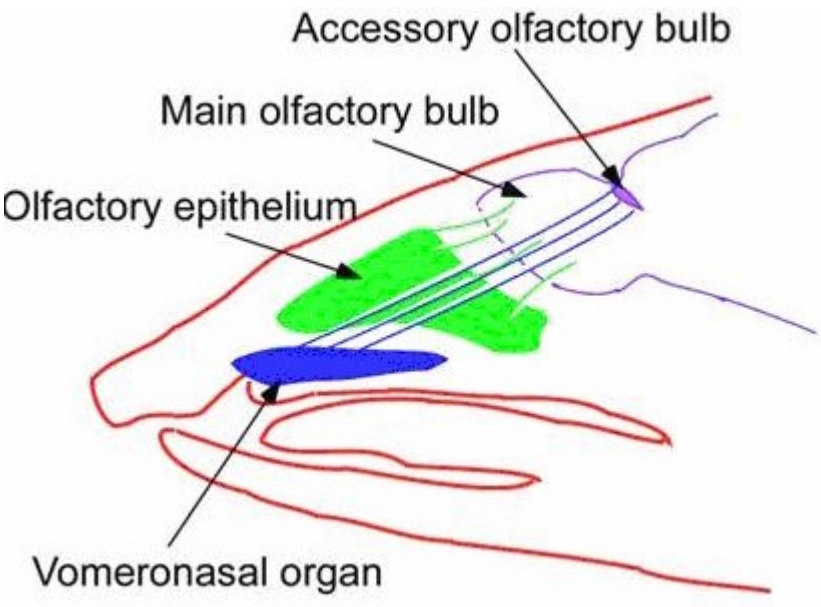



**Figure 1-7 A side view of the vomeronasal organ and olfactory and accessory olfactory bulbs in the possum (Halpern, Daniels, & Zuri, 2005).**

The vomeronasal organs are two bilateral tubular pits inside each nasal septum, rather like pouches. They are located in the upper part of the nose and lined by sensory cells, which are very sensitive to low concentrations of pheromone molecules. The axons of these sensory cells project to the accessory olfactory bulb (Tronson, 2001).

Neurons project from the AOB to the medial nucleus of the amygdala via the accessory olfactory tract and then to the hypothalamus, which then sends signals via the endocrine system. Neurons from the AOB are completely separate from the olfactory system. If the VNO is destroyed in guinea pigs or mice, the animals can still smell odours via the olfactory route but they cannot mate successfully. The interaction of the VNO with the endocrine system means that pheromones can change endocrine states (Keverne, 2002). However, although olfactory bulbectomy in the rat or the gerbil impairs common olfactory behaviours, such as investigation of con-specifics and food-seeking behaviours, it does not prevent normal mating and insemination of mature female gerbils by males. This was exemplified when five male gerbils received complete olfactory bulbectomies by removal of the main and accessory bulbs. The success of the operation was confirmed after the study by histological examination of the gerbil brains. All of these gerbils mated with normal females and produced normal healthy off-spring, even though none of them responded to either food odour (a sunflower seed) or the odour of strange male con-specifics. The authors explained this mating behaviour by the theory of multisensory dependence of mammals; the gerbils were housed together for a few weeks, which enabled the males to investigate the females using other sensory systems to initiate and support mating behaviour (Cheal & Domesick, 1979). In a further experiment, cuts to the vomeronasal nerve prevented male gerbils from copulating during brief, rather than prolonged, encounters with strange females (l'Hommedieu & Hull, 1978).

There is evidence for the presence of a very small vomeronasal organ in humans. However, there is no evidence to show that it is lined with active sensory neurons and therefore it has been assumed to be vestigial (Stockhorst & Pietrowsky, 2004). Recent studies looking at the expression of vomeronasal receptor molecules in humans found VIr receptors, which are pheromone receptors (Rodriguez, Greer, Mok, & Mombaerts, 2000). These are expressed in the human olfactory mucosa but not the human

vomeronasal pits, possibly indicating that pheromones in humans mediate their effects via the olfactory pathways (Rodriguez et al., 2000).

Although humans do not appear to have a separate VNO system, research has demonstrated that they do respond to pheromone-like compounds. Putative pheromones in humans have been shown to be involved in a variety of endocrine responses and social behaviours. One example is the McClintock effect in which the menstrual cycles of women living in close proximity synchronise. This effect can be replicated by exposing women to the underarm secretions taken from other women (Olsson, Lundstrom, Diamantopoulou, & Esteves, 2006).

Furthermore, androstenone odour, an androgen-like compound, increases hypothalamic activity in women; whereas, estratetraenol, an oestrogen like substance found in female urine, boosts hypothalamic activity in men's brains (Savic 2001). Interestingly, in laboratory studies androstendione can positively enhance mood in heterosexual women if a man, but not a woman, is running the study. The same is true, but in reverse, when heterosexual men are exposed to estratetraenol (Olsson et al., 2006).

There is some evidence that olfaction rather than a vomeronasal pathway might be involved in this sex-specific behaviour in humans. The congenital disorder, Kallman's syndrome, links an absence of the sense of smell to hypogonadism (Hardelin, 2001). In these patients, olfactory structures in the brain are lacking and this coincides with small testes in individuals. Studies have also revealed a link between gonadotropin-releasing hormone and the embryonic olfactory system (Hardelin, 2001; Swaab, 2004).

It has also been suggested that the VNO might mediate its effects via the trigeminal nerve, cranial nerve 5 (Watson, 2001), as discussed briefly below.

### 1.6.3 Trigeminal pathway

Odorants sometimes have an ability to cause cold or burning sensations when they are sniffed, for example, minty odours can cause a cooling sensation in the nose

(Dragich & Halpern, 2008). These effects are mediated by cranial nerve V, the trigeminal nerve (Doty, 2001). This nerve-set is responsible for the sensations of touch, movement, pain, and temperature being relayed from the head and facial areas to the trigeminal nucleus in the brain stem (Valls-Sole, 2005). The trigeminal nerve also has a role in modifying nasal potency, nasal secretion and respiratory processes. It also modulates elements of odour perception (Savic, 2001; Yousem et al., 1997). The trigeminal system is involved in the activation of many areas of the brain, particularly the ones involved in pain perception, as well as the trigeminal nucleus in the brain stem (Tronson, 2001). Parts of the limbic system are also activated by the trigeminal nerve (Iannilli, Gerber, Frasnelli, & Hummel, 2007).

Odorants and other lipophilic volatile molecules might also cross the blood brain barrier themselves, rather than mediating their effects via CNS pathways, in order to exert a direct effect on the brain and then behaviour (Buchbauer, Jirovetz, Jager et al., 1993; Illum, 2004).

### 1.6.4 Other routes of odorants to the brain

On inhalation, not all odours bind to the olfactory, vomeronasal or trigeminal receptors. Some molecules inevitably pass to the lungs and enter the bloodstream. Others might pass through the nasal endothelium, by-passing the blood-brain barrier, and thus enter the CNS (Illum, 2003; Hanson & Frey, 2007; Costantino, Illum, Brandt, Johnson, & Quay, 2007). Some drugs act in this way, for example cocaine (Stathis et al., 1995). Similarly, components of EOs have lipophilic properties, which could facilitate this effect (Buchbauer & Jirovetz, 1994; Buchbauer, Jirovetz, Jager et al., 1993). As already discussed in section 1.4.2.2, detection of an odorant is not required for it to be registered in the brain. Even the brains of anosmic patients are active in the same way as normosmics in the presence of an odorant; perhaps detection of the odour is not needed for an odour to exert behavioural effects (Nasel et al. 1994; Sobel et al., 1999).

Researchers have looked at the potential of using the olfactory route to deliver drugs to the brain, especially where delivery by other routes is not possible and a rapid onset of action of the drug is needed. The uptake of drugs into the brain via this route

has been found to be dependent on molecular mass and lipophilicty, and therefore EOs are suitable candidates (Illum, 2003). Because of the potential speed of action of nasally-delivered drugs, pharmaceutical companies are aiming to develop drugs that can be administered in this way to treat anxiety disorders, such as panic attacks (Illum, 2003). Migraine drugs such as sumatripan, zolmitriptan and ergotamine are already delivered via this route (Illum, 2003).

It must be pointed out that even though an olfactory route to the brain appears the most likely mechanism for EOs to exert their effects, injected and orally-administered EOs often have the same effects as EOs administered as an odour (Kovar, 1987; Umezu et al., 2006). Likewise, if lavender is exerting its effects via pheromonal systems, then it might be possible that any anxiolytic effects will be more pronounced in species with an active VNO system. Although humans have not yet been shown to have an active VNO, it has been demonstrated that odours have a profound effect on human physiology and behaviour, presumably via the olfactory route. Thus, perhaps the two systems have integrated in humans. Some evidence for this might be provided by VIr pheromone receptors, which are expressed in the olfactory mucosa rather than any VNO type pits in the nose.

## 1.7 RESEARCH QUESTIONS

Human studies show effects for lavender, but, with one exception, they are confounded by the possibility that effects are cognitive or associative rather than pharmacological in origin. Animal studies can eliminate the possibility of cognitive effects, but have only been studied with acute doses of lavender. Cognitive mechanisms of odour transduction often confound pharmacological effects in humans. It could be argued that if the effects of lavender odour are purely due to odour hedonics, then they would only be transitory in nature until habituation to that odour takes place. Studies in humans using the odour are confounded because of the psychological effects associated with odours. Using animals naïve to the odour of lavender to investigate lavender's effects overcomes some of the difficulties presented in the human studies mentioned earlier. This thesis attempts to answer two questions (1.7.1 and 1.7.2):

### 1.7.1 Does lavender relieve anxiety following longer-term administration in animal models of anxiety?

From the odour studies described in humans and in animals, only the acute effects of lavender have been examined. When examining the effects of odours, it has been suggested that any effects due to an odour will rapidly diminish as habituation to the odour takes places (Dalton, 2000). A meta-analysis conducted by Cooke and Ernst (2000) would provide evidence for this, as after examining the results of a small number of published studies that examined the effects of EOs on anxiety, they concluded that EOs have a small but transient anxiolytic effect.

Testing odour effects in animals allows for a more controlled approach, because laboratory-reared animals have no expectation effects and, if testing is conducted in animals naïve to the odour there will be no memory or expectation effects caused by the odour. Unlike humans, laboratory animals generally live in very controlled and unchanging environments, making it easier to expose the laboratory animal to odour over a prolonged period. Therefore, the first questions posed by this thesis are whether lavender odour has an anxiolytic effect in animal models of anxiety, and whether this effect continues, or even potentiates, with prolonged exposure or alternatively diminishes as habituation to the odour takes place.

It has been noted in Umezu's studies that acutely administered lavender had anti-conflict effects in two different conflict tests: Vogel's test and the Geller-Siefter test (Umezu, 2000; Umezu 2006). Of the other essential oils that they tested, only rose oil also had an anti-conflict effect (Umezu, 1999; Umezu et al., 2002). Therefore, it would also be interesting to compare prolonged lavender odour administration with the effects of prolonged rose odour administration and the commonly prescribed anxiolytic, diazepam. If, following longer-term exposure, there is an anxiolytic effect in response to either odour, then it would be of interest to ascertain whether the behavioural profiles are similar to each other and whether they are similar to that of diazepam; this might suggest similar mechanisms of action.

Finally, most work in animals has been conducted in the males of the species, even though anxiety in humans is more prevalently reported in females (Kinrys & Wigant, 2005). Therefore, it is of interest to examine the effects of lavender in both male and

female animals to see if these treatments, but in particular lavender EO odour, are more effective in either males or females.

Secondly, if the results in animals indicate that in controlled environment prolonged lavender odour administration does have anxiolytic effects, then it would be of worth to see if these effects translate into humans in a placebo-controlled and randomised trial.

### 1.7.2 Are lavender's effects anxiolytic in anxious humans when tested in a randomised placebo-controlled double-blind trial?

Following on from part one of the thesis, if lavender proves to be anxiolytic in the animal models then, prior to doing further *in-vivo* and *in-vitro* studies, it is important to establish whether its effects are also truly anxiolytic in humans by using a controlled and validated test of anxiety, such as a laboratory-based study. The second part of this thesis examines the question; if lavender works to relieve anxiety in animals then does it also work in humans?

As discussed, the problem when working with odours in an experimental setting is mainly one of blinding to the odour. It is very difficult to blind participants to an odour such as lavender in a placebo-controlled and double-blind trial. In fact, the only reported study that has been conducted in humans, which controls for the odour, examined only the effects of one component of lavender (acute dermally administered linalool) rather than the whole oil (Heuberger et al., 2004). Importantly, this study looked at only relaxed participants, participants were presumably relaxed as the linalool was massaged into their abdomen, and the study did not include an intervention to measure linalool's effectiveness to relieve anxiety in anxious or stressed participants. However, the results of the physiological measures taken, that relate to the somatic symptoms of anxiety, indicated that linalool might have specific pharmacologically anxiolytic properties besides any effects caused by its odour.

Since an administration route such as massage might confound any attempts to create anxiety in participants, an alternative approach is via oral-administration in sealed capsule. At first consideration, this route of administration might seem confounded

because oral administration introduces the complication of the digestive system, where essential oil components, such as linalool, might be degraded by the acid in the stomach and then rendered inactive by the enzyme systems in the liver following first-pass metabolism. However, although not in human studies, studies in mice that had been fed linalool, in order to determine linalool's metabolic fate, indicated that only 57% of the linalool is eliminated following treatment by the liver. It follows that the other 43% might be pharmacologically available to exert its effects (Letizia et al., 2003). In addition, in some branches of aromatherapy it is acceptable to administer the oils orally (Schnaubelt, 1995). Furthermore, animal studies provide evidence that injected and orally-administered EOs often have similar effects to EOs administered in odour form (Kovar, 1987; Umezu et al., 2006). There are a few published studies that have been conducted using sealed capsule administration of essential oils other than lavender; these show some success in elucidating effects that have been traditionally attributed to the odour of the oil rather than from eating it (Kennedy et al., 2002; Tildesley et al., 2003).

As discussed earlier, some studies that have attempted to show that lavender can relieve anxiety in a stressful situation have used tasks that might not be anxiety eliciting. Prior to examining longer-term effects, it is important to have a valid laboratory test of anxiety and to ascertain whether acute lavender has any anxiolytic effects. Therefore, it is planned in a second study to develop a laboratory test of anxiety and then to test the anxiolytic effects of whole lavender oil in humans by acute oral administration in a randomised double-blind placebo-controlled trial (RCT).

Finally, it was decided to include both male and female participants in the studies conducted here. This was because anxiety is more prevalent in females (Kinrys & Wigant, 2005). In addition, females are reported to make more use of aromatherapy than males (Greenfield, Innes, Allan & Wearn, 2002; Risberg et al., 2004). There have been studies conducted examining lavender's effects on anxiety in both males and females, and there have been no reported differences, (Leherner et al., 2006). However, lavender has been shown to have potentially estrogenic effects (Henley, Lipson, Korach, & Bloch, 2007), and, as has been mentioned previously, estrogens and other neurosteroids could be implicated in the pathophysiology of anxiety (Toufexis et al., 2006).

The thesis that follows is in two sections: part one examines the prolonged effects of lavender odour administration in two animal models of anxiety. Part two examines the effects of lavender in a dose response, placebo-controlled, and randomised study, in which lavender is administered to human particpants orally in sealed capsules.

# CHAPTER 2 ANIMAL MODELS OF ANXIETY

## 2.1 OVERVIEW OF ANIMAL MODELS

One of the first stages in validating a new drug for treatment of a condition is to test its effects in animal models; no drug marketed in the UK can be licensed without undergoing rigorous tests on mammals other than humans (Dixon & Fisch, 1998). In an academic setting, animal models play a necessary and important role in understanding the aetiology and treatment of disease. In the pharmaceutical industry they are mainly used in the validation and testing of drugs and in drug discovery.

One of the basic tenets of ethological (behavioural) theory is that all mammals share a common phylogenetic history, and all systems, including the CNS, are similar (Belzung & Philippot, 2007). In other words the human brain contains recognisable circuits that are also found in other mammals, including rodents and primates. This might be because these circuits are necessary for the survival and reproduction of the species, as suggested by Darwinian theories of evolution, (Wilner, 1991; Blanchard, Hynd, Minke, Minemoto, & Blanchard, 2001). Although speech plays a large part in human behaviour, human non-verbal behaviour can be compared to animal behaviour and changes in normal behaviour can be an indicator of maladaptive psychiatric/mental behaviour (Dixon & Fisch, 1998; Lawford et al., 2003; Franzini & Spears, 2003). For example, Table 2.1 illustrates the strong behavioural similarities in response to anxiety in both animals and humans; these lend weight to the use of animal models as pre-clinical models of drugs to treat human anxiety.

**Table 2.1 Comparison of anxiety symptoms (in all types of anxiety disorders) in animals and humans.**

| Behaviour | Humans | Animals |
|---|---|---|
| Fear (excessive)- (more specific to phobia and panic than GAD) | √ | √ |
| Worry (for no reason) | √ | |
| Guilt (for no reason) | √ | |
| Feelings of failure | √ | |
| Subjective anxiety | √ | |
| Defence | √ | √ |
| Avoidance | √ | √ |
| Escape (attempt to) | √ | √ |
| Non-verbal vocalisation | √ | √ |
| Hypervigilance | √ | √ |
| Inhibition of exploratory behaviour/suppression of ongoing behaviour | √ | √ |
| Freezing immobility | √ | √ |
| Flight | √ | √ |
| Risk-assessment behaviours | √ | √ |
| Increase in heart-rate, blood pressure, urination, defecation, muscle tension, respiration (i.e. autonomic hyperactivity-sweating increased skin conductance) | √ | √ |
| Increase startle response | √ | √ |
| Increase plasma Cortisol/ Corticosterone. | √ | √ |
| Insomnia | √ | √ |
| Distractibility | √ | √ |

(Green & Hodges, 1991; Hodges & Joseph, 1992; Nutt, 1990).

Animal models need to be validated before they can be reliably used to measure conditions such as anxiety (Rodgers, 2006). Validation usually involves demonstrating similarities between the animal system and the human system, using concepts that can be measured and analysed, (McKinney, Gardner, Barlow, & McGuire, 1994; Dixon & Fisch, 1998; Blanchard et al., 2001). For example, face validity, which as the name indicates, is a visual measure of validity and measures visual symptoms relevant to the condition. For example, in depression, visual symptoms include lethargy, weight loss, sleep alterations and lack of movement. However, face validity is the weakest form of validity. For example, monkeys have a facial expression which looks like a human smile; however, in monkeys this expression uses very different muscles, and although it looks the same as a human smile, it is an indicator of aggression and not happiness or joy (Blanchard, Blanchard, Griebel & Nutt, 2008).

Good predictive validity is when the model behaves in the way one would expect when treated with drugs that make the condition worse or better and the dosage (mg/kg) corresponds to the dose that would be used to treat the same condition in humans.

Construct validity demands that the model demonstrates a good theoretical rationale (Starkey, 2000), in that the same underlying mechanisms that cause the symptoms in humans cause the homologous symptoms in the model (Dixon & Fisch, 1998). From an evolutionary perspective, whatever the behaviours involved in anxiety within a species, the underlying neurological and endocrine pathways are conserved between species e.g. rodents and humans (Blanchard, et al., 2008). Thus, different behaviours can be seen as representing homologous traits between species. It is these types of homologous traits, or behaviours, that animal models of anxiety seek to measure. Models with good construct validity are usually based on a well-developed theory such as, for example Gray and McNaughton's septohippocampal theory of anxiety (2003) (see section 1.2.2).

Reproducibility in other laboratories and independently agreed criteria for the measurement/interpretation of behaviour are also vital in the validation of a model, according to the criteria of (Bunney & McKinney, 1969).

One problem with animal models of anxiety is that the aetiology of anxiety in humans is far from straightforward (see Chapter 1 section 1.2). It occurs at a variety of levels and symptoms are very wide ranging and overlap with other illnesses. In addition, the animals used in these models are 'normal' and anxiety is induced by controlled environmental challenges inherent in the model. For example, risk-assessment behaviours by rodents in response to cat odour is a normal healthy self-defence behaviour, but can be used as a measure of the effect of an anxiolytic. This type of behaviour is very different from the pathological anxiety exhibited in a human when there is no stressor present, even though the symptoms are similar in both cases. Furthermore, humans verbalise and animals cannot, although ultrasonic vocalisation in rats has been used as a measure of anxiety (Sanchez, 2003). Ethological signs in animals are open to the interpretation of the onlooker (Archer, 1973) and might not mean the same as in the human. Hence, validation, especially construct validity, is important. Behaviours in the model need to be homologous with the behaviour in the human condition; that is they occur for the same reasons. For example, maternal behaviour might not present in the same way in a rat compared with a human, but has the same underlying mechanism and neurotransmitter pathway. Face validity alone is not enough and predictive validity might represent a good screen test, but not necessarily a good model. Construct validity is the most important as it ensures that the model is asking the correct questions (Overall, 2000; Wilner, 1991).

## 2.2 BEHAVIOURAL ANIMAL MODELS OF ANXIETY

Behavioural animal models of anxiety can be divided into two types conditioned and unconditioned.

**Conditioned Models:** animals require training or treatment prior to the start of the experiment. The training usually involves pavlovian or operant conditioning measures, which involve aversive stimuli e.g. mild electric shock to condition the avoidance of normally 'ongoing' behaviour. In other words, the animal avoids being punished because it remembers that it hurt the last time and is frightened of it happening again, giving rise to anxiety in the animal. These models might also

involve food or water deprivation regimes, which add additional confounds when trying to interpret results. For example, in the Vogel water-lick conflict, electric shocks to rats reduce the number of licks compared to controls and anxiolytics increase the rates of responding. In the Geller-Seifter conflict test, electric shocks and an increased availability of food are signalled by a tone and response rates during this period are increased by known anxiolytics such as chlordiazepoxide and barbiturates (Weiss, Lightowler, Stanhope, Kennett, & Dourish, 2000). Given the specific conditioning effects of treatments it has been suggested these models might be more representative of phobia rather than general anxiety (Gray & McNaughton, 2003).

**Unconditioned models**: Models measuring unconditioned or spontaneous behaviour in which animals are exposed to a variety of novel situations are known as unconditioned models. This type of model generally measures the conflict between exploration of a new environment and the fear that being in a new environment might bring threats to survival, in other words, approach-avoidance conflicts (Hendrie, Weiss, & Eilam, 1996). These situations create a conflict between exploration and defensive reactions. Some examples are new environments, or the presence of cage mates that could represent a threat to survival; these promote the FFFS response, hypervigilance and fear. All of these can be compared to human symptoms of anxiety (see Table 2.1 above). These paradigms are thought mainly to model generalized anxiety disorder and aspects of panic, for example, escape from the open arms in the case of the elevated plus-maze (Cheeta, Kenny, & File, 2000; Cole & Rodgers, 1995). These, models are generally held to have good theoretical rationale for studying anxiety and thus good construct validity.

Some advantages of these models are that they are related to the animal's natural behaviour and so provide some ecological validity; they are cheap, easy to use and ethically more acceptable. As mentioned, exploratory models, in particular the elevated plus-maze but also the black white box, have been well validated and shown to have good predictive and construct validity (see sections 2.2.1 and 2.2.2, below). Some disadvantages are that the tests are not considered specific enough, as they measure exploration, which is controlled by several factors, as well as anxiety (Archer, 1973). However, ethological analysis provides a basis for good construct validity (Blanchard et al., 2008). An example of this is risk-assessment behaviour, which is the assessment of whether it is safe to move or whether a predator is still

lurking. This behaviour is forward looking in modality and utilises the same brain areas as human worry. Therefore, a detailed ethological analysis needs to be carried out with all of these models (Bourin, 1997).

Ethological analysis involves counting when and how often in a specific situation a participant displays certain behavioural elements. Cluster elements are identified statistically, providing a behavioural 'fingerprint' that can be used to compare different treatments allowing behaviours to be compared within different species. An example of this is defence of individual space, which is similar in rats and humans (Dixon & Fisch, 1998).

Different models seem to represent different types or different aspects of anxiety and most of the models of anxiety measure more than one factor, usually including general anxiety disorder. Some of the models, particularly the conditioned ones, are more closely related to specific disorders. Examples include phobic disorder in electric shock conditioned tests, post-traumatic stress in the uncontrolled shock model, obsessive-compulsive disorder in ultrasonic vocalisation, general anxiety disorder in the exploratory models, as well as panic in the elevated plus-maze exploratory model (Bourin, 1997; Cheeta et al., 2000; Cole & Rodgers, 1995). It is therefore important when using these models that a number of different models are used.

Unconditioned models have the best ecological validity, as they represent circumstances that the animal is likely to find itself in and so are the most realistic. Two of these types of models have been widely validated for use in rodents and have been shown to have good construct validity with human correlates of anxiety (Blanchard et al., 2008). These are, the Elevated plus-maze, (EPM) and the Black/White box (BWB).

### 2.2.1 Elevated plus-maze (EPM)

The EPM was first introduced by Handley and Mithaney for use with rats. It was based on work by Montgomery (1955) and was later adapted by Lister for use with mice (1990). More recently, it was adapted for use with the mongolian gerbil (Varty, Morgan, Cohen-Williams, Coffin, & Carey, 2002). The EPM is a cross shape, elevated 50cm above the ground. It consists of two opposite, walled arms with the other two opposing arms left open. Ledges, 1cm high, have been added to these arms to prevent animals falling off during exploration and to encourage exploration of the open arms (Fernandes & File, 1996). The underlying principle is that elevated open arms are more anxiety-provoking, and therefore rodents would prefer to spend more time in the closed arms (Rodgers & Dalvi, 1997). The time spent in the open or closed arms and the number of entries into the closed or open arms reflects the level of anxiety being experienced by the animals. If anxious, a rodent will spend more time in the closed arms and enter them more. If the animal has been treated with an anxiolytic, for example, non-sedative doses of diazepam or chlordiazepoxide, it will spend considerably more time on the open arms and enter the open arms more frequently (Handley & Mithani, 1984, Green & Hodges, 1991; Weiss et al., 2000). In contrast, if treated with an anxiogenic, such as yohimbine or caffeine, then the opposite will occur and the animal will spend more time in the closed arms, while entries to and time spent on the open arms will be less (Pelow, Chopin, File & Briley, 1985).

However, a weakness of the EPM is that, although it is particularly sensitive to the anxiolytic effects of BDZ related drugs, which are effective in treating anxiety in humans, the EPM does not consistently give anxiolytic results when testing more modern anxiolytic drugs such as the 5-HT drug, buspirone. In order to refine the test further and overcome this problem, workers have included ethological, behavioural measures, for example, measures of risk-assessment (stetch-attend postures) and exploration (head-dipping and rearing), as well as behaviours such as grooming, immobility, and locomotor activity in each area of the maze (Blanchard, Blanchard, Rodgers, & Weiss, 1990; Blanchard, Yudko, Rodgers, & Blanchard, 1993; Cole & Rodgers, 1995; Rodgers & Johnson, 1995; Wall & Messier, 2000; Carobrez & Bertoglio, 2005).

Generally, when rodents are exposed to a stressor such as a predator or predator odour, they first freeze and orient themselves towards the danger and then they carry out risk-assessment behaviours, such as stetch-attending: slowly approaching the dangerous area, in what is called a flat back approach (Blanchard et al., 1991). Risk-assessment behaviour is forward-oriented approach behaviour. It involves checking if the coast is clear and if it is safe to explore; it has been likened to worry-type anxiety in humans (Blanchard & Blanchard, 1989; Blanchard, Blanchard, Griebel, & Nutt, 2008) and is different from normal exploration. This type of behaviour is thought to reflect the conflict behaviour mentioned in Gray's septohippocampal theory of anxiety (2003) and might either facilitate ongoing behaviour or cause behavioural inhibition depending on the outcome of the risk-assessment. As mentioned in section 1.2.2 and 1.2.3) anxiety is a complex and not unitary condition and different types of anxiety respond to different drugs. Risk-assessment behaviours are reported to be more sensitive to anxiolytic drugs and to the type of anxiety found in general anxiety disorder in humans (Carobrez & Bertoglio, 2005; Blanchard, et al., 2008). In contrast, when a rodent remains in the closed arm the type of anxiety elicited can be likened to avoidance, which is more similar to phobia. Also, escape from an open to a closed arm has been compared to panic and Gray's FFFS (Blanchard et al., 2008; Gray & McNaughton, 2003). Extensive work involving lesion studies and injecting 5HT anxiolytics, and other drugs, into various parts of the brain have confirmed these findings (Blanchard et al., 2008).

Ethological behavioural analysis has proved to be more sensitive to non-BDZ anxiolytics in detecting serotonergic type drugs, for example buspirone, which are only effective in GAD at anxiolytic doses (Setem, Pinheiro, Motta, Morato, & Cruz, 1999; Carobrez & Bertoglio, 2006; Varty, Morgan, Cohen-Williams, Coffin, & Carey, 2002). It is also sensitive to the anxiolytic effects of hormones and neurotransmitters such as corticotropin releasing hormone (CRH) (Schmidt & Muller, 2006) and other novel anxiolytics such as neurokinin 1 receptor agonists (Varty et al., 2002), as well as to anxiogenics such as caffeine (El Yacoubi, Ledent, Parmentier, Costentin, & Vaugeois, 2000). This model has been extensively validated in both rats and mice (Albrechet-Souza, Borelli, & Brandao, 2008; Cruz, Frei, & Graeff, 1994; Pellow, Chopin, File, & Briley, 1985; Wall & Messier, 2001; Wall & Messier, 2000; Fernandes & File, 1996; Rodgers & Johnson, 1995). It is popular because it is

sensitive to the effects of both anxiolytic and anxiogenic agents and results are quickly obtained.

It is important to note that the effects of drugs on locomotor activity could potentially confound the results in terms of measuring anxiety. Factor analysis of behavioural data has shown that measures of closed-arm entry frequency reflect motor activity (Rodgers & Johnson, 1995; Fernandes & File, 1996; Cruz, Frei, & Graeff, 1994). Even so, locomotor behaviour can still confound results, making it difficult to distinguish between sedative effects of treatment and anxiogenic/anxiolytic effects, so immobility and locomotor activity are often also measured when using this test.

Factor analysis has been conducted on large amounts of data generated using the EPM in both rats (Cruz et al., 1994; Fernandes, Gonzalez, Wilson, & File, 1999; Ohl, Toschi, Wigger, Henniger, & Landgraf, 2001; Ramos, Berton, Mormede, & Chaouloff, 1997) and mice (Rodgers & Johnson, 1995; Wall & Messier, 2000) revealing that these behaviours factor on to two, three or up to six factors. However, while it is considered good to measure a wide variety of behaviours, there is some question as to what exactly such behaviours are measuring. In order to refine these factor analytic models further and reduce the large number of factors to more meaningful constructs, Wall & Messier (2000) used 'confirmatory' factor analysis to suggest a vastly reduced two-factor model. They suggested that one factor, unprotected exploration, consisted of head-dipping (head and shoulders exploring over the sides of the open arm and from the centre square), percentage open arm entries and open arm duration. The other factor represented 'protected exploration,' which consisted of percentage closed arm entries and time spent on the closed arms, as well as stetch-attending (stretching the body forwards to see if it is safe to progress while leaving the hind quarters stationary) and vertical stretches, rearing (standing on hind legs).

Studies looking at changes in an animal's behaviour over the time of its first exposure to the EPM have shown changes in preference for the open or closed arms, leading to suggestions that the EPM is a model of state rather than trait anxiety (Andreatini & Bacellar, 2000). It has been shown that, on second exposure, the animals spend more time in the closed arms, even when drugs that had an anxiolytic effect in the first trial are administered in the second trial. Experiments exposing rats injected with

chlordiazepoxide to repeated exposures on the EPM, with and without ledges on the open arms, have suggested that, in the first exposure, the open space of the open arms creates the aversive environment. In contrast, fear in the second exposure was thought to be caused by the elevation. Therefore, the two exposures are measuring different forms of fear (Fernandes & File, 1996). The second exposure fear has been likened more to that caused by phobias in humans (File, 2001). Only animals naïve to the maze will be used in studies described here.

Quite often, the EPM is not the only test used experimentally. It has been shown that it is important when using a battery of tests, to test using the EPM early on in the study. Evidence from studies with mice have shown that prior-maze exposure, to any kind of exploratory maze can detract from the novelty of the EPM and thus limit their activity in the EPM (Rodgers & Cole, 1993). Therefore, in the studies described here the EPM model will always be used first prior to the BWB.

The EPM like many exploratory models of anxiety is also sensitive to sex differences; male rats are more sensitive to factors affecting anxiety rather than motor activity. By contrast, female rats are more sensitive to factors affecting motor activity rather than anxiety (Fernandes et al., 1999; Archer, 1975). It has been suggested that oestrogens increase exploratory behaviour in females (Archer, 1975). Studies have shown that the EPM might have different effects on females depending on the stage of oestrous of the rodent being tested (Koss, Gehlert, & Shekhar, 2004; Weiser, Foradori, & Handa, 2008). Likewise, progesterones also have anxiolytic effects on EMP-induced anxiety (Gomez, Saldivar-Gonzalez, Delgado, & Rodriguez, 2002; Löfgren, Johansson, Meyerson, Lundgren, & Backstrom, 2006).

### 2.2.2 Black white box (BWB)

The BWB consists of a Perspex box with two compartments, one clear and brightly illuminated and the other dark. Separating these two compartments is a wall with a small aperture/doorway, no larger than 7cm wide, in the middle of it. Hence, it is also known as the light dark box (Bourin & Hascoet, 2003). Usually, the dark side is one third and the light side is two-thirds of the total volume. Nocturnal rodents prefer to stay in the safe dark side of the box, rather than the aversive bright side (Bourin & Hascoet, 2003). It was initially validated in mice (Crawley & Goodwin, 1980;

Costall, Jones, Kelly, Naylor, & Tomkins, 1989; Kilfoil, Michel, Montgomery, & Whiting, 1989; Sanchez, 1997); and later in rats, (Chaouloff, Durand, & Mormede, 1997; Chaouloff, Kulikov, Sarrieau, Castanon, & Mormede, 1995; Kulikov, Castanon, & Mormede, & Chaouloff, 1995; Chaouloff, Castanon, & Mormede, 1994; Smythe, Bhatnagar, Murphy, Timothy, & Costall, 1998; Smythe, Murphy, Bhatnagar, Timothy, & Costall, 1996; Sanchez, 1996). There has also been a short paper published of a validation in male gerbils (Lapiz & Hogg, 2001).

The stressors in this model are the novel environment and the light (Hascoet & Bourin, 2003). The time spent in each compartment is measured; more time spent in the light compartment, along with more crossings into the light compartment, is interpreted as anxiolytic-like behaviour. More time spent in the dark compartment is interpreted as anxiogenic-like behaviour. As with the other exploratory models, exploratory behaviours, such as rearing and sniffing, have also been taken into account as measures of anxiolysis (Sanchez, 1997). In addition to these measures, the rodent is usually placed in the light-side of the box and the length (latency) of time taken to enter the dark-side is measured. A longer latency is interpreted as anxiolysis or sedation, depending on the effects of the treatment (Bourin & Hascoet, 2003). In summary, anxiolysis in this test is interpreted as a longer latency to enter the black box, more exploration, a higher number of transitions between compartments, locomotion and time spent in the light box (Hascoet & Bourin, 2003). Anxiogenic behaviour is generally characterised by more time spent in the dark compartment, a shorter latency to enter the dark compartment, and less exploration and time spent in the light-side (Bourin & Hascoet, 2003). It has been argued that locomotor effects in this model factor on to measures of anxiety and are not linked to drug-induced locomotor behaviour (Hascoet & Bourin, 1998).

This model is also similar to the other exploratory models, in that the genetic strain of the animal and pre-test manipulations such as handling, housing conditions and acute stressors, has an effect on baseline results, and therefore can increase the robustness of the models as a test of anxiolysis (Sanchez, 1997).

Drug effects in the black white box: as with all of the other exploratory models, BDZs generally tend to have an anxiolytic effect, and high doses can induce sedation, which affects locomotor activity and thus the number of transitions between

compartments (Crawley & Goodwin, 1980; Hascoet & Bourin, 1998). The BWB has been shown to be sensitive to the anxiolytic effects of 5HT-1A agonist, buspirone (Shimada et al., 1995; Sanchez, 1996). However, mixed results have been obtained with other drugs acting at serotonin receptor sites. Likewise, the SSRIs, popularly used to treat anxiety disorders, give mixed results when tested acutely in the BWB (Bourin & Hascoet, 2003; Sanchez & Meier, 1997).

Neuropeptide receptor ligands, which have been implicated in anxiety, have also been tested for effects in the BWB with mixed results. Examples of neuropeptides tested in the BWB are cholecystokinin (Acosta, 1998), CRF (Griebel, Perrault, & Sanger, 1998), neurokinin (Zernig, Troger, & Saria, 1993) and dopamine antagonists (Timothy, Costall, & Smythe, 1999) (see also (Bourin & Hascoet, 2003)). Other agents tested include the stimulant and anxiogenic drug caffeine, which is an A2 adenosine receptor antagonist (El Yacoubi et al., 2000), and the synthetic amphetamine, MDMA, also known as ‘ecstasy’ (Maldonado & Navarro, 2000).

In all of the exploratory models, factors such as pre-test handling, cage position, exposure to predator odour, or other aversive circumstances prior to testing, can affect the outcome of results (Sanchez, 1996; Izidio, Lopes, Spricigo, Jr., & Ramos, 2005). Also, all of the exploratory models of anxiety are affected by the age and weight of the animals being tested (Hascoet, Colombel, & Bourin, 1999) and by environmental factors, such as the amount of illumination present in the light areas of the mazes (Blanchard et al., 2008).

### 2.2.3 Comparison of the two models

Although both models are exploratory models of anxiety with good construct validity, there have been some studies that indicate that they are modelling different aspects of the anxiety response (Belzung & Le Pape, 1994). For example, in lesion studies of the hippocampus and amygdala, only rodents in the BWB responded in an anxiolytic fashion to both lesions to the amygdala and to the hippocampus. By contrast, the successive alleys test (a modified version of the EPM) was only responsive to lesions to the hippocampus on first exposure (McHugh, Deacon, Rawlins, & Bannerman, 2004). The second exposure to the successive alleys test was more like the BWB, in that it was responsive to lesions to the amygdala and to the anxiolytic effects of BDZs

perfused directly into the amygdala (Do-Rego et al., 2006). Likewise, the BWB, but not the EPM on first exposure, is responsive to the anxiolytic effects of BDZ infusions directly into the entire amygdala (Gray & McNaughton, 2003). Therefore, perhaps the BWB is measuring anxiety-related to avoidance (phobic type anxiety) which, according to Gray and McNaughton's (2003) model of anxiety, is controlled by the amygdala. In contrast, first exposure to the EPM might be more reflective of behavioural inhibition, which is thought to be controlled by the septohippocampal loop and is responsible for scanning behaviour such as risk-assessment (see Gray & McNaughton, 2003).

This suggestion here is in contrast with the alternative suggestions, that the approach-avoidance paradigm created by the open arms of the EPM is more representative of panic disorder. The open arms are thought to create a fight-flight situation where the rodent can escape from the aversive stimuli of the open arms, to the closed arms (Cheeta, Kenny, & File, 2000; Cole & Rodgers, 1995). However, when risk-assessment behaviour is included as a measure, the EPM becomes responsive to behavioural inhibition type behaviour; this has been likened to worry and is seen in GAD (Blanchard & Blanchard, 1989b; Blanchard, Yudko et al., 1993; Cole & Rodgers, 1994; Rodgers & Dalvi, 1997; Griebel, Rodgers et al., 1997; Griebel, Blanchard et al., 1997; Rodgers, 1997; Carobrez & Bertoglio., 2005). This type of anxiety is thought to be controlled by the septohippocampal loop system (Gray & McNaughton, 2003). Although both of these models are measuring constructs related to anxiety, with the addition of ethological measures the EPM appears to be a model of GAD and anticipatory type anxiety; whereas, the BWB appears to be measuring avoidance.

Both of these models have, to some extent, been validated in the Mongolian gerbil, *Meriones uguiculatus* (see Varty et al., 2002; and Lapiz & Hogg, 2001), which is becoming an increasingly popular choice because of its ease of handling and breeding. They are generally quite placid creatures. In addition, some of their receptors such as the NK1 receptor molecules share more homology with the human NK1 receptor than do those of more commonly tested rodents, such as rats or mice. The next section will discuss the ethology of the gerbil, as this is the animal used in the animal models of anxiety studies described in part I of this thesis.

# CHAPTER 3 THE GERBIL (*MERIONES UNGUICULATUS*)

The Mongolian gerbil, *Meriones unguiculatus*, cited as far back as 1965 as a promising new laboratory animal because of its docile nature and ease of breeding (Marston & Chang, 1965), is being increasingly used in laboratory studies of anxiety. Gerbils share neuro-endocrinological similarities with humans. The gerbil neurokinin I (NK1) receptor is more similar to the human NKI receptor than that of rats or mice. NK1 receptors are widely distributed in brain regions that are implicated in the pathogenesis of anxiety; agonists at these sites mimic the autonomic and behavioural effects of anxiety (Holmes et al., 2003). Acute stress enhances NK1 receptor occupation by substance P in limbic regions (Ebner et al., 2004; Ebner & Singewald, 2006). Mice lacking the gene for substance P, or conversely lacking the NK1 receptor gene, are less anxious than wild-type mice and show anxiolysis when tested in models of anxiety (Brocco et al., 2008).

As existing drug treatments are not effective for all patients, the search for better anxiolytic drugs with fewer side-effects continues. Recently, drugs which target the neurokinin system, in particular NK1 receptors, have been the focus of intensive clinical and preclinical study (Ebner & Singewald, 2006; Gobbi & Blier, 2005; Holmes et al., 2003), particularly since there is some evidence that drugs that act on the NK1 receptor have an effect on human anxiety. For example, the NK1 antagonist, GR205, 171, relieves social phobia (Furmark et al., 2005).

However, current models of anxiety that employ rodents, such as rats and mice, have limited utility when testing these newer potential anxiolytics. This is because there are species differences in the affinity of non-peptide NK1 receptor antagonists for the NK1 receptor (Engberg et al., 2007). Gerbil NK1 receptors have a very similar affinity for non-peptide NK1 antagonists to that of human NK1 receptors (Duffy et al., 2002). This is because of identical primary structures in the seven trans-membrane domains of the NK1 G-protein trans-membrane receptor linked structure, and consequently gerbils share a more similar pharmacology when compared with human NK1 receptors than do rat or mouse NK1 receptors (Beresford et al., 1991; Duffy et al., 2002; Engberg et al., 2007). As a result, this species is now being used more

frequently in anxiety research. Work has shown that gerbils respond in a similar way to rats and mice when tested in animal models of anxiety in response to drugs commonly tested in these models (Varty et al., 2002; File, Cheeta, Akanezi, 2001). Added to this similarity, the main stress hormone in humans, cortisol, would also appear to be one of the main stress hormones in gerbils, rather than corticosterone which is the main stress hormone in rats and mice (Fenske, 1991; Morse & Davis, 1990; Oliver & Peron, 1964). Gerbils also form pair-bonds for life, a behaviour that is more akin to that of humans when compared to other rodents (Marston & Chang 1965; Starkey 2000; Ågren, Zhou, & Zhong, 1989; Agren, 1984). These facts serve to increase the construct validity of models that use gerbils and which seek to model human symptoms of anxiety.

The facts that they are not nocturnal, thought to be crepuscular (more active in the morning and evening (Refinetti, 2006; Brain, 1999)), breed easily and readily form colonies in captivity (Thiessen & Yahr, 1977), all add to their attractiveness as a laboratory animal. They have a typical lifespan of about four years, reaching sexual maturity at 10-12 weeks. They can have as many as 10-12 litters in their lifetime; the average litter size is 4.5, and weaning takes place at 21-25 days (Thiessen & Yahr 1977). Typical of a desert rodent, their food consumption is 5-8g/day and water consumption 4-7ml/day on average. The average body weight of an adult male and female are 100g and 85g respectively (Thiessen & Yahr 1977).

In their natural habitats Mongolian gerbils are burrowing, desert rodents, living in deserts and semi-deserts from South Africa to Mongolia and Manchuria. Illinger first described the Genus Meriones of the order Rodentia in 1811 and '*Meriones unguiculatus*' was first identified as a species by Milne-Edwards in 1867. A distinctive feature of the Mongolian gerbil is its propensity to exhibit seizures; these occur spontaneously in response to various stimuli, such as stress, or stroking the back of the neck. These seizures are because of synaptic ultra-structural changes in the hippocampus of the gerbil (Brain, 1999; Loskota, Lomax, & Rich, 1974; Cutler & Piper, 1990). Research that is more recent has revealed that seizure-sensitive gerbils have altered expression of the $GABA_A$ receptor in the hippocampus (Hwang et al., 2004). Additionally, decreases in BDZ receptor binding sites in the substantia nigra and periaqueductal gray, midbrain regions, have been shown in seizure sensitive gerbils relative to normal controls (Olsen, Walmsley, Lee & Lomax, 1986). To

minimise the number of gerbils excluded from the studies here, and to ensure GABA receptor distribution was as similar as possible to rats, the seizure resistant (SR) strain of gerbils were used in the current study.

Gerbils that are currently used in laboratories are from a very limited population of five females and four males, first introduced into the USA in 1954 by Victor Schwentker. Hence, there is very little genetic variation between these animals. Tests show that there is no behavioural or physiological indication that these animals are in any way abnormal (Thiessen & Yahr 1977).

A number of animal models of anxiety using gerbils have been validated in various laboratories. These validations show that these animals behave in a manner comparable to rats and mice, which have been widely validated for use in these models of anxiety, for example, studies looking at the ethological gerbil EPM suggest that it is sensitive to a range of anxiolytic agents and not just BDZ related drugs (Varty, Cohen-Williams, Morgan, Pylak 2002; Varty, Morgan, et al. 2002). Similar results have been obtained with the BWB (Lapiz & Hogg 2002) and the social interaction model (File, Cheeta, & Akanezi, 2001). In addition, like mice, they are sensitive to the locomotor effects of SSRIs in novel situations (Prinssen, Ballard, Kolb, & Nicolas, 2006).

Although gerbils rely heavily on scent to demarcate their territory and to advertise their reproductive status to their mates, studies have shown that olfactory bulbectomised male gerbils still mate normally (Cheal & Domesick 1979); this is possibly because saliva is the main attractant between male and female gerbils (Smith & Block, 1991).

The fact that gerbils are macrosomatic, rely heavily on odour for their normal behaviour and have relatively large olfactory bulbs compared to their size, and appear to have an active vomeronasal organ, could potentially confound any odour studies that are investigating potential anxiolytics for humans, because humans are classed as microsomatic and do not have an active vomeronasal organ. However, as discussed earlier, human behaviour has also been shown to be influenced by pheromone-like chemicals (Berliner, 2003; Berliner, 1996; Jacob, Garcia, Hayreh, & McClintock, 2002; Jacob & McClintock, 2000; Yasumatsu, Uchida, Sugano, & Suzuki, 1994) and

odours do influence human behaviour (see section 1.6). The rationale for these studies is that we still use EO odours to alleviate anxiety, even though, as humans we are classed as being microsomatic. Furthermore, the studies of Kovar et al. (1987) in mice provided evidence for orally delivered and inhaled effects of EOs being similar. Likewise, injected EOs also appear to have potent effects (Umezu et al., 2006), which would argue for a mechanism of action that does not necessarily include the olfactory and vomeronasal organs in the action of EOs on the brain. Extrapolating from this therefore, gerbil models of anxiety ought still to be valid tests of the anxiolytic properties of EOs in so far as they are valid screen tests of any other type of postulated anxiolytic.

In order to use the gerbil EPM and BWB in our laboratory the animals first need to be shown to be sensitive to the anxiolytic effects of drugs used to treat anxiety, such as the BDZs and drugs which act on the serotonin receptors system; for example, diazepam and buspirone. Consequently, the chapters that follow describe firstly the validation procedures of the models and the results of these validations. These are followed by the use of the validated models to test the effects of prolonged lavender odour, and to compare this with prolonged exposure to rose EO, another pleasant smelling odour, as well as chronically administered diazepam.

# CHAPTER 4 GERBIL STUDIES: MATERIALS AND METHODS

## 4.1 ANIMAL HUSBANDRY

Mongolian gerbils (*Meriones unguicalatus*), from a breeding stock at UCLan, which was originally purchased from Huntingdon Life Sciences (Huntingdon, Cambridgeshire), were weaned at three weeks of age and housed in unrelated same-sex groups of 3-4. Cages were of a standard size (33x25x19.5 cm). Animals were kept under a 12 hour light cycle (lights on 0700- 1900 h) and in constant temperature conditions (21+/-1$^{o}$ C). Food, CRMP (supplied by Special Dietary Services, Witham, Essex, CM8 3RD), and water were available *ad libitum*. Each cage was supplied weekly with a supplementary food, which included sunflower seeds and fruit, and a cardboard tube and wooden block for environmental enrichment purposes. The gerbils were at least 10 -12 weeks old at testing. All animals were maze-naïve and used only once on each maze. All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. Ethical approval was obtained from the University's Animal Procedures Committee.

## 4.2 GERBIL MODELS OF ANXIETY

### 4.2.1 Elevated plus-maze (EPM)

The EPM, (see Figures 4.1 and 4.2) relies on the natural anxiety-related behaviour of rodents to remain in shadow, close to walls (thigmotaxis) and to avoid heights. The maze provides free access to either a protected dark and enclosed environment or an unprotected brightly lit, open and elevated environment (Albrechet-Souza, et al., 2005). The rationale behind the EPM is that it creates a conflict between the animal's desire to explore the maze and its fear of bright open spaces and heights. Thus, a frightened gerbil will try to avoid the open, brightly lit, and high-up areas and remain in the relative safety of the dark, enclosed areas, sticking closely to the walls, thigmotaxis. A gerbil exhibiting less 'anxious-type' behaviour will explore more and will spend more time than it otherwise would in the open areas of the maze.

Structurally, the elevated plus-maze used here was based on the one developed by Montgomery (1955) and then adapted for rats (Handley & Mithani, 1984) and for mice by Lister, (1990) (Rodgers & Dalvi 1997). The EPM used in this study was made of Perspex, formed in a plus-shape, with four perpendicular arms set around a centre square and elevated 50cm above the ground on a podium. The arm dimensions were 40cm length and 8cm width with a centre square (7.5 cm x 7.5 cm). Two of the opposing arms had walls 28.5 cm high, blackened with 80% windscreen tint and the other two were open, apart from the addition of a 1cm deep ledge around the open arms (Fernandes et al., 1996). The floor of the maze was made of black melamine. Light levels were at 270 Lux. The maze was surrounded on the floor by a black and white checked cloth around the bottom of the maze; this was to discourage gerbils from jumping off the mazes (Figure 4.1).

**Figure 4-1 A Mongolian gerbil on open arm of the elevated plus-maze.**

Studies on the visual depth perception and acuity of gerbils found that a checked cloth lessened the likelihood of their jumping from the maze (personal communication Dr. N Starkey based on unpublished data). Two cameras, placed on either side of the maze, recorded gerbil behaviour on VHS tape for future analysis (Figure 4.2).

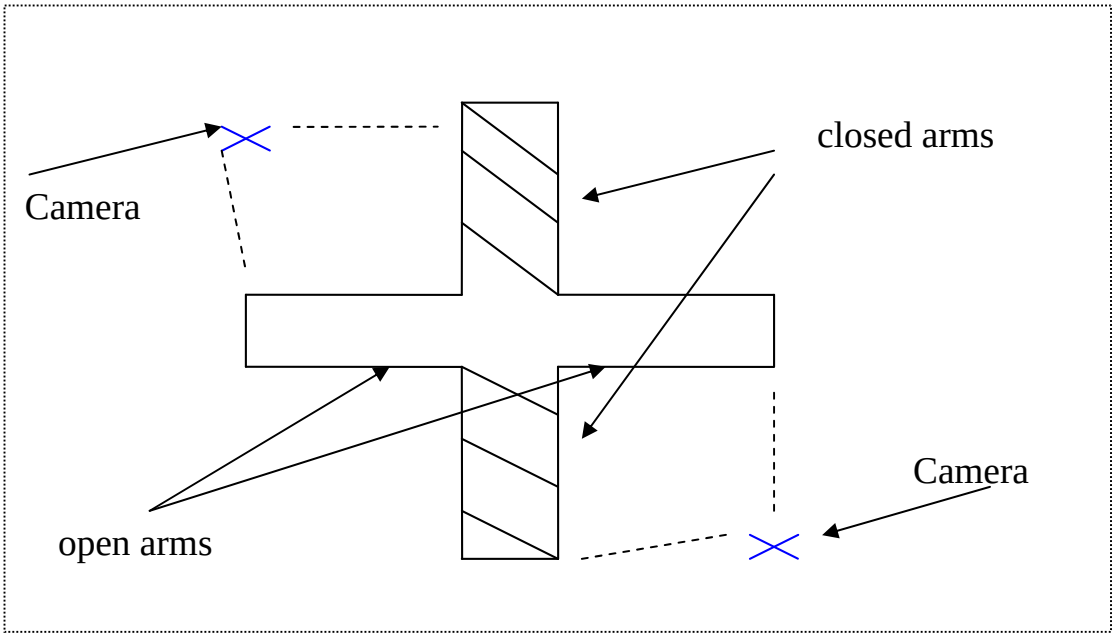



**Figure 4-2 Schematic of camera positions around the EPM.**

### 4.2.2 Black and White Box (BWB)

The rationale behind the BWB is similar to the EPM. When gerbils are anxious they enter quickly and remain in the darker, smaller section of the box where they can hide, and when they are less anxious they spend more time in exploration, particularly in the light side of the box.

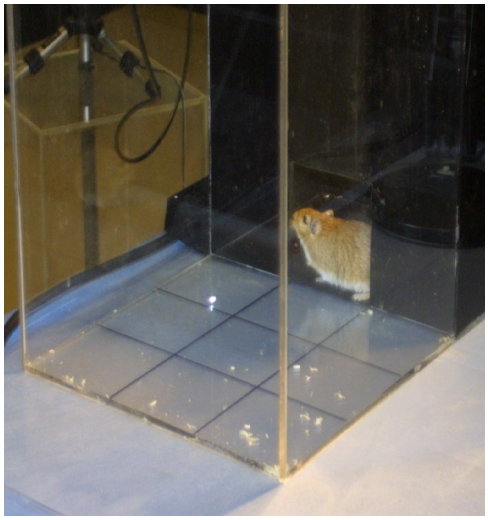


**Figure 4-3 A Mongolian gerbil in the dark compartment of the black-white box, looking into the white compartment.**

The black white box (BWB) used here was based on the one validated by Costall and colleagues (1989). It was a Perspex box of dimensions: 30cm width x 40cm height x 51cm length; divided into two separate compartments, 1/3 and 2/3 of the volume. The compartments were separated by a black wall with an aperture (small doorway) in its centre (10cm x 7cm) to allow the gerbils access to both compartments of the box (see Figure 4.3). The walls of the larger 2/3 compartment were transparent and open to the light in the room. An angle-poise lamp containing a 60 W bulb was angled over this side to make the light side aversive and to reduce shadows. The smaller compartment, one third of the total volume, was painted black to make it dark, and the dimmed overhead lights helped create shadow in this section. Two cameras recorded activity, one above the box to record gross movement and transitions between compartments and the other camera, which was perpendicular to the light side, recorded fine activity in the white side and at the crossing point (see Figure 4.4).

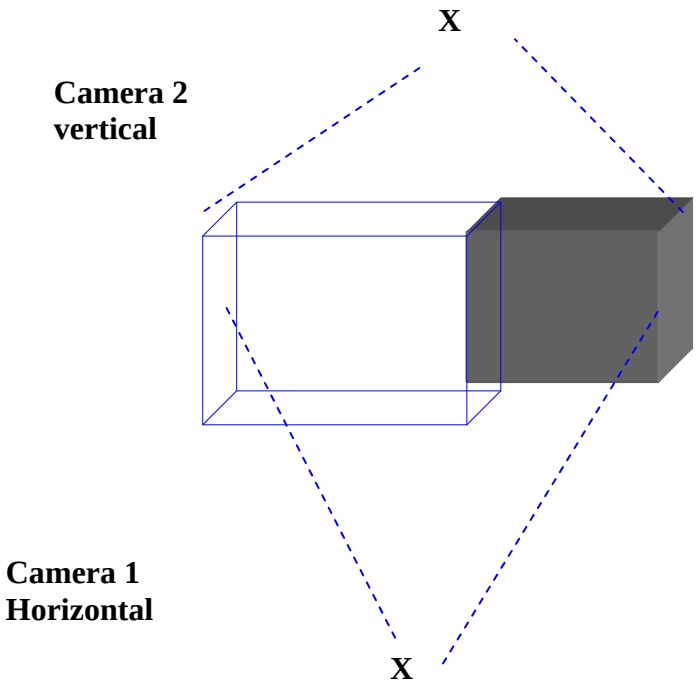



**Figure 4-4 Schematic of the gerbil black-white box showing camera positions.**

# 4.3 BEHAVIOURAL MEASURES

## 4.3.1 Behavioural measures in the EPM

The EPM maze is designed to discriminate anxiolytic and anxiogenic responses from purely locomotor effects. This is achieved by examining animals' preferences for unprotected (open) and protected (closed) environments, and observing their ethology in these areas (Cruz et al, 1994). These should be interpreted in the context of the full behavioural profile (Cruz et al., 1994). All tests were videoed for subsequent scoring using Hindsight 1.5 (ethological analysis software, Scott Weiss, Leeds).

**Behaviours and locations scored**

These measures were based on conventional measures described elsewhere (Frey, Löscher, Reiche, & Schulz, 1983; Varty, et al., 2002; Wall & Messier 2001) and are described in Table 4.1 below:

**Table 4.1 Description of behaviours measured in the EPM**

| Behaviour | Description |
|---|---|
| **Open entry** | All four paws in the open arm |
| **Closed entry** | All four paws in the closed arm |
| **Total entries** | The frequency of open plus the frequency of closed entries. |
| **Centre square** | Not in open or closed arm but on the maze |
| **Rearing** | Upright posture with gerbil's forelegs moving into the air or leaning against a wall |
| **Locomotor** | Any movement around the maze |
| **Immobile** | No visible movement |
| **Headdip** | Scanning over the sides of the maze towards the floor; this can be protected, occurring on a closed arm or centre square or unprotected, occurring from the open arm. |
| **Stetch-attend** | Head and shoulders stretched forward without moving the whole body forwards then retracting back to original position. |
| **Seizures/fits** | Twitching of vibrissae and ears; motor arrest with general myoclonic jerks, and sudden extreme spontaneous motor movement/loss of motor control generally followed by a period of immobility |

**Derived Measures**

In addition to frequency and duration of the measures in Table 4.1, percentage measures were also calculated. Percentages are a more robust measure as they provide a reference of one measure compared to another; whereas, absolutes only give an arbitrary measure which, when not compared to anything, have no point-of-reference component. For example, open entries could increase, which indicates a decrease in anxiety. However, at the same time closed entries could also increase indicating an increase in anxiety. In fact, these increases actually reflect an increase in locomotor activity, which is confounding the interpretation of these two measures. Percentage open entries take into account locomotor activity and hence, any increase in open entries when expressed as a percentage of total entries can be interpreted as a true increase and therefore a decrease in anxiety. Derived measures included the following: percentage open arm entries: ((open entries/total entries) x 100); percentage closed arm entries; percentage open arm duration, i.e. (open arm time /300) x 100); percentage closed arm duration. Percentage protected head-dips any that occurred on the centre or from the closed arms as a fraction of the total head-dips: (((closed arm head-dips) + (centre square head-dips)) / (total head-dips) x100), and percentage protected stretch-attends (((closed arm stretch-attend) + (centre stretch-attend)) / (total head-dips) x100).

Behaviours were coded as low anxiety, anxious behaviour, or drug effects, as seen in sedation or increased locomotor activity (Rodgers & Dalvi, 1997; Rodgers & Johnson, 1995; Salome et al., 2002) as follows:

**Low anxiety behaviours**: increased frequency and duration of open entries, increased percentage open entries, percentage open arm time, total head-dipping, decreased closed arm duration and decreased percentage duration.

**Anxious behaviours**: increased closed arm duration, increased percentage closed arm time and risk-assessment behaviours; percentage stetch-attends and percentage protected head-dipping ((head-dipping occurring in the closed arms and centre square) / total head-dips) x100) (Rodgers & Johnson 1995) and, seizures.

**Locomotor behaviours:** measures of drug effects on general locomotor activity include changes closed arm entries and total entries (Rodgers & Johnson, 1995). Although total entries is a mixed measure consisting of open and closed entries and, thus, also loads onto the anxiety factor in addition to locomotor behaviour and immobility (Rodgers & Johnson, 1995).

## 4.3.2 Behavioural measures in the BWB

### Behaviours and locations scored

Behaviours measured include:

Time spent in each compartment (all four paws in each compartment) and around the crossing point

Movement between the two compartments (crossing frequency)

Frequency and duration of:

Rearing

Sniffing

Locomotor

Freezing (immobility)

Derived measures were:

Latency black (the time taken after initial placement of a gerbil in the white side facing the entrance to the black side to move into the less aversive black side compartment)

Percentage entries to the white and black sides

Exploration a composite score of rearing and sniffing (rear/sniff) frequency and duration, representing environmental exploration of the maze (exploratory frequency and duration)

These were based upon conventional measures previously described (Smythe, Murphy, Timothy, & Costall, 1997; Chaouloff, 1994; Chaouloff et al., 1994; Costall et al., 1989; Bridges & Starkey, 2004).

An anxiolytic response would be represented by more exploration, for example: more rearing, sniffing, and locomotor activity particularly in the white side, larger percentage duration white, and, more movement between the two compartments (crossing frequency). More freezing behaviour in this model generally indicates anxiety but it could also be indicative of drug-induced sedation, particularly if it takes place in the white side. Higher anxiety is represented by less time spent and less activity in the white side, and, more time spent in the black side. Coding of behaviours followed that established by other workers for this test (Bridges & Starkey 2004; Chaouloff 1994; Costall et al. 1989).

In addition, seizures were recorded in both models. Seizures were defined as, twitching of vibrissae and ears, motor arrest with general myoclonic jerks, sudden extreme spontaneous motor movement and, loss of motor control, generally followed by a period of immobility (Frey et al., 1983).

## 4.4 PROCEDURES

### 4.4.1 General procedure for gerbil maze studies

For two weeks prior to the validations, the experimental gerbils were weighed, scruffed, and, handled, as they would be during injection in the treatment room. The purpose was to habituate them to the environment and handling by the experimenter during experimental procedures and, in order to lessen seizures in the validation procedure (Frey et al., 1983).

Since gerbils are not nocturnal (Refinetti, 2006) testing was conducted between the hours of 8:00 and 14:00, lights on. Testing procedure was as follows: at approximately 8:00 on the day of testing the gerbils were moved from their holding rooms to the pre-experimental room, in their home cages and racks, and left to acclimatize for one hour (see Figure 4.5 for a schematic layout of the experimental suite).

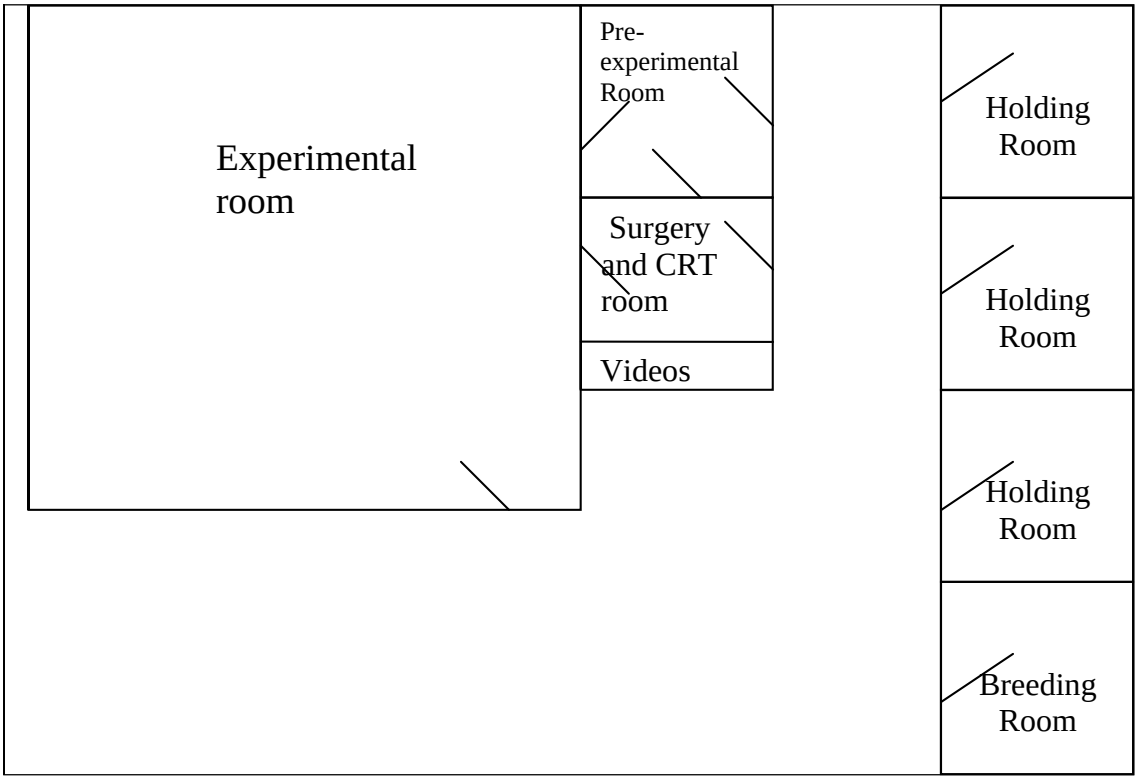



**Figure 4-5 Schematic layout of the gerbil experimental suite and holding rooms.**

On testing, each gerbil was placed firstly on the EPM in the centre square, facing an open arm and left to explore for five minutes. Gerbils that fell off the maze were returned only after the first time of falling off. Following any subsequent fall they were returned to their cage until it was time to go in the BWB. Any gerbils off the maze for longer than 100 seconds were excluded from the analysis. Immediately after this, gerbils were tested in the BWB. The gerbil was placed in the centre of the light-side of the BWB, facing the dark-side, and left to explore for a further five minutes. Between the testing of each gerbil, faecal boli were removed and the mazes washed and dried thoroughly by using a detergent routinely used to clean the cages in the animal house, any possible pheromone/drug-urine odour effects were overcome (Dixon, Huber, & Kaesermann, 1984; Halpin, 1976).

Behaviours were recorded on video tape. Two trained observers that were blind to experimental conditions later scored the videos using ‘Hindsight‘ (Scott Weiss, Leeds University), a computer assisted scoring program (inter-rater reliability and intra-rater reliability was > 0.9). Hindsight converted behaviours and locations in each of the mazes to frequency and duration of behaviour and location on each maze.

## 4.5 DATA ANALYSIS

Data from gerbils that had seizures were excluded from the main analysis (Bridges & Starkey, 2004). Seizure data were analysed separately for association between seizure occurrence and experimental condition.

Occasionally, gerbils jumped from the EPM and animals were returned to the EPM as quickly as possible. When this occurred, data were included in the subsequent analysis providing the gerbil had spent at least two thirds of the test session on the maze (i.e. 200 seconds). The data from these animals were corrected to standardise the data to five minutes (300s) using the following formula:

(Dependent variable / (300- time off the maze)) x 300.

Although fit data were not included in the main analysis they were analysed separately, using the Chi squared test for association, firstly for all gerbils and then separately for each sex.

# CHAPTER 5 AN EXAMINATION OF THE EFFECTS OF TWO ANXIOLYTIC AND TWO ANXIOGENIC DRUGS IN THE GERBIL ELEVATED PLUS-MAZE AND BLACK-WHITE BOX AS A VALIDATION OF THEIR USEFULNESS.

## 5.1 INTRODUCTION

In order to determine if the EPM and BWB are useful tests of anxiety in gerbils, it was necessary to compare the effects of each of two anxiolytics, diazepam and buspirone, and two anxiogenics, caffeine and FG7142, on those behaviours that are typically used to reflect anxiety in other rodents tested in these models. The structures of these drugs are as shown in Figures 5.1 to 5.4.

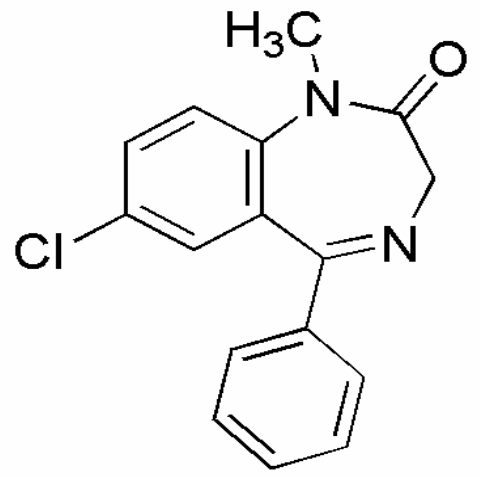



**Figure 5-1 The structure of diazepam**

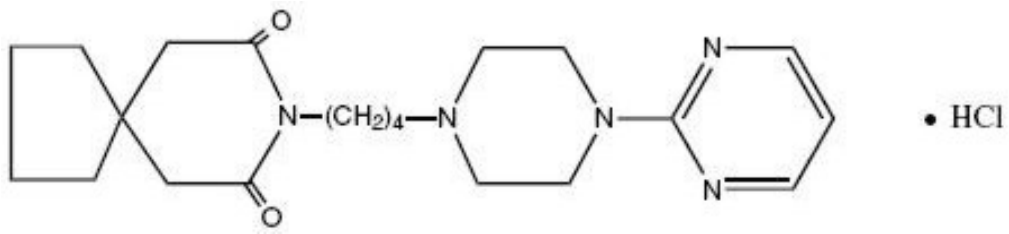



**Figure 5-2 The structure of buspirone**

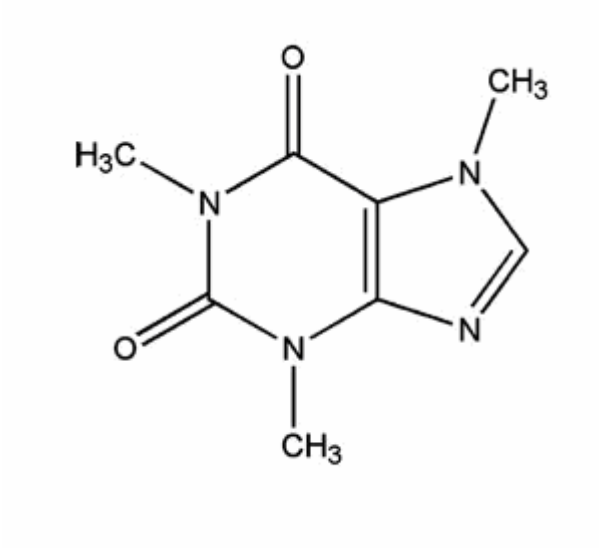



**Figure 5-3 The structure of caffeine**

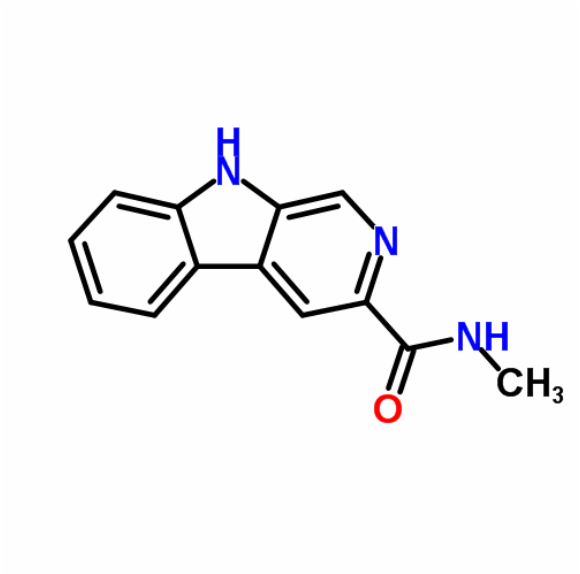



**Figure 5-4 the structure of FG7142 (N-methyl-ß-carboline-3-carboxamide)**

# 5.2 GERBIL MAZE VALIDATION METHODS

## 5.2.1 Experimental design

The design of the experiment was a between-groups one, with a minimum of 10 gerbils per group, using stratified random sampling to achieve the required number of gerbils per group, for each sex and drug group tested. In order to minimise pheromone effects

and for logistical reasons, randomisation of testing took place between cages; thus all cage mates were tested with the same drug (cage mates were non-related gerbils separated at weaning). Hence, the design of the study took the form of a between-subjects randomised block design.

### 5.2.2 Drugs used in the validations

Diazepam (0.05-1mg/kg), caffeine (0.05-3mg/ml), buspirone (1-30mg/kg) and FG7142 (1-30mg/kg) were purchased from Sigma- Aldrich, Poole.

The drugs were dissolved as follows: diazepam, caffeine and buspirone in distilled water with 50 μl of Tween-20 and sonicated for twenty minutes; FG7142, in distilled water with a drop of glycerol and sonicated for 20 minutes. Drug doses were chosen from doses used in similar studies using rats, mice or gerbils (Baldwin, Johnston, & File, 1989; Stanford, Baldwin, & File, 1989; Ballard, Sanger, & Higgins, 2001; Barry, Costall, Kelly, & Naylor, 1987; Chaouloff et al., 1997; Costall et al., 1989; Varty, Morgan, et al., 2002). Drug doses were as follows: diazepam: vehicle, 0.05mg/kg, 0.1mg/kg, 0.5mg/kg, and 1mg/kg; caffeine: vehicle, 0.5mg/kg, 5mg/kg, 15mg/kg, 30mg/kg; buspirone**:** vehicle, 1mg/kg, 3mg/kg, 10mg/kg, 30mg/kg; Fg7142: vehicle, 1mg/kg, 3mg/kg, 10mg/kg, 30mg/kg.

Each drug was dissolved in one of two vehicles. Hence, there were two vehicle controls: distilled water with 50 μl of Tween-20 as the control for diazepam, caffeine and buspirone, and distilled water with a 50 μl of glycerol as the control for FG7142.

Drugs were administered by intra-peritoneal (i.p.) injection, using a size 27 gauge needle at a dose volume of 1ml/100g thirty minutes prior to placing the gerbil on the EPM.

The numbers of gerbils in each drug group was as follows. Controls: female, Tween-20, n = 23, glycerol, n = 19. Male: Tween-20, n = 19, glycerol, n = 21. Each drug dose group consisted of n = 12 males or n = 12 females, thus in total there were 60 males and 60 females tested for each individual drug.

## 5.2.3 Protocol and order of testing

On the day of testing, gerbils were moved to the pre-experimental room in their home cages and left for at least an hour. Thirty minutes prior to testing on the mazes each gerbil was removed from its home cage, weighed, injected (intra-peritoneal) with vehicle or drug and then singly housed in the pre-experimental room until testing on the mazes.

Each drug type was tested separately and males and females were tested on separate days to minimise drug urine and pheromone effects (Dixon et al., 1984; Halpin 1976). Females were tested first for each drug. Testing began with the lowest concentration of drug and followed in consecutive order of increasing dose ending with the highest concentration of drug.

Work in other laboratories (Cruz-Morales, Santos, & Brandao, 2002; Frussa-Filho & Ribeiro, 2002) has revealed that using rodents twice (re-testing) on the EPM confounds results. Therefore, in this validation, each animal was used only once: first, on the EPM for five minutes test duration followed by the BWB for five minutes test duration.

## 5.2.4 Data analysis

There were only nine dependent variables, which are too few cases in each cell to warrant using a MANOVA. In addition, the data were skewed and kurtosed. Therefore, the analyses that were conducted were non-parametric. The Jonckheere-Terpstra trend test, which has more power when comparing ordered alternatives, as in the case of increasing drug doses, than the more frequently used Kruskall-Wallace non-parametric ANOVA , was used (Siegel & Castellan, 1998).

### 5.2.4.1 Missing data

#### EPM

Gerbils which had seizures were as follows: vehicle (combined) males, 5; females, 7.
For data on seizures in gerbils that had been treated with anxiolytic or anxiogenic, drugs see Table 5.1 below.

**Table 5.1 Number of seizures in gerbils on the EPM in response to diazepam, buspirone, caffeine and FG7142**

| Dose | Diazepam | | Buspirone | | Caffeine | | FG7142 | |
|---|---|---|---|---|---|---|---|---|
| | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** |
| **1** | 2 | 4 | 1 | 8 | 11 | 12 | 7 | 5 |
| **2** | 1 | 1 | 3 | 5 | 6 | 8 | 1 | 3 |
| **3** | 1 | 0 | 7 | 6 | 14 | 9 | 3 | 6 |
| **4** | 3 | 0 | 3 | 7 | 7 | 17 | 3 | 4 |

m = males; f= females.

Diazepam: Dose 1: 0.05mg/kg; dose 2 0.1mg/kg; dose 3 0.5mg/kg, dose 4:1mg/kg. Buspirone: Dose 1 1mg/kg; dose 2 3mg/kg; dose 3 10mg/kg, dose 4 30mg/kg.

Caffeine: Dose 1 0.5mg/kg, dose 2 5mg/kg, dose 3 15mg/kg, dose 4 30mg/kg.

FG7142: Dose 10.5mg/kg, dose 2 5mg/kg, dose 3 15mg/kg, dose 4 30mg/kg.

Chi square analysis of fit data, using a 2 (fit /no fit) x 5 (drug dose) design, indicated that seizures were not significantly associated with differing doses of diazepam in all gerbils ($\chi 2$ (4) = 7.09, p n/s), or male gerbils when tested ($\chi 2$ (4) = 4.14, p = n/s). However, in females, there was a significant association between increasing dose and a lower seizure frequency ($\chi 2$ (4) = 9.66, p >0.05). There was no relationship between buspirone dose and fit occurrence in all gerbils ($\chi 2$ (12) = 17.95, p n/s), or males or females when tested separately (males $\chi^2$ (8) = 12.77; females $\chi 2$ (12) = 13.47).

Increasing caffeine dose was associated with a higher frequency of fits in all gerbils ($\chi 2$ (12) = 57.48, p < 0.001), causing increased seizures in both males and females ($\chi^2$ (8) = 34.48, p < 0.001; $\chi^2$ (12) = 34.69, p < 0.001). Likewise, there was a significant relationship between FG7142 dose and fit occurrence in all gerbils ($\chi 2$ (4) = 8.82, p < 0.05 (one tailed)) and in males ($\chi 2$ (4) = 8.37, p < 0.05 (one tailed)). In females there was no significant relationship ($\chi 2$ (4) = 3.71, p n/s) between fits and caffeine dose.

**BWB**

The number of gerbils excluded based on seizures was as follows: vehicle control: males, 1; females, 3. For data on seizures in gerbils treated with anxiolytic and anxiogenic drugs see Table 5.2 below

**Table 5.2 Number of seizures in gerbils on the BWB in response to diazepam, buspirone, caffeine and FG7142**

| Drug dose | Diazepam | | Buspirone | | Caffeine | | FG7142 | |
|---|---|---|---|---|---|---|---|---|
| | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** |
| **1** | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| **2** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **3** | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| **4** | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 1 |

m = males; f = females

Diazepam: Dose 1: 0.05mg/kg; dose 2 0.1mg/kg; dose 3 0.5mg/kg, dose 4:1mg/kg. Buspirone: Dose 1 1mg/kg; dose 2 3mg/kg; dose 3 10mg/kg, dose 4 30mg/kg,.

Caffeine: Dose 1 0.5mg/kg, dose 2 5mg/kg, dose 3 15mg/kg, dose 4 30mg/kg.

FG7142: Dose 10.5mg/kg, dose 2 5mg/kg, dose 3 15mg/kg, dose 4 30mg/kg.

Data from these were treated separately, using the Chi squared test for associations of fit frequency of seizures versus drug dose. There were no significant associations between drug dose and fit occurrence for any of the drugs tested.

# 5.3 RESULTS

## 5.3.1 EPM validation results

### 5.3.1.1The effects of diazepam on male and female gerbil behaviour in the EPM

Median, inter-quartile ranges and J-T trend test result for male and female gerbils in response to each dose are presented in Table 5.3.

To test for differences between the sexes, male and female data were compared pair-wise using the Mann-Whitney U test for each dose prior to analysis in the Jonckheere-Terpstra trend test for ordered alternatives. Results indicate that locomotor duration ($U = 6$, $p < 0.05$) at 0.05 mg/kg and immobile duration ($U = 9$, $p < 0.05$) at 0.1mg/kg were higher in females than males, while rear frequency ($U = 12.5$, $p < 0.05$) at 0.1mg/kg was higher in males. These variables were analysed separately for each sex. (See Table 5.1 for median and inter-quartile ranges and J-T trend test results)

Data from both sexes of gerbils were combined in the analysis of the remaining variables. Results of this analysis were as follows: at 0.05mg/kg there was an increase in exploration; head-dip frequency was higher than in vehicle control animals ($U = 96.5$, $p < 0.05$). At 0.5mg/kg total entries ($U = 15.5$, $p < 0.05$), percentage open entries ($U = 127$, $p < 0.01$), percentage open duration ($U = 146$, $p < 0.05$) and head-dip frequency ($U = 80$, $p < 0.001$) were higher; while percentage closed entries ($U = 147$, $p < 0.05$) and percentage closed duration ($U = 145$, $p < 0.05$) were lower than controls. At 1mg/kg the anxiolytic profile was further strengthened, total entries ($U = 118$, $p < 0.05$), percentage open entries ($U = 89.5$, $p < 0.01$), percentage open duration ($U = 94$, $p < 0.01$), and head-dip frequency ($U = 53.5$, $p < 0.001$) were higher than controls; while percentage closed entries ($U = 103.5$, $p < 0.01$), percentage closed duration ($U = 81$, $p < 0.01$) and stretch-attend frequency ($U = 131.5$, $p < 0.05$) were less than vehicle controls at this dose.

In males alone, immobile duration was significantly lower at 0.1mg/kg ($U = 15$, $p < 0.01$) and 0.5mg/kg ($U = 17.5$, $p < 0.05$), while in females, rear frequency was significantly lower at the top dose (1mg/kg) ($U = 32$, $p < 0.05$) (See Table 5.1 for median inter-quartile ranges and J-T results).

**Table 5.3 Effects of acute diazepam (i.p.) on male and female gerbil behaviour in the elevated plus-maze. (Median and inter-quartile ranges)**

| Dependent variable | Diazepam dose (mg/kg) | Median and inter-quartile ranges | | | J-T statistic |
|---|---|---|---|---|---|
| | | All gerbils | Males | Females | |
| **Total entries** | **vehicle** | 17.09, **28.50**, 35.00 | 19.54, **25.00**, 30.50 | 17.50, **31.00,** 38.07 | |
| | **0.05** | 25.00, **2.97** 34.69 | 26.55, **32.44,** 34.32 | 25.00, **33.88,** 42.00 | |
| | **0.1** | 27.00, **3.44**, 41.07 | 31.00, **34.22,** 41.07 | 16.63, **39.00,** 41.46 | [i]3.63*** |
| | **0.5** | 30.00, **36.50***,, 44.44 | 30.00,**35.00,** 38.00 | 30.70, **40.04,** 49.62 | |
| | **1** | 32.75**, 41.70***, 48.00 | 38.12, **40.83,** 50.50 | 28.10, **41.83,** 47.00 | |
| **Percentage open entries** | **vehicle** | 36.36, **44.44**, 56.52 | 41.47,**44.44,** 55.86 | 34.01, **44.44,** 55.53 | |
| | **0.05** | 48.15, **54.14**, 62.50 | 47.75, **50.81,** 62.02 | 48.15, **56.90,** 68.18 | |
| | **0.1** | 41.18, **50, 00**, 62.50 | 41.18, **49.42,** 55.56 | 37.79, **54.55,** 64.58 | [i] 3.91*** |
| | **0.5** | 51.14, **57.14****, 66.23 | 52.27, **61.91,** 66.67 | 51.32, **56.25,** 62.90 | |
| | **1** | 57.50**, 60.38****, 70.27 | 54.49**, 60.38,** 69.17 | 57.50, **62.43,** 70.27 | |
| **Percentage closed entries** | **Vehicle** | 40.00, **52.78**, 60.00 | 44.14, **55.56,** 58.54 | 41.74, **48.39,** 62.65 | |
| | **0.05** | 37.50, **45.86**, 51.85 | 37.98, **49.19,** 52.25 | 31.82, **43.10,** 51.85 | |
| | **0.1** | 37.50, **50.00**, 58.82 | 44.44, **50.58,** 58.82 | 35.42, **45.45,** 62.21 | [i] -3.47** |
| | **0.5** | 33.77, **42.86***, 48.86 | 33.33, **38.09,** 47.73 | 37.10, **43.75,** 48.68 | |
| | **1** | 29.73, **39.62****, 42.50 | 30.83, **39.63,** 45.51 | 29.73, **37.57,** 42.50 | |
| **Percentage open duration** | **vehicle** | 18.30, **34.83**, 57.02 | 25.65, **29.34,** 43.57 | 16.23, **43.36,** 59.61 | |
| | **0.05** | 41.07, **49.95**, 60.55 | 38.48, **53.51,** 60.25 | 44.09, **47.65,** 62.85 | |
| | **0.1** | 30.78, **38.48**, 56.01 | 31.48, **43.21,** 54.58 | 13.99, **33.18,** 63.69 | [i] 3.71*** |
| | **0.5** | 41.25, **56.39***, 63.05 | 45.24, **59.65,** 69.88 | 43.18, **52.61,** 59.24 | |
| | **1** | 55.92, **60.64****, 69.32 | 46.77, **60.00,** 67.05 | 55.92, **62.14,** 69.32 | |
| **Percentage closed duration** | **vehicle** | 23.81, **33.19**, 39.29 | 28.15, **32.29,** 37.44 | 23.59, **34.08,** 38.94 | |
| | **0.05** | 19.36**, 25.18**, 32.97 | 20.41, **23.59,** 34.89 | 19.27, **26.28,** 28.15 | |
| | **0.1** | 21.20, **31.09**, 38.60 | 21.19, **30.33,** 38.60 | 19.64, **31.09,** 37.39 | [i] -3.87*** |
| | **0.5** | 14.77, **23.46***, 31.21 | 11.88, **21.25,** 26.61 | 23.18, **23.71,** 32.99 | |
| | **1** | 13.98, **18.63****, 23.20 | 16.30, **21.63**24.77 | 12.84, **17.21**21.51 | |
| **Rear frequency** | **vehicle** | 7.59, **16.00**, 24.88 | 7.59, **16.00,** 20.00 | 10.50, **18.91,** 28.46 | |
| | **0.05** | 10.76, **16.46**, 20.00 | 8.78, **15.93,** 23.50 | 12.13, **16.46,** 19.66 | |
| | **0.1** | 12.83, **24.00**, 25.51 | 17.22, **24.50,** 25.67 | 7.01, **17.00,** 24.75 | [i i] -0.23<br>[i i i] -2.34* |
| | **0.5** | 5.05, **12.32**, 19.31 | 5.00, **11.00,** 14.00 | 7.86, **16.89,** 21.02 | |
| | **1** | 7.30, **8.94**, 11.00 | 10.00, **16.44,** 18.23 | 5.43, **7.91*,** 8.94 | |
| **Immobile duration** | **vehicle** | 3.58, **20.42**, 48.88 | 13.52,**33.22,** 96.29 | 3.27, **12.79,** 28.29 | |
| | **0.05** | 1.37, **8.65**, 20.33 | 1.32, **9.89,** 19.23 | 2.15,**8.65,** 20.44 | [i i] -2.16*<br>[i i i] -1.02 |
| | **0.1** | 0.00**, 2.61**, 13.67 | 0.00, **1.29****, 2.61 | 11.77, **20.97**, 23.30 | |
| | **0.5** | 0.00, **1.92**, 9.33 | 1.27, **1.76*,** 3.41 | 0.00, **2.08,** 11.37 | |
| | **1** | 2.30, **5.43**, 13.90 | 3.26, **5.43,** 9.69 | 2.30,**7.48,** 85.62 | |
| **Locomotor duration** | **vehicle** | 56.79, **105.14**, 125.26 | 77.86, **104.12,** 115.43 | 44.33, **12.89,** 30.62 | |
| | **0.05** | 93.86, **103.96**, 112.04 | 89.62, **95.47,** 103.96 | 110.17, **13.28,** 25.07 | |
| | **0.1** | 84.01**, 94.54**, 102.60 | 84.01, **91.31,** 102.27 | 58.11, **95.88,** 09.79 | [i i] 1.13<br>[i i i] -1.23 |
| | **0.5** | 90.24, **101.95**, 115.36 | 96.32, **101.28,** 106.12 | 84.88, **03.41,** 18.07 | |
| | **1** | 90.34, **102.28**, 112.28 | 103.77,**105.41,**115 | 85.93, **92.81**, 110.59 | |
| **Head-dip Frequency** | **vehicle** | 13.00**, 27.00**, 43.77 | 21.49, **26.00,** 33.50 | 13.00, **34.67,** 47.66 | |
| | **0.05** | 35.00, **49.00***, 52.67 | 30.98, **41.28,** 50.44 | 48.56, **52.34,** 55.83 | |
| | **0.1** | 21.00, **37.00**, 49.00 | 30.99, **40.05,** 49.00 | 13.41, **21.00,** 44.38 | [i] 5.54*** |
| | **0.5** | 44.32, **58.50*****, 72.34 | 40.00, **58.00,** 69.00 | 50.32, **59.46,** 72.34 | |
| | **1** | 57.02, **66.43*****, 83.69 | 55.29, **63.90,** 75.00 | 57.02, **68.58,** 86.00 | |
| **Stretch-** | **vehicle** | 1.32, **4.80,** 12.00 | 1.00, **3.65,** 6.50 | 2.66, **8.51,** 16.34 | [i] -2.69** |

| | | | | | |
|---|---|---|---|---|---|
| **attend frequency** | **0.05** | 3.59, **5.71**, 8.57 | 1.13, **5.19,** 10.67 | 4.21,**6.31,** 8.57 | |
| | **0.1** | 0.00, **2.00**, 5.93 | 0.00, **1.51,** 3.00 | 0.00,**4.51,** 5.96 | |
| | **0.5** | 0.00, **3.15**, 6.84 | 1.00, **4.00,** 6.00 | 0.00,**2.11,** 6.84 | |
| | **1** | 0.00, **2.41*,** 4.00 | 0.00, **2.41,** 4.00 | 1.00, **2.28,** 5.00 | |
| **Percent protected head-dips** | **vehicle** | 5.00, **8.96**, 23.08 | 2.50, **11.77,** 20.79 | 5.69, **8.41,** 22.33 | |
| | **0.05** | 3.57, **7.28**, 11.90 | 2.73, **6.43,** 8.59 | 5.66, **10.76,** 16.28 | |
| | **0.1** | 4.44, **11.11**, 18.42 | 7.41, **11.81,** 18.42 | 2.22, **5.36,** 22.14 | -1.09 |
| | **0.5** | 3.41, **6.83**, 13.96 | 3.79, **6.76,** 7.02 | 1.90, **8.57,** 16.64 | |
| | **1** | 3.85**, 6.41,** 11.54 | 2.83, **6.82,** 19.88 | 3.85, **5.71,** 11.54 | |

Number of gerbils in each group was all gerbils: vehicle n = 26, 0.01 mg/kg n= 14, 0.05 mg/kg n = 17, 0.1mg/kg n = 20, 1mg/kg n = 17. Male gerbils: vehicle n = 11, 0.01 mg/kg n= 8, 0.05 mg/kg n = 10, 0.1mg/kg n = 9, 1mg/kg n = 7. Female gerbils: vehicle n = 15, 0.01 mg/kg n= 6, 0.05 mg/kg n = 7, 0.1mg/kg n = 11, 1mg/kg n = 10. [i] J-T results for all gerbils; [ii] results for male gerbils, [iii] results for female gerbils. Levels of significance: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

### 5.3.1.2 The effects of buspirone on male and female gerbil behaviour in the EPM

Median, inter-quartile ranges and J-T trend test results for male and female gerbils in response to each dose are presented in Table 5.4

Pair-wise comparisons of male and female gerbils revealed that at 3mg/kg head-dip frequency ($U = 6$ $p < 0.05$) and immobile duration ($U = 2$, $p < 0.05$) were less in females than males; whereas, rear frequency ($U = 0.5$, $p < 0.05$) at 10mg/kg was greater in females than males. Data for these variables were analysed separately for males and females. (See Table 5.2 for median, inter-quartile range and J-T trend test results).

For all other variables, data from males and females were combined for analysis in the J-T test. At 1mg/kg total entries were significantly higher than vehicle-treated controls ($U = 97$, $p < 0.05$); while at 3mg/kg percentage open entries ($U = 87.5$, p, 0.05), percentage open duration ($U = 67$, $p < 0.01$) and locomotor duration ($U = 19$, $p <0.001$) were higher than vehicle controls. Also at this dose, total entries ($U = 97$, $p < 0.05$), percentage closed entries ($U = 87.5$, $p < 0.05$) and percentage closed duration ($U = 100.5$, $p < 0.05$) were less. At 10mg/kg percentage open duration ($U = 54$, $p < 0.01$) and locomotor duration ($U = 65$, $p < 0.05$) were higher, and percentage closed duration ($U = 38$, $p < 0.01$) was less, than vehicle control. At 30mg/kg total entries ($U = 19$, $p <0.001$), percentage closed entries ($U = 63$, $p < 0.05$) and percentage closed duration ($U = 61$, $p < 0.01$) were all lower, and percentage open entries ($U = 63$, $p < 0.01$) were higher, than vehicle control.

In males, pair-wise analysis revealed that rear frequency was significantly less when compared with control at 30mg/kg ($U = 16$, $p < 0.05$).

**Table 5.4 Effects of acute buspirone (i.p.) on male and female gerbil behaviour in the elevated plus-maze. (Median and inter-quartile ranges)**

| Dependent variable | Buspirone Dose (mg/kg) | Median and inter-quartile ranges | | | J-T Statistic |
|---|---|---|---|---|---|
| | | All gerbils | Males | Females | |
| **Total entries** | **vehicle** | 17.09, **28.50**, 35.00 | 19.54, **25.00,** 30.50 | 17.50, **31.00,** 38.07 | [i] -5.65*** [ii] -4.43*** [iii] 3.37*** |
| | **1** | 26.19,**29.00*,** 34.00 | 20.09, **26.19,** 30.09 | 17.50, **31.00,** 38.06 | |
| | **3** | 14.00, **20.70*,** 23.32 | 9.50, **17.35,** 26.15 | 29.00, **31.00,** 34.40 | |
| | **10** | 4.00, **12.68,** 14.00 | 1.69, **2.37,** 7.68 | 19.00, **22.00,** 22.00 | |
| | **30** | 1.11, **2.00***,** 4.38 | 1.11, **2.00,** 2.79 | 9.83, **14.00,** 15.50 | |
| **Percentage open entries** | **vehicle** | 39.29, **44.44,** 56.52 | 41.47, **44.44,** 55.81 | 1.16, **4.69,** 14.52 | [i] 3.64*** [ii] 3.08** [iii] 2.37** |
| | **1** | 30.77, **43.67,** 64.71 | 46.64, **64.71,** 72.39 | 36.99, **44.44,** 55.53 | |
| | **3** | 50.00, **54.55*,** 66.67 | 53.37, **64.37,** 71.46 | 33.13, **35.48,** 43.66 | |
| | **10** | 46.15, **54.41,** 70.00 | 73.08, **100.00,** 100.00 | 50.00, **52.00,** 52.63 | |
| | **30** | 66.07, **100.00**,** 100.00 | 60.00, **100.00,** 100.00 | 45.83, **50.00,** 61.55 | |
| **Percentage closed entries** | **vehicle** | 43.48, **55.56,** 60.71 | 44.14, **55.56,** 58.53 | 66.07, **87.50,** 100.00 | [i] -3.64*** [ii] -3.08** [iii] -2.37** |
| | **1** | 35.29, **56.33,** 69.23 | 27.65, **35.29,** 53.36 | 44.47, **55.56,** 63.01 | |
| | **3** | 33.33, **45.45*,** 50.00 | 28.57, **35.63,** 46.63 | 56.33, **64.52,** 66.87 | |
| | **10** | 30.00, **45.59,** 53.85 | 0.00, **0.00,** 26.92 | 47.37, **48.00,** 50.00 | |
| | **30** | 0.00, **0.00*,** 33.93 | 0.00, **0.00,** 40.00 | 38.45, **50.00,** 54.16 | |
| **percent open duration** | **vehicle** | 17.60, **32.08,** 47.07 | 25.65, **29.34,** 43.57 | 0.00, **12.50,** 33.92 | [i] 3.32*** [ii] 2.14* [iii] 2.54** |
| | **1** | 21.73, **47.60,** 63.95 | 36.50, **51.27,** 57.66 | 14.78, **36.20,** 46.60 | |
| | **3** | 44.68, **50.47**,** 72.81 | 47.27, **71.85,** 77.39 | 31.53, **43.93,** 53.94 | |
| | **10** | 45.33, **56.05**,** 81.48 | 24.76, **45.33,** 70.83 | 44.68, **46.15,** 49.35 | |
| | **30** | 7.67, **59.91,** 85.14 | 29.22, **59.91,** 90.39 | 51.46, **60.53,** 76.07 | |
| **percent closed duration** | **vehicle** | 23.37, **32.22,** 36.82 | 28.15, **32.29,** 37.44 | 7.10, **38.85,** 73.44 | [i] -4.65*** [ii] -3.49*** [iii] -3.23** |
| | **1** | 15.46, **23.90,** 34.45 | 11.10, **15.46,** 20.12 | 18.90, **32.15,** 36.37 | |
| | **3** | 8.95, **22.47*,** 32.10 | 4.04, **11.41,** 29.19 | 28.73, **34.45,** 44.08 | |
| | **10** | 5.80, **11.91**,** 18.62 | 0.00, **0.00,** 9.65 | 22.47, **25.91,** 32.97 | |
| | **30** | 0.00, **0.00**,** 10.20 | 0.00, **0.00,** 45.21 | 9.58, **12.98,** 16.64 | |
| **rear frequency** | **vehicle** | 7.59, **16.00,** 22.00 | 7.59, **16.00,** 20.00 | 0.00, **0.96,** 10.20 | [i] -2.37** [ii] -2.64* [iii] -0.82 |
| | **1** | 14.00, **20.50,** 26.00 | 13.11, **14.00,** 20.00 | 10.50, **17.00,** 26.34 | |
| | **3** | 10.00, **15.00,** 23.95 | 4.00,**13.00,** 22.27 | 20.50, **24.00,** 25.08 | |
| | **10** | 12.00, **13.08,** 17.51 | 4.06, **7.12,** 9.56 | 11.00, **17.00,** 23.94 | |
| | **30** | 0.51, **2.51,** 8.98 | 0.00, **1.51*,** 6.16 | 13.08, **14.43,** 19.25 | |
| **immobile duration** | **vehicle** | 3.58, **23.84,** 51.36 | 13.52, **33.22,** 96.27 | 3.33, **6.00,** 19.10 | [i] 0.68 [ii] 1.82* [iii] -1.14 |
| | **1** | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 6.03 | 3.27, **12.79,** 40.78 | |
| | **3** | 0.00, **3.29,** 16.50 | 2.22, **12.21,** 39.95 | 0.00, **0.00,** 2.25 | |
| | **10** | 0.00, **9.56,** 36.74 | 68.56, **121.68,** 158.94 | 0.00, **0.00,** 10.07 | |
| | **30** | 62.96, **83.19,** 157.64 | 71.59, **88.06,** 157.63 | 6.24, **46.77,** 142.02 | |
| **locomotor** | **vehicle** | 74.46, **106.11,** 125.26 | 77.86, **104.12,** 115.45 | 83.39, **112.89,** 130.61 | [i] 2.13* |

| | | | | | |
|---|---|---|---|---|---|
| **duration** | **1** | 130.34, **157.67,** 159.28 | 144.81, **159.28,** 169.35 | 136.04, **156.09,** 157.67 | [ii] 0.27<br>[iii] 3.17** |
| | **3** | 152.50,**155.37,** 167.61*** | 141.79, **158.70,** 182.59 | 152.50, **154.62,** 162.50 | |
| | **10** | 101.88, **150.62,** 167.35* | 65.12, **76.75,** 124.39 | 137.02, **157.36,** 163.40 | |
| | **30** | 72.05, **105.78,** 147.65 | 72.05, **84.56,** 123.22 | 94.61, **147.65,** 177.67 | |
| **head-dip frequency** | **vehicle** | 13.00, **27.00,** 40.00 | 21.49, **26.00,** 33.50 | 13.00, **27.71,** 40.88 | [i] -1.22<br>[ii] -1.37<br>[iii] -0.10 |
| | **1** | 30.00, **38.50,** 53.00 | 41.50, **53.00,** 60.54 | 29.63, **36.00,** 38.50 | |
| | **3** | 27.00, **28.47,** 33.51 | 27.73, **32.75,** 36.37 | 25.00, **27.00,** 28.00 | |
| | **10** | 13.06, **31.50,** 45.00 | 9.53, **13.06,** 22.02 | 24.50, **37.07,** 46.92 | |
| | **30** | 5.23, **13.26,** 29.75 | 3.41, **11.62,** 26.74 | 9.65, **18.63,** 31.50 | |
| **stretch-attend frequency** | **vehicle** | 1.32, **4.00,** 12.00 | 1.00, **3.65,** 6.50 | 1.88, **7.88,** 14.00 | [i] 1.85*<br>[ii] 3.06**<br>[iii] -0.40 |
| | **1** | 3.00, **4.65,** 14.00 | 3.25, **3.49,** 8.74 | 2.91, **5.82,** 9.90 | |
| | **3** | 8.33, **10.35,** 11.00 | 7.50, **10.68,** 13.00 | 8.33, **10.00,** 11.00 | |
| | **10** | 5.00, **13.19,** 18.00 | 13.50, **14.00,** 20.64 | 3.11, **6.00,** 15.69 | |
| | **30** | 3.51, **4.87,** 15.47 | 4.41, **8.47,** 23.00 | 2.50, **3.51,** 5.32 | |
| **percent protected head-dips** | **vehicle** | 0.00, **8.93,** 18.52 | 2.50, **11.76,** 20.79 | 2.63, **8.33,** 16.38 | [i] 1.52<br>[ii] 0.13<br>[iii] 2.03* |
| | **1** | 16.67, **27.31,** 50.00 | 27.31, **28.21,** 45.76 | 13.21, **16.67,** 33.33 | |
| | **3** | 11.11, **22.22,** 25.00 | 8.47, **13.37,** 24.39 | 22.22, **24.00,** 25.00 | |
| | **10** | 8.11, **20.49,** 38.46 | 12.90, **25.81,** 49.26 | 12.01, **18.75,** 30.34 | |
| | **30** | 6.62, **14.82,** 28.21 | 2.78, **14.63,** 21.53 | 10.10, **22.92,** 51.66 | |

Group sizes: all gerbils: vehicle n = 26, 1mg/kg n = 6, 3mg/kg n = 13,10mg/kg n= 10, 30mg/kg n =12. Male gerbils: vehicle n= 11, 1mg/kg n=3, 3mg/kg n = 8, 10mg/kg n = 3, 30mg/kg n = 8. Female gerbils: vehicle n=15,1mg/kg n= 3, 3mg/kg n = 5,10mg/kg n= 7, 30 mg/kg n= 4

[i] J-T results for all gerbils; [ii] results for male gerbils, [iii] results for female gerbils. Levels of significance: p< 0.05*, p < 0.01**, p < 0.001

#### 5.3.1.3 The effects of caffeine on male and female gerbil behaviour in the EPM

Median, inter-quartile ranges and J-T trend test results for male and female gerbils in response to each dose are presented in Table 5.5

Pair-wise comparisons of data from male and female gerbils revealed that percentage open entries was higher in females than males ($U = 0$, $p < 0.05$), while percentage closed entries was higher in males than females ($U = 0$, $p < 0.05$) at 0.5mg/kg. None of the other measured variables differed between the sexes and so were combined (see Table 5.3 below for median, inter-quartile range and J-T results). Only percentage open duration and head-dip frequency showed significant dose-related trends (see Table 5.3). Percentage open duration ($U = 46$, $p < 0.05$) and head-dip frequency ($U = 42$, $p < 0.05$) were significantly less at 30mg/kg when compared with vehicle control gerbils. For median, inter-quartile ranges and J-T results see Table 5.5 below.

**Table 5.5 Effects of acute caffeine on male and female gerbil behaviour in the elevated plus-maze (Median and inter-quartile ranges).**

| Dependent variable | Drug Dose (mg/kg) | Median and inter-quartile ranges | | | J-T statistic |
|---|---|---|---|---|---|
| | | All gerbils | Males | Females | |
| **Total entries** | **vehicle** | 17.09, **29.00,** 35.00 | 17.09,**25.50,** 32.00 | 17.50, **31.00,** 38.07 | [i] -1.68 |
| | **0.5** | 16.58, **34.19,** 36.39 | 6.50, **12.00,** 27.12 | 27.68, **34.38,** 36.39 | |
| | **5** | 12.62, **19.00,** 31.00 | 8.50, **17.41,** 21.50 | 12.62, **31.00,** 37.98 | |
| | **15** | 5.16, **24.05,** 38.16 | 10.55, **18.11,** 25.99 | 5.95, **30.00,** 42.45 | |
| | **30** | 9.71, **12.56,** 22.50 | 10.50, **20.00,** 22.50 | 9.71, **12.55,** 26.53 | |
| **Percent open entries** | **vehicle** | 39.29, **44.44,** 56.52 | 40.63, **44.10,** 60.00 | 36.99, **44.44,** 55.53 | [i] -0.35<br>[i i i] 0.63 |
| | **0.5** | 28.86, **40.00,** 51.88 | 12.20, **24.39,** 28.86 | 41.88, **51.88,** 66.67 | |
| | **5** | 45.16, **50.00,** 54.17 | 51.92, **54.17,** 60.15 | 35.29, **44.80,** 50.00 | |
| | **15** | 33.75, **46.05,** 64.37 | 31.25, **37.50,** 52.08 | 42.11, **50.00,** 62.07 | |
| | **30** | 35.00, **40.54,** 54.00 | 15.00, **30.00,** 39.00 | 40.27, **50.27,** 80.00 | |
| **Percent closed entries** | **vehicle** | 43.48, **55.56,** 60.71 | 40.00, **55.90,** 59.38 | 44.47, **55.56,** 63.01 | [i i] 0.35<br>[i i i] -0.64 |
| | **0.5** | 48.13, **60.00,** 71.14 | 71.14, **75.61,** 87.80 | 33.33, **48.13,** 58.13 | |
| | **5** | 45.83, **50.00,** 54.84 | 39.85, **45.83,** 48.08 | 50.00, **55.20,** 64.71 | |
| | **15** | 35.63, **53.95,** 66.25 | 47.92, **62.50,** 68.75 | 37.93, **50.00,** 57.89 | |
| | **30** | 46.00, **59.46,** 65.00 | 61.00, **70.00,** 85.00 | 20.00, **49.73,** 59.73 | |
| **Percent open duration** | **vehicle** | 17.60, **30.70,** 46.14 | 24.69, **28.13,** 36.76 | 14.78, **36.20,** 46.60 | [i] -2.00* |
| | **0.5** | 19.10, **23.95,** 46.80 | 9.86, **19.72,** 21.83 | 29.01, **46.80,** 62.41 | |
| | **5** | 11.06, **28.16,** 41.74 | 19.61, **37.42,** 42.47 | 5.80, **27.43,** 41.74 | |
| | **15** | 4.56, **14.28,** 24.85 | 10.36, **14.17,** 14.28 | 2.57, **19.43,** 30.27 | |
| | **30** | 12.69, **16.93*,** 21.63 | 8.47, **16.93,** 24.82 | 12.69, **17.48,** 21.63, | |
| **Percent closed duration** | **vehicle** | 23.81, **32.29,** 36.82 | 30.85, **33.78,** 39.58 | 18.90, **32.15,** 36.37, | [i] 1.63 |
| | **0.5** | 26.23, **38.43,** 57.55 | 39.13, **55.55,** 77.15 | 25.67, **34.09**, 48.98 | |
| | **5** | 27.20, **39.94,** 52.85 | 17.78, **27.20,** 42.01 | 39.94, **44.36,** 89.94 | |
| | **15** | 32.37, **42.13,** 71.85 | 37.57, **59.54,** 71.85 | 36.22, **39.79,** 44.47 | |
| | **30** | 23.12, **43.98,** 69.21 | 23.12, **25.37,** 61.09 | 21.99, **49.50,** 69.21 | |
| **Rear frequency** | **vehicle** | 7.59, **16.00,** 21.00 | 7.58, **13.50,** 19.00 | 10.50, **17.00,** 26.35 | [i] -1.25 |
| | **0.5** | 5.41, **18.23,** 22.04 | 5.00, **8.00,** 22.55 | 10.53, **19.82,** 22.04 | |
| | **5** | 5.00, **9.00,** 12.44 | 3.31, **8.00,** 9.50 | 5.00, **14.50,** 21.22 | |
| | **15** | 1.86, **9.96,** 35.42 | 1.63, **2.26,** 9.60 | 2.98, **30.83,** 40.00 | |
| | **30** | 3.77, **11.00,** 14.14 | 5.50, **11.00,** 11.50 | 3.77, **11.91,** 21.28 | |
| **Immobile duration** | **vehicle** | 3.58, **21.30,** 51.36 | 7.51, **37.40,** 143.71 | 3.27, **12.79,** 40.78 | [i] -0.24 |
| | **0.5** | 0.00, **0.00,** 58.66 | 0.00, **0.00,** 139.24 | 0.00, **0.00,** 58.66 | |
| | **5** | 0.00, **0.00,** 65.18 | 0.00, **0.00,** 32.59 | 0.00, **0.31,** 08.61 | |
| | **15** | 0.00, **58.81,** 172.17 | 57.63, **115.27,** 136.86 | 0.00, **0.36,** 85.88 | |
| | **30** | 10.17,**138.80,** 193.07 | 9.15, **17.09,** 103.51 | 71.03, **67.51,** 10.76 | |
| **Locomotor duration** | **vehicle** | 74.46,**106.16,** 125.26 | 56.79, **105.14,** 118.21 | 83.39, **12.89,** 30.62 | [i] 0.95<br>[i i] 0.83<br>[i i i] 0.61 |
| | **0.5** | 110.73,**151.06,** 176.90 | 77.70, **151.06,** 165.90 | 110.73, **56.43,** 76.90 | |
| | **5** | 71.87, **156.21,** 181.09 | 104.95,**156.21,** 185.20 | 30.51, **53.12,** 81.09 | |
| | **15** | 41.79, **111.71,** 156.46 | 62.49, **82.26,** 153.28 | 40.86, **41.15,** 41.20 | |
| | **30** | 39.20, **52.92,** 137.81 | 76.35, **135.90,** 137.81 | 39.20, **52.89,** 30.54 | |
| **Head-dip frequency** | **vehicle** | 13.00, **27.00,** 38.00 | 18.99, **26.00,** 27.00 | 13.00, **27.71,** 40.88 | [i] -2.54* |
| | **0.5** | 17.61, **23.70,** 30.86 | 11.50, **23.00,** 23.35 | 20.23, **30.86,** 43.53 | |
| | **5** | 12.44, **18.74,** 26.81 | 12.72, **14.00,** 21.37 | 4.21, **25.90,** 27.00 | |
| | **15** | 4.63, **10.19,** 25.47 | 4.63, **7.00,** 7.73 | 11.91, **23.00,** 27.93 | |

| | | | | | |
|---|---|---|---|---|---|
| | **30** | 9.41, **14.00*,** 16.98 | 7.50, **14.00,** 14.50 | 0 .41, **15.13**, 22.07 | |
| **Stretch-attend frequency** | **vehicle** | 1.32, **4.00,** 12.00 | 1.00, **2.78,** 4.00 | 1.88,**7.88,** 14.00 | [i] 0.11 |
| | **0.5** | 2.55, **4.32,** 11.78 | 5.67, **11.33,** 15.67 | 2.55,**3.57,**8.28 | |
| | **5** | 4.00, **7.00,** 11.00 | 6.00, **9.37,** 10.48 | 1.00,**4.50,** 14.52 | |
| | **15** | 0.00, **1.91,** 8.26 | 1.91, **2.82,** 3.68 | 0.00,**0.00,** 12.00 | |
| | **30** | 2.09, **3.77,** 15.39 | 12.00, **22.00,** 26.00 | 1.96,**2.98,**6.27 | |
| **percent protected head-dips** | **vehicle** | 0.00 ,**9.52,** 18.52 | 0.00, **14.22,** 23.08 | 2.63,**8.33,** 16.39 | [i] 1.68 |
| | **0.5** | 10.32, **16.13,** 38.04 | 13.04, **26.09,** 43.48 | 10.32, **13.38,** 33.06 | |
| | **5** | 0.00, **11.11,** 33.33 | 8.85, **14.29,** 36.67 | 0.00,**5.56,** 25.00 | |
| | **15** | 10.00, **43.79,** 87.50 | 28.57, **57.14,** 78.57 | 20.00, **30.43,** 75.00 | |
| | **30** | 5.56, **14.29,** 39.71 | 33.81, **53.33,** 76.67 | 0.00, **5.56,** 18.60 | |

Group sizes: all gerbils: vehicle n = 25, 1mg/kg n = 7, 3mg/kg n = 13, 10mg/kg n= 8, 30mg/kg n = 7. Male gerbils: vehicle n= 10, 1mg/kg n= 3, 3mg/kg n = 7, 10mg/kg n = 3 , 30mg/kg n = 3 . Female gerbils: vehicle n= 15,1mg/kg n= 4, 3mg/kg n = 6,10mg/kg n= 5, 30 mg/kg n= 4.

[i] J-T results for all gerbils; [ii] results for male gerbils, [iii] results for female gerbils. Levels of significance: p< 0.05*, p < 0.01**, p < 0.001***

### 5.3.1.4 The effects of FG7142 on male and female gerbil behaviour in the EPM

Median, inter-quartile ranges and J-T trend test results for male and female gerbils in response to each dose are presented in Table 5.6.

Pair-wise comparisons of male and female gerbils revealed that there were differences between the sexes; percentage open entries were higher in males than females at 30mg/kg ($U = 0$, $p < 0.05$). In contrast, percentage closed entries and duration were higher in females at the 30m/kg dose ($U = 0$, $p < 0.05$ and $U = 0$, $p < 0.05$, respectively). For all other variables, data from male and female gerbils were combined. Only locomotor duration (J-T = -2.84, $p < 0.01$) and stretch-attend frequency (J-T = 1.97, $p < 0.05$) showed dose-related trends. Overall, locomotor duration at 5mg/kg ($U = 35$, $p < 0.05$) and at 30mg/kg ($U = 16$, $p < 0.01$) were significantly less, whereas stretch-attend frequency was significantly higher, than vehicle at 30mg/kg ($U = 16.5$, $p < 0.01$). For median, inter-quartile ranges and J-T results see Table 5.4 below.

Variables that differed between males and females were analysed separately. In females at 30mg/kg percentage open entries ($U = 1$ $p < 0.05$) were significantly lower than, and closed entries significantly higher than ($U = 1$, $p < 0.05$), vehicle control treated gerbils.

**Table 5.6 Effects of acute FG7142 on male and female gerbil behaviour in the elevated plus-maze (Median and inter-quartile ranges).**

| Dependent variable | Drug dose (mg/kg) | Median and inter-quartile ranges | | | J-T results |
|---|---|---|---|---|---|
| | | All gerbils | Males | Females | |
| **Total entries** | **vehicle** | 20.84, **26.00**, 33.42 | 23.00, **25.97**, 32.00 | 18.37, **33.00,** 43.31 | |
| | **0.5** | 25.00, **27.00**, 36.00 | 13.50, **24.50**, 31.01 | 27.00, **36.00,** 38.00 | |
| | **5** | 26.00, **27.00**, 28.74 | 26.00, **26.07**, 27.41 | 27.50, **28.00,** 35.34 | [i] -0.9 n/s |
| | **15** | 11.48, **22.00**, 34.25 | 15.39, **22.00**, 27.75 | 7.28, **29.43,** 53.15 | |
| | **30** | 13.86, **24.00**, 33.05 | 22.00, **32.29**, 37.82 | 12.88, **21.50,** 30.76 | |
| **Percentage open entries** | **vehicle** | 41.21, **48.08**, 60.43 | 35.29, **45.45**, 60.87 | 44.95, **55.56,** 57.88 | |
| | **0.5** | 48.15, **55.56**, 59.09 | 50.64, **56.11**, 69.55 | 38.89, **55.56,** 56.00 | [i i] -0.07 n/s |
| | **5** | 45.45, **50.00**, 54.17 | 46.15, **51.00**, 54.17 | 42.37, **50.00,** 53.01 | [i i i] -1.81* |
| | **15** | 50.00, **51.47**, 60.36 | 41.67, **53.55**, 60.36 | 50.00, **50.00,** 60.00 | |
| | **30** | 33.33, **42.86**, 58.62 | 37.50, **54.55**, 58.62 | 24.32, **36.02*,** 52.68 | |
| **Percentage closed entries** | **vehicle** | 39.57, **51.92**, 58.79 | 39.13, **54.55**, 64.71 | 42.12, **44.44,** 55.05 | |
| | **0.5** | 40.91, **44.44**, 51.85 | 30.45, **43.89**, 49.36 | 44.00, **44.44,** 61.11 | [i i] 0.07 n/s |
| | **5** | 45.83, **50.00**, 54.55 | 45.83, **49.00**, 53.85 | 46.99, **50.00,** 57.63 | [i i i] 1.81** |
| | **15** | 39.64, **48.53**, 50.00 | 39.64, **46.45**, 58.33 | 40.00, **50.00,** 50.00 | |
| | **30** | 41.38, **57.14**, 66.67 | 41.38, **45.45**, 62.50 | 47.32, **63.98*,** 75.68 | |
| **Percentage open duration** | **vehicle** | 14.17, **33.92**, 53.10 | 8.92, **25.34**, 54.88 | 18.09, **41.60,** 49.15 | |
| | **0.5** | 36.15, **51.14,** 65.28 | 43.65, **58.21**, 72.37 | 26.34, **50.45,** 56.77 | |
| | **5** | 26.22, **35.23**, 44.64 | 26.22, **34.59**, 38.24 | 23.90, **37.39,** 46.83 | [i] -0.9 |
| | **15** | 16.93, **48.96**, 58.05 | 34.46, **55.03**, 62.83 | 10.50, **20.35,** 35.08 | |
| | **30** | 12.49, **33.45**, 45.80 | 33.45, **38.88**, 56.34 | 10.99, **19.73,** 34.17 | |
| **Percentage closed duration** | **vehicle** | 20.18, **35.42**, 48.85 | 20.02, **34.49**, 51.23 | 22.72, **36.34,** 42.53 | |
| | **0.5** | 20.21, **21.94**, 29.06 | 10.61, **20.97**, 21.84 | 26.03, **29.06,** 41.29 | [i i] 1.26 n/s |
| | **5** | 26.74, **33.19**, 43.65 | 26.01, **31.65**, 41.11 | 28.18, **33.19,** 49.42 | [i i i] 1.24 |
| | **15** | 9.47, **21.12**, 37.31 | 9.47, **16.63**, 25.02 | 14.70, **36.81,** 67.39 | |
| | **30** | 16.62, **27.25**, 37.34 | 16.62, **23.32,** 33.27 | 14.25, **37.68,** 42.26 | |
| **Rear frequency** | **vehicle** | 9.68, **17.00**, 23.50 | 12.00, **17.00**, 24.00 | 9.18, **17.00,** 20.27 | |
| | **0.5** | 9.85, **11.00**, 18.00 | 4.93, **10.43**, 14.50 | 11.00, **17.00,** 22.00 | |
| | **5** | 15.00, **20.47**, 25.00 | 13.80, **17.49**, 25.00 | 20.08, **21.00,** 23.00 | [i] 0.16 |
| | **15** | 2.65, **11.86**, 16.00 | 4.35, **13.40**, 16.00 | 1.00, **7.36,** 14.86 | |
| | **30** | 7.98, **16.38**, 21.00 | 7.98, **15.00**, 17.19 | 8.09, **16.65,** 24.00 | |
| **Immobile duration** | **vehicle** | 1.90, **18.26**, 50.97 | 6.38, **18.57**, 44.28 | 0.00, **17.95,** 50.97 | |
| | **0.5** | 0.00**, 1.75**, 14.94 | 088, **8.35**, 21.50 | 0.00, **1.26,** 5.82 | |
| | **5** | 1.92, **6.59**, 11.26 | 2.57, **5.77**, 11.97 | 1.84, **6.59,** 11.03 | [i] -1.47 |
| | **15** | 1.29, **5.72**, 121.92 | 1.89, **5.72,** 22.00 | 0.41, **110.20,** 234.94 | |
| | **30** | 6.30, **15.46**, 68.99 | 6.30, **9.28,** 25.30 | 9.08, **19.29,** 148.89 | |
| **Locomotor duration** | **vehicle** | 83.39, **111.33**, 130.16 | 92.33, **98.46,** 118.72 | 91.72, **128.43,** 149.52 | |
| | **0.5** | 96.05, **103.14***, 105.83 | 83.10, **102.86,** 104.35 | 96.05, **105.83,** 106.52 | |
| | **5** | 79.48, **91.54,** 95.96 | 78.25, **81.81,** 93.42 | 91.88, **93.61,** 99.81 | [i] -2.84** |
| | **15** | 54.25, **92.46**, 106.24 | 78.84, **92.46**, 102.44 | 22.40, **83.13,** 138.44 | |
| | **30** | 66.36, **88.72****, 101.83 | 66.36, **89.20,** 102.89 | 47.44, **79.78,** 97.08 | |
| **Headdip frequency** | **vehicle** | 14.87, **27.60**, 39.36 | 11.74, **22.00,** 38.72 | 19.53, **27.95,** 40.50 | [i] -0.54 |
| | **0.5** | 39.34, **47.07**, 55.00 | 39.50, **45.53,** 49.53 | 39.34, **55.00,** 58.00 | |
| | **5** | 22.00, **31.42**, 46.00 | 22.00, **31.71,** 34.49 | 20.50, **28.99,** 47.00 | |
| | **15** | 20.97, **37.29**, 49.12 | 29.00, **44.00,** 52.41 | 11.47, **21.68,** 35.71 | |

| | | | | | |
|---|---|---|---|---|---|
| | **30** | 18.00, **23.00**, 45.61 | 23.00, **31.00,** 46.96 | 13.46, **19.16,** 22.07 | |
| **Stretch-attend frequency** | **vehicle** | 2.09, **6.00**, 10.33 | 3.21, **6.00,** 8.00 | 2.09, **6.00,** 11.67 | [i] 1.97* |
| | **0.5** | 8.00, **18.00**, 21.00 | 13.00, **19.50,** 21.45 | 6.00, **8.00,** 18.58 | |
| | **5** | 6.00**, 9.00**, 14.00 | 6.85, **11.00,** 15.00 | 5.59, **6.82,** 11.00 | |
| | **15** | 3.69, **7.62**, 13.00 | 4.69, **10.87,** 14.38 | 3.50, **5.17,** 7.62 | |
| | **30** | 2.97, **10.31****, 18.12 | 2.34, **10.31,** 12.00 | 4.01, **10.50,** 19.03 | |
| **percent protected head-dips** | **vehicle** | 0.00, **7.08,** 18.63 | 0.00, **7.50,** 18.75 | 1.92, **7.02,** 15.14 | [i] 1.63 |
| | **0.5** | 5.08, **9.09**, 30.23 | 4.55, **10.31,** 20.89 | 5.08, **5.17,** 30.56 | |
| | **5** | 6.25, **15.63**, 26.32 | 15.63, **22.84,** 26.67 | 2.17, **6.25,** 9.45 | |
| | **15** | 8.58, **14.07**, 23.09 | 8.58, **13.33,** 15.92 | 7.61, **22.61,** 35.00 | |
| | **30** | 5.66, **16.22,** 26.32 | 5.66, **9.68,** 20.00 | 8.11, **21.85,** 30.56 | |

Group sizes: all gerbils: vehicle n = 15, 1mg/kg n = 7, 3mg/kg n = 11,10mg/kg n= 6, 30mg/kg n = 7. Male gerbils: vehicle n= 9 , 1mg/kg n= 3, 3mg/kg n = 6 , 10mg/kg n = 4 , 30mg/kg n = 4 . Female gerbils: vehicle n= 6, 1mg/kg n= 4 , 3mg/kg n = 5, 10mg/kg n= 2 , 30 mg/kg n= 3.

[i] J-T results for all gerbils; [ii] results for male gerbils, [iii] results for female gerbils. Levels of significance: p< 0.05*, p < 0.01**, p < 0.001***.

## 5.3.2 BWB validation results

### 5.3.2.1 The effects of diazepam on male and female gerbil behaviour in the BWB

Median, inter-quartile ranges and J-T trend test results for male and female gerbils in response to each dose are presented in Table 5.7.

There were sex differences in the percentage of time spent in the black compartment following treatment with diazepam. More specifically, following 0.1mg/kg diazepam, males spent significantly longer in the black compartment compared to females ($U = 27$, $p < 0.05$). At 0.5mg/kg ($U = 20$, $p < 0.01$) and 1mg/kg (U 26, $p< 0.05$) this situation was reversed, i.e. females spent significantly longer in the black compartment compared to males. Thus, analyses were conducted separately for each sex for this variable. (See Table 5.5 for median, inter-quartile ranges and J-T results).

Pair-wise comparisons of diazepam dose versus vehicle, for variables that showed a significant dose-related trend, revealed that at 0.05mg/kg more time was spent in exploration (exploration duration white $U = 264$, $p <0.05$) and less time in freezing/ sedation (immobile duration white $U = 272$, p, 0.05), in the white compartment. At 0.1mg/kg exploratory behaviour was higher than vehicle-treated animals (exploration frequency white $U = 264.5$, $p < 0.05$; exploration duration white $U = 211.5$, $p < 0.01$). Again, at 0.5mg/kg exploration was higher than vehicle indicated by more traversing between compartments (crossing frequency $U = 303$, $p < 0.05$ and exploration duration white $U = 305$, $p < 0.05$). This anxiolytic profile was strengthened still further at 1mg/kg with higher crossing frequency ($U = 189.5$, $p < 0.001$), exploration frequency white ($U = 181.5$, $p < 0.001$), exploration duration white ($U = 215$, $p < 0.01$) and less immobile duration white ($U = 272.5$, $p < 0.05$) than vehicle. See Table 5.7 for median, inter-quartile range and J-T results.

Trend analysis revealed a significant dose-related trend in the time spent in the black compartment by male gerbils (males J-T = -3.57, $p < 0.001$; females J-T = 0.43, n/s). In males, pair-wise percentage duration black at 0.5mg/kg and 1mg/Kg were significantly less when compared to vehicle-treated gerbils (0.5 mg/kg, $U = 45$, $p < 0.01$; 1mg/kg , $U = 34$, $p < 0.01$).

**Table 5.7 The effects of diazepam on gerbil behaviour in the black-white box. Results are presented as median and inter-quartile ranges.**

| Dependent Variable | Diazepam dose (mg/kg) | Median and inter-quartile ranges | | | J-T Statistic |
|---|---|---|---|---|---|
| | | All gerbils | Male gerbils | Female gerbils | |
| **Latency black** | **Vehicle** | 4.56, **8.24,** 22.08 | 6.64, **10.07,** 22.08 | 3.90, **7.77,** 22.63 | |
| | **0.05** | 5.11, **7.39,** 14.03 | 5.21, **8.54,** 45.42 | 4.01, **7.20,** 9.01 | |
| | **0.1** | 3.73, **11.37,** 19.06 | 3.73, **8.23,** 19.06 | 6.72, **15.76,** 19.78 | [i] -0.78, n/s |
| | **0.5** | 4.29, **7.00,** 20.59 | 5.82, **7.52,** 22.24 | 3.49, **5.33,** 14.94 | |
| | **1** | 4.66, **6.75,** 13.46 | 5.71, **7.58,** 10.96 | 2.37, **6.21,** 23.40 | |
| **% White duration** | **vehicle** | 40.69, **46.39,** 56.86 | 39.89, **46.39,** 48.80 | 42.13, **47.68,** 58.04 | |
| | **0.05** | 37.11, **43.76,** 51.08 | 38.20, **42.43,** 53.04 | 36.44, **43.90,** 49.11 | |
| | **0.1** | 41.16, **48.35,** 56.27 | 35.52, **44.94,** 51.11 | 44.62, **53.71,** 62.51 | [i] -0.67, n/s |
| | **0.5** | 39.61, **46.80,** 49.64 | 46.09, **47.60,** 51.10 | 38.38, **40.99,** 48.57 | |
| | **1** | 44.63, **47.22,** 52.73 | 45.84, **48.70,** 54.93 | 42.72, **45.40,** 49.44 | |
| **%Black duration duration** | **vehicle** | 32.40, 39.98, 44.78 | 35.24, **41.51**, 46.70 | 30.43, **36.35**, 42.65 | |
| | **0.05** | 32.85, **38.11**, 44.43 | 35.59, **39.59**, 43.21 | 30.12, **37.93**, 45.65 | |
| | **0.1** | 29.10, **34.37**, 39.73 | 34.37, **37.96**, 44.99 | 28.63, **30.68**, 35.81 | [i i]-3.57*** [i i i]0.43, n/s |
| | **0.5** | 28.81, **37.65**, 42.52 | 28.32, **32.27**, 36.61* | 39.22, **40.82**, 45.74 | |
| | **1** | 31.45, **33.63**, 36.37 | 29.58, **32.22**, 33.86** | 33.63, **36.37**, 44.14 | |
| **Crossing frequency** | **vehicle** | 36.00, **42.50,** 51.00 | 38.00, **42.50,** 50.00 | 35.00, **46.50,** 54.00 | |
| | **0.05** | 38.00, **44.00,** 52.00 | 36.00, **41.00,** 45.00 | 41.00, **51.50,** 59.00 | |
| | **0.1** | 41.00, **44.00,** 53.00 | 43.00, **45.00,** 47.00 | 38.50, **51.00,** 56.00 | [i] 3.57*** |
| | **0.5** | 39.00, **49.00** 57.00 ***** | 40.50, **53.00,** 55.50 | 40.50, **47.00,** 61.00 | |
| | **1** | 48.00,**56.00,**67.00******* | 49.00, **58.00,** 66.00 | 47.00, **54.50,** 73.00 | |
| **Mobile Duration white** | **vehicle** | 56.69, **64.80,** 72.57 | 56.69, **64.80,** 72.57 | 55.84, **63.13,** 82.25 | |
| | **0.05** | 42.91, **53.45**, 62.20 | 42.91, **53.45,** 62.20 | 45.38, **56.11,** 68.22 | |
| | **0.1** | 38.96, **51.04**, 55.26 | 38.96, **51.04,** 55.26 | 55.50, **62.02,** 65.55 | [i] -1.78 |
| | **0.5** | 51.14, **57.43,** 60.57 | 51.14, **57.43,** 60.57 | 43.91, **50.88,** 57.97 | |
| | **1** | 62.74, 63.97, 69.76 | 62.74, **63.97,** 69.76 | 45.88, **52.44,** 55.82 | |
| **Exploration frequency white** | **vehicle** | 50.00, **74.00,** 82.00 | 50.00, **74.00,** 82.00 | 56.50, **72.50,** 89.50 | |
| | **0.05** | 71.00, **84.00**, 114.00 | 71.00,**84.00,** 114.00 | 63.00, **74.50,** 83.00 | |
| | **0.1** | 78.00, **88.00,** 96.00***** | 78.00, **88.00,** 96.00 | 67.50, **80.00,** 92.00 | [i] 3.12** |
| | **0.5** | 77.00, **82.00,** 87.00****** | 77.00, **82.00,** 87.00 | 56.00, **60.00,** 90.50 | |
| | **1** | 87.00,**101.00,**109.5****** | 87.00, **101.00,** 109.50 | 76.00, **86.00,** 98.00 | |
| **Exploration Duration white** | **vehicle** | 40.91, **51.27,** 57.85 | 36.76, **52.37,** 58.46 | 42.04, **50.45,** 55.37 | |
| | **0.05** | 50.60, **54.38,** 66.74 | 51.88, **54.19,** 66.88 | 48.51, **55.30,** 66.61 | |
| | **0.1** | 52.90, **62.08**, 71.49****** | 53.90, **64.18,** 71.17 | 50.62, **60.43,** 71.93 | [i] 3.06** |
| | **0.5** | 47.36, **57.84,** 64.69 | 55.42, **63.99,** 65.52 | 39.80, **48.22,** 63.57 | |
| | **1** | 53.72, **61.43,** 73.64****** | 57.67, **66.51,** 76.60 | 45.77, **59.60,** 69.12 | |
| **Immobile Duration white** | **vehicle** | 0.00, **3.38**, 7.36 | 0.00, **3.38,** 7.36 | 0.00, **1.79,** 7.52 | |
| | **0.05** | 0.00, **0.00**, 0.99 | 0.00, **0.00,** 0.99 | 0.00, **0.33,** 2.03 | |
| | **0.1** | 0.00, **0.00**, 0.88 | 0.00, **0.00,** 0.88 | 0.31, **1.37,** 5.46 | [i] -2.3* |
| | **0.5** | 0.31, **1.16**, 1.62 | 0.31, **1.16,** 1.62 | 0.00, **0.00,** 2.88 | |
| | **1** | 0.00, **0.00,** 0.19 | 0.00, **0.00,** 0.19 | 0.00, **0.00,** 2.03 | |

Group sizes All gerbils: vehicle n= 38, 0.05mg/kg n= 20, 0.1 mg/kg n= 21, 0.5 mg/kg n= 22, 1mg/kg n= 21. Males: vehicle n= 18, 0.05mg/kg n= 10, 0.1 mg/kg n= 10, 0.5 mg/kg n= 11, 1mg/kg n= 11.Females: vehicle n= 20, 0.05mg/kg n= 10, 0.1 mg/kg n= 11, 0.5 mg/kg n= 11, 1mg/kg n= 10.Levels of significance for J-T test and Mann Whitney U pair-wise comparisons with vehicle * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

#### 5.3.2.2 The effects of buspirone on male and female gerbil behaviour in the BWB

Median, inter-quartile ranges and J-T trend test results for male and female gerbils in response to each dose are presented in Table 5.8.

Analysis revealed significant sex differences for all of the behavioural measures in the BWB following the two highest doses of buspirone (10mg/kg & 30mg/kg). Closer inspection of the data revealed that these differences were largely a result of male gerbils failing to enter the black compartment of the box during the test session following these two doses of buspirone. Thus, there were significant differences between male and female gerbils in their latency to enter the black compartment (10mg/kg ($U = 13$, $p < 0.001$; 30mg/kg ($U= 23.5$, $p < 0.05$)). Males spent a significantly greater percentage of the test session in the white compartment compared to females (10mg/kg ($U = 13$, $p < 0.01$; 30mg /kg ($U = 23.5$, $p < 0.05$)). In contrast, females spent a significantly greater proportion of their time in the black compartment of the BWB compared to males (10mg/kg ($U = 0$, $p < 0.001$); 30mg/kg ($U = 21.5$, $p < 0.05$)). Consistent with this, females showed a significantly higher frequency of crossing between the compartments compared to males (10mg/kg ($U = 0$, $p < 0.001$); 30mg/kg ($U =19.5$, $p < 0.05$)). In addition to these differences, males showed a significantly greater duration of immobility in the white compartment compared to females following 30mg/kg ($U = 1$, $p < 0.001$). Given these sex differences, subsequent analyses were carried out separately for male and female gerbils.

Treatment with buspirone had significant effects on most of the behaviours measured in male and female gerbils in the BWB (these data are summarised in Table 5.8). The exceptions to this were latency to enter the black compartment (J-T =-3.1, $p < 0.05$ for males, J-T = 0.61, p n/s for females) and mobile duration in the white compartment (J-T = 1, p n/s for males, J-T = 0.29, n/s for females). Hence, in males at 1mg/kg exploration frequency and duration white ($U = 39.5$, $p < 0.01$, $U = 44$, $p < 0.05$) were higher, while the time spent in the black compartment (percentage duration black $U= 61$, $p < 0.05$) and the time spent in freezing/ sedation ($U= 27.5$, $p < 0.001$) were less than vehicle controls. At 3mg/kg latency black ($U = 49.5$, $p < 0.05$) was higher, while time spent crossing between compartments (crossing frequency ($U = 14$, $p < 0.001$)) and time spent

in the black compartment (percentage black duration ($U = 47.5$, $p < 0.05$)) were significantly less than male vehicle controls.

In male gerbils treated with 10mg/kg and 30 mg/kg buspirone, latency black ($U = 22$, $p < 0.001$; $U = 22.5$, $p < 0.001$ respectively) was higher than vehicle-treated gerbils. While, percentage black duration at 10mg/kg ($U = 5$, $p < 0.001$) and 30mg/kg ($U = 5.5$, $p < 0.001$), crossing frequency at 10mg/kg ($U = 1$, $p < 0.001$) and 30mg/kg ($U = 0$, $p < 0.001$), exploration frequency at 10mg/kg ($U = 3$, $p < 0.001$) and 30mg/kg ($U = 11$, $p < 0.001$) and exploration duration at 10mg/kg ($U = 12$, $p < 0.001$) and 30mg/kg ($U = 12$, $p < 0.001$) were all significantly lower than vehicle controls. In contrast, time spent in the white compartment (percentage duration white at 10mg/kg ($U = 19$ $p < 0.001$) and 30mg/kg ($U = 2$, $p < 0.001$) and time spent immobile at 10mg/kg, (immobile duration at 10mg/kg ($U = 5$, $p < 0.001$) and 30mg/kg ($U = 9$, $p < 0.001$)) was higher than vehicle controls.

In females at 1mg/kg, 3mg/kg, 10mg/kg and 30mg/kg movement between the two compartments was less than in vehicle control gerbils (crossing frequency at 1mg/kg ($U = 44.5$, $p < 0.05$), 3mg/kg ($U = 19.5$, $p< 0.01$), 10mg/kg ($U = 16.5$, $p < 0.001$) and 30mg/kg ($U = 0$, $p < 0.001$)). Exploration frequency was also lower at 10mg/kg ($U = 93.5$, $p < 0.05$) than in vehicle-treated gerbils.

Again at 30mg/kg, percentage black duration ($U = 30$, $p < 0.01$), crossing frequency ($U = 0$, $p < 0.001$), exploration frequency ($U = 30.5$, $p < 0.01$) and exploration duration ($U = 36$, $p < 0.05$) were also less than in vehicle control gerbils. Additionally, sedation, immobile duration, ($U = 21.5$, $p < 0.001$) and time spent in the white compartment, percentage duration white ($U = 31$, $p < 0.001$) was higher than vehicle control gerbils.

**Table 5.8 The effect of buspirone on male and female gerbil behavior in the black-while box (Table of medians and inter-quartile ranges).**

| Dependent variable | Buspirone dose (mg/kg) | Median and inter-quartile ranges | | J-T Statistic |
|---|---|---|---|---|
| | | Males | Females | |
| **Latency black** | **vehicle** | 6.64, **10.07,** 22.08 | 4.23, **8.15,** 25.59 | [i i] -3.1*<br>[i i i] 0.61 n/s |
| | **1** | 4.58, **7.42,** 8.15 | 2.93, **5.43,** 19.97 | |
| | **3** | 14.33, **22.62,** 41.36* | 8.28, **10.57,** 21.20 | |
| | **10** | 300+ (did not enter)** | 8.28, **17.74,** 25.49 | |
| | **30** | 300+ (did not enter)*** | 0.00, **10.24,** 56.12 | |
| **% White Duration** | **vehicle** | 39.88, **46.38,** 48.80 | 42.44, **47.67,** 57.89 | [i i] 5.06***<br>[i i i] 1.92* |
| | **1** | 39.82, **45.01,** 49.93 | 45.75, **57.12,** 63.11 | |
| | **3** | 41.08, **62.36,** 81.91 | 40.02, **48.59,** 70.83 | |
| | **10** | 99.62, **100.00,** 100.00** | 37.46, **44.09,** 68.44 | |
| | **30** | 99.61, **100.00,** 100.00*** | 75.66, **87.84,** 100.00** | |
| **% Black duration** | **vehicle** | 35.24, **41.50,** 46.70 | 30.62, **36.35,** 41.48 | [i i] -6.46***<br>[i i i] -1.97* |
| | **1** | 31.34, **33.19,** 38.06* | 27.21, **31.94,** 39.81 | |
| | **3** | 6.85, **20.33,** 36.85* | 22.43, **35.41,** 45.93 | |
| | **10** | 0.00, **0.00,** 0.00*** | 16.36, **47.22,** 48.63 | |
| | **30** | 0.00, **0.00,** 0.00*** | 0.00, **8.34,** 13.75** | |
| **Crossing frequency** | **vehicle** | 36.00, **41.50**, 49.00 | 39.00, **47.00,** 54.00 | [i i] -6.8**<br>[i i i] -6.59*** |
| | **1** | 35.00, **38.00,** 46.00 | 27.50, **35.00,** 43.50** | |
| | **3** | 7.00, **21.00,** 25.50*** | 28.00, **31.00,** 31.00*** | |
| | **10** | 0.00, **0.00,** 0.00*** | 16.00, **27.00,** 28.00*** | |
| | **30** | 0.00, **0.00,** 1.00*** | 0.00, **5.50,** 12.00*** | |
| **Mobile Duration white** | **vehicle** | 56.69, **64.80**, 72.57 | 57.75, **65.63,** 84.14 | 1.00, n/s<br>0.29, n/s |
| | **1** | 55.25, **64.51**, 79.13 | 65.68, **80.23,** 85.24 | |
| | **3** | 68.64, **82.14**, 102.09** | 55.58, **77.01,** 96.26 | |
| | **10** | 15.59, **18.12**, 49.92 | 60.39, **71.41,** 80.27 | |
| | **30** | 42.52, **83.86**, 143.95 | 24.78, **68.12,** 125.46 | |
| **Exploration frequency white** | **vehicle** | 50.00, **74.00**, 82.00 | 65.00, **75.50,** 91.00 | [i i] -4.65***<br>[i i] -3.09** |
| | **1** | 88.50, **97.00**, 103.00** | 64.50, **77.00,** 109.00 | |
| | **3** | 39.00, **50.00**, 69.00 | 63.00, **72.50,** 87.00 | |
| | **10** | 1.00, **3.00**, 12.00*** | 52.00, **68.00,** 72.00 | |
| | **30** | 8.00, **15.50**, 25.00*** | 6.00, **21.50,** 57.00** | |
| **Exploration Duration white** | **vehicle** | 36.76, **52.37**, 58.46 | 42.58, **51.44,** 55.71 | [i i] 4.03***<br>[i i i] -1.81* |
| | **1** | 63.15, **68.67**, 72.20** | 46.29, **59.49,** 79.85** | |
| | **3** | 32.39, **44.37**, 57.48 | 53.26, **58.24,** 74.09* | |
| | **10** | 2.47, **5.16**, 15.05*** | 36.66, **51.36,** 56.34 | |
| | **30** | 7.37, **21.28**, 22.28*** | 4.83, **21.62,** 41.00* | |
| **Immobile Duration white** | **vehicle** | 0.00, **3.38**, 7.36 | 0.00, **1.79,** 6.54 | [i i] 4.39***<br>[i i i] 2.32* |
| | **1** | 0.00, 0.00, 0.00 | 0.00, **0.00,** 2.44 | |
| | **3** | 0.66, **8.89**, 102.36 | 0.00, **0.00,** 8.62 | |
| | **10** | 203.82, **234.53**, 279.40*** | 0.00, **0.00,** 32.33 | |
| | **30** | 115.74, **170.23**, 239.79*** | 11.74, **97.58,**224.71*** | |

Group sizes Males: vehicle n = 18; 0.5 mg/kg n = 11; 5mg/kg n = 11; 15 mg/kg n = 9; 30 mg/kg n = 10. Females: vehicle n = 18; 0.5 mg/kg n = 11; 5mg/kg n = 10; 15 mg/kg n = 13; 30 mg/kg n = 10. Levels of significance for J-T test and Mann Whitney U pair-wise comparisons with vehicle * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

#### 5.3.2.3 The effects of caffeine on male and female gerbil behaviour in the BWB

Median, inter-quartile ranges and J-T trend test results for male and female gerbils in response to each dose are presented in Table 5.9.

Sex differences were apparent for all variables. At 0.5mg/kg caffeine, crossing frequency ($U = 20.5$, $p < 0.01$), percentage duration in the white compartment ($U = 29$, $p < 0.05$), locomotor activity (mobile duration white ($U = 17$, $p< 0.05$)) and exploration in the white compartment (frequency ($U = 12$, $p < 0.01$) duration ($U = 26$, $p < 0.01$)) were significantly lower in females compared to males. Following the same dose of caffeine, females also spent a significantly greater proportion of the test session in the black side ($U = 28$, $p < 0.05$) and significantly longer immobile in the white side of the BWB compared to males ($U = 32.5$, $p < 0.05$). The only other sex differences were observed following 5mg/kg caffeine, which resulted in females taking significantly less time to enter the black compartment ($U = 21$, $p < 0.05$). Hence, all data for males and females were analysed separately.

For median, inter-quartile ranges and trend test data see Table 5.9.

In males, at 0.5mg/kg time spent in the white compartment (percentage duration white ($U = 55$, $p< 0.05$)) and locomotor activity were higher (mobile duration white ($U = 34$, $p< 0.001$)), while time spent in the black compartment (percentage duration black ($U = 34$, $p < 0.001$)) and in freezing/sedation (immobile duration white ($U = 39.5$, $p < 0.05$) were less than vehicle controls. At 5mg/kg, locomotion (mobile duration white ($U= 20$, $p < 0.001$) was higher, while percentage duration black ($U = 52$, $p < 0.05$) and immobile duration white ($U = 50$, $p < 0.05$) were lower, than vehicle controls. At 15mg/kg latency black ($U = 53$, $p < 0.05$), percentage duration white ($U = 53$, $p < 0.05$), mobile duration white ($U = 56$, $p < 0.05$) and immobile duration white ($U = 62.5$, $p < 0.05$) were higher than vehicle, while, percentage duration black ($U = 43$, $p < 0.01$) was lower. Finally, at 30mg/kg, percentage duration black ($U = 22.5$, $p < 0.001$) and crossing frequency ($U = 26.5$, $p < 0.01$) were less, and percentage duration white ($U = 51$, $p < 0.05$) was higher, than vehicle.

In female gerbils at 5mg/kg and 30mg/kg, mobile duration white ($U = 51$, $p < 0.05$ and $U = 39$, $p < 0.05$, respectively) was higher than vehicle control females; whereas,

crossing frequency at 15 and 30mg/kg ($U = 58.5$, $p < 0.05$ and $U = 31.5$ $p < 0.01$, respectively) was less.

**Table 5.9 The effect of caffeine on male and female gerbil behavior in the black-while box. (Table of medians and inter-quartile ranges).**

| Dependent variable | Caffeine dose (mg/kg) | Median and inter quartile ranges | | J-T Results |
|---|---|---|---|---|
| | | Males | Females | |
| **Latency black** | **vehicle** | 6.64, **10.07**, 22.08 | 3.90, **7.77,** 22.63 | [ii] 2.48** [iii] 0.09 |
| | **0.5** | 6.86, **15.43,** 27.58 | 1.59, **3.38,** 11.56 | |
| | **5** | 8.62, **18.62,** 27.44 | 5.33, **6.02,** 13.13 | |
| | **15** | 16.09, **20.66*,** 96.56 | 5.41, **10.16,** 20.11 | |
| | **30** | 6.86, **36.30,** 85.05 | 2.80, **3.63,** 17.19 | |
| **% white duration** | **vehicle** | 39.89, **46.39,** 48.80 | 42.13, **47.68,** 58.04 | [ii] 3.1** [iii] 0.81 |
| | **0.5** | 46.53, **53.51*,** 57.97 | 35.48, **39.08,** 50.33 | |
| | **5** | 44.01, **56.34,** 62.93 | 40.28, **47.72,** 56.78 | |
| | **15** | 52.64, **61.69*,** 81.53 | 34.02, **48.73,** 79.02 | |
| | **30** | 51.23, **59.21*,** 93.77 | 44.95, **66.25,** 83.57 | |
| **% black duration** | **vehicle** | 35.24, **41.51,** 46.70 | 30.43, **36.35,** 42.65 | [ii] -4.7*** [iii] -1.4 |
| | **0.5** | 26.21, **30.45,** 37.40** | 32.32, **45.06,** 49.78 | |
| | **5** | 21.21, **29.92,** 40.30* | 24.58, **34.49,** 47.32 | |
| | **15** | 7.60, **19.90,** 27.43* | 16.07, **35.78,** 41.67 | |
| | **30** | 2.99, **14.89,** 27.29** | 7.58, **15.11,**40.36 | |
| **Crossing frequency** | **vehicle** | 36.00, **41.50,** 49.00 | 34.50, **45.50,** 53.00 | [ii] -3.86*** [iii] -3.1*** |
| | **0.5** | 40.00, **49.50,** 53.00 | 26.00, **34.00,** 40.00** | |
| | **5** | 34.00, **38.00,** 45.50 | 31.00, **37.50,** 45.00 | |
| | **15** | 18.50, **25.00,** 38.50* | 9.00, **29.00,** 38.50** | |
| | **30** | 4.00, **17.00,** 29.00*** | 14.00, **28.00,** 32.00** | |
| **Mobile Duration white** | **vehicle** | 56.69, **64.80,** 72.57 | 55.84, **63.13,** 82.25 | [ii] 1.97* [iii] 2.11* |
| | **0.5** | 81.52, **85.55**, 92.86*** | 56.04, **66.65,** 81.05 | |
| | **5** | 77.90, **85.77,**109.60*** | 74.34, **84.31,** 99.69* | |
| | **15** | 64.19, **76.83,** 100.11 | 43.47, **62.03,** 86.57 | |
| | **30** | 53.24, **66.19**, 95.17 | 78.33, **95.97**, 109.88* | |
| **Exploration frequency White** | **vehicle** | 50.00, **74.00,** 82.00 | 56.50, **72.50,** 89.50 | [ii] 1.19 [iii] -0.76 |
| | **0.5** | 67.00, **101.50,** 110.00* | 32.00, **46.50,** 61.50** | |
| | **5** | 82.00, **96.00,** 100.50* | 72.00, **85.50,** 108.00 | |
| | **15** | 61.00, **85.00,** 99.50 | 35.00, **72.00,** 84.00 | |
| | **30** | 35.00, **43.00,** 58.50* | 36.00, **62.00,** 69.00 | |
| **Exploration Duration White** | **vehicle** | 36.76, **52.37,** 58.46 | 42.04, **50.45,** 55.37 | [ii] 0.4 [iii] 0.43 |
| | **0.5** | 46.73, **63.92,** 72.12 | 30.92, **45.25,** 51.36 | |
| | **5** | 50.20, **65.00,** 73.91 | 46.52, **61.02,** 74.04 | |
| | **15** | 48.85, **61.80,** 79.71 | 35.30, **51.35,** 57.69 | |
| | **30** | 34.84,**45.12,** 60.55 | 37.77, **47.75,** 72.12 | |
| **Immobile Duration White** | **vehicle** | 0.00, **3.38,** 7.36 | 0.00, **1.79,** 7.52 | [ii] 1.81* [iii] 0.1 |
| | **0.5** | 0.00, **0.00,** 0.00* | 0.00, **0.30,** 5.52 | |
| | **5** | 0.00, **0.00,** 0.61* | 0.00, **0.00,** 0.00* | |
| | **15** | 2.70, **9.34,** 53.84 | 0.00, **0.00,** 53.99 | |
| | **30** | 1.51, **31.53,** 148.98 | 0.00, **10.22,** 51.41 | |

Group sizes All gerbils: vehicle n = 38; 0.5mg/kg n= 22; 5mg/kg n = 21; 15mg/kg n = 22; 30mg/kg n = 20. Males: vehicle n = 18; 0.5mg/kg n= 10; 5mg/kg n = 11; 15mg/kg n = 11; 30mg/kg n = 11. Females: vehicle n = 20; 0.5mg/kg n= 12; 5mg/kg n = 10; 15mg/kg n = 11; 30mg/kg n = 9.Levels of significance for J-T test and Mann Whitney U pair-wise comparisons with vehicle * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

### 5.3.2.4 The effects of FG7142 on male and female gerbil behaviour in the BWB.

Median, inter-quartile ranges and J-T trend test results for male and female gerbils in response to each dose are presented in Table 5.10.

There were differences between male and female gerbils in their response to Fg7142. At 3mg/kg the crossing frequency between compartments ($U = 26.5$, $p < 0.05$) was significantly higher in females than males. Mirroring this, females spent significantly less time immobile in the white compartment compared to males ($U = 25$, $p < 0.01$). Following 10mg/kg, Fg7142 females were significantly more active (mobile duration ($U = 29$, $p < 0.05$)) and less immobile in the white zone than males ($U = 23$, $p < 0.001$). At 30mg/kg females took significantly longer to enter the black zone ($U = 31$, $p < 0.05$), spent significantly longer in the white side ($U = 23$, $p < 0.01$) and were more active (mobile duration ($U = 28$, $p < 0.05$)) compared to males. These variables were analysed separately for each sex and are summarised in Table 5.10 below.

When data from each dose were compared with vehicle for each of the behaviours that did not differ between the sexes, there were consistent trends (see Table 5.10 below). Gerbils spent less time in the black side, percentage black duration (1mg/kg $U = 181$, $p < 0.05$; 10mg/kg $U = 282$, $p < 0.05$; 30mg/kg; $U = 313$, $p <0.05$) and more time exploring the white compartment, exploration frequency (1mg/kg $U = 235.5$, $p < 0.01$; 3mg/kg $U= 283$, $p < 0.05$) and duration (1mg/kg $U = 194$, $p < 0.01$; 3mg/kg $U = 198.5$ $p < 0.01$; 10mg/kg $U = 284$, $p< 0.05$; 30mg/kg $U = 274$, $p < 0.01$) than vehicle-treated controls.

Variables that differed between males and females were analysed separately and compared to their own vehicle for each drug dose. In males, at 1mg/kg, latency black was higher than vehicle ($U = 61$, $p < 0.05$). At 3mg/kg, locomotor activity was less than vehicle (mobile duration white $U = 59$, $p < 0.01$). Similarly, at 10mg/kg, both locomotor activity (mobile duration white $U = 41.5$, $p < 0.01$) and freezing/ sedation ($U = 46.5$, $p < 0.01$) were less than vehicle-treated animals. Again, at 30mg/kg, mobile duration white was lower than vehicle-treated gerbils ($U = 52$, $p < 0.01$).

In females, latency black was higher at the top dose 30mg/kg ($U = 52$, $p < 0.05$) than vehicle-treated gerbils.

**Table 5.10 The effect of FG7142 on male and female gerbil behaviour in the black-while box. (Table of medians and inter-quartile ranges).**

| Dependent variable | FG7142 dose (mg/kg) | Median and inter-quartile ranges | | | J-T Result |
|---|---|---|---|---|---|
| | | all gerbils | males | females | |
| **Latency black** | **vehicle** | 4.56, **8.24,** 22.08 | 6.64, **10.07**, 22.08 | 3.90, **7.77**, 22.63 | [i i] -2.11* [i i i] 2.07* |
| | **1** | 8.60, **13.84,** 23.07 | 13.49, **15.15**, 23.07* | 3.85, **10.05**, 18.18 | |
| | **3** | 5.94, **9.01,** 12.85 | 6.23, **9.72**, 12.90 | 5.00**, 7.36**, 11.10 | |
| | **10** | 4.8, **9.20,** 21.53 | 4.31, **6.81**, 13.26 | 8.79, **15.60**, 27.43 | |
| | **30** | 4.99, **9.56,** 19.28 | 1.59, **6.97**, 11.70 | 9.45, **13.02**, 27.28* | |
| **% White duration** | **vehicle** | 40.69, **46.39,** 56.86 | 39.89, **46.39**, 48.80 | 42.13, **47.68,** 58.04 | [i i] -1.17 [i i i] 1.19 |
| | **1** | 48.89, **53.08,** 62.26 | 47.20, **50.83**, 59.52 | 50.79, **58.83**, 68.45 | |
| | **3** | 39.80, **44.03,** 47.54 | 42.90, **44.03**, 48.21 | 39.29, **42.65**, 47.54 | |
| | **10** | 44.51, **48.03,** 55.64 | 42.37, **45.70**, 47.62 | 49.45, **54.92**, 56.52 | |
| | **30** | 41.00, **48.59,** 59.61 | 38.13, **41.00**, 47.48 | 49.44, **51.28**, 66.60 | |
| **% Black Duration** | **vehicle** | 32.40, **39.98,** 44.78 | 35.24, **41.51,** 46.70 | 30.43, **36.35,** 42.65 | [i] -1.76* |
| | **1** | 25.76,**28.30,** 34.20* | 26.98, **32.27,** 34.16 | 25.34, **28.12,** 33.82 | |
| | **3** | 32.58, **39.44,** 41.21 | 30.64, **40.53,** 41.70 | 36.10, **38.85,** 41.20 | |
| | **10** | 27.06,**32.72,** 40.26* | 32.72, **35.10,** 40.96 | 26.11, **28.59,** 34.50 | |
| | **30** | 25.17,**37.64,** 39.15* | 34.19, **37.73,** 40.87 | 24.44, **28.29,** 38.32 | |
| **Crossing frequency** | **vehicle** | 37.00, **44.00,** 51.00 | 36.00, **41.50**, 49.00 | 34.50, **45.50**, 53.00 | [i i] -0.12 [i i i] -1.34 |
| | **1** | 32.00, **42.50,** 49.50 | 37.00, **45.00**, 48.50 | 22.00, **38.00**, 48.00 | |
| | **3** | 37.00, **42.00,** 50.00 | 35.50, **38.00**, 41.50 | 40.00, **49.00**, 52.00 | |
| | **10** | 38.00, **41.00,** 52.00 | 37.00, **40.00,** 47.00 | 35.50, **47.00**, 49.50 | |
| | **30** | 34.50, **39.00,** 48.00 | 35.00, **44.50**, 47.50 | 26.50, **34.00**, 44.50 | |
| **Mobile Duration White** | **vehicle** | 56.10, **64.25,** 73.30 | 56.69, **64.80**, 72.57 | 55.84, **63.13**, 82.25 | [i i] -3.85*** -1.29 |
| | **1** | 58.83, **66.78,** 74.41 | 60.52, **67.59**, 72.92 | 57.17, **63.20**, 78.37 | |
| | **3** | 48.56, **54.61,** 57.64 | 50.52, **54.61,** 57.04** | 47.26, **54.92**, 57.64* | |
| | **10** | 49.50, **52.84,** 61.82 | 46.11, **50.22**, 54.18** | 51.04, **55.64**, 67.14 | |
| | **30** | 46.84, **55.01,** 62.54 | 42.01, **48.87**, 58.12* | 54.08, **60.32**, 65.29 | |
| **Exploration frequency White** | **vehicle** | 56.00, **73.50,** 84.00 | 50.00, **74.00,** 82.00 | 56.50, **72.50,** 89.50 | [i] 1.34 |
| | **1** | 70.50,**87.50,**108.** | 63.00, **84.00,** 100.50 | 84.00, **106.00,** 124.00 | |
| | **3** | 68.00,**80.00,** 93.00* | 68.50, **78.00,** 92.50 | 68.00, **85.00,** 111.00 | |
| | **10** | 63.00,**77.00,** 100.00 | 72.50, **79.00,** 92.50 | 53.50, **73.00,** 109.00 | |
| | **30** | 70.50, **79.00,** 97.50 | 67.50, **78.00,** 90.50 | 73.50, **81.00,** 102.00 | |
| **Exploration Duration White** | **vehicle** | 40.91, **51.27,** 57.85 | 36.76, **52.37,** 58.46 | 42.04, **50.45,** 55.37 | [i] 4.21*** |
| | **1** | 55.98,**63.82,**78.46* | 50.44, **62.23,** 71.50 | 58.68, **64.71,** 87.39 | |
| | **3** | 51.79,**63.76,**69.42* | 52.02, **65.08,** 68.80 | 50.58, **62.98,** 71.62 | |
| | **10** | 46.00,**64.49,** 71.68* | 50.47, **68.99,** 71.64 | 36.21, **58.43,** 69.93 | |
| | **30** | 49.19,**61.47,**69.38* | 45.77, **58.21,** 66.91 | 56.14, **64.84,** 77.59 | |
| **Immobile Duration White** | **vehicle** | 0.00, **1.92,** 7.36 | 0.00, **3.38**, 7.36 | 0.00, **1.79**, 7.52 | [i i] -2.45** 1.09 |
| | **1** | 0.00, **2.01,** 12.49 | 0.00, **1.38**, 12.49 | 0.00, **4.51**, 6.22 | |
| | **3** | 0.00, **0.00,** 0.66 | 0.00, **0.66**, 2.20 | 0.00, **0.00**, 0.00** | |

| | | | | | |
|---|---|---|---|---|---|
| | **10** | 0.00, **0.33,** 7.75 | 0.00, **0.00**, 0.00 | 0.88, **2.47,** 12.08 | |
| | **30** | 0.00, **1.49,** 8.46 | 0.00, **0.00**, 5.03 | 1.18, **3.50**, 9.67 | |

Group sizes: All gerbils: vehicle n = 38, 1mg/kg  n = 20, 3mg/kg n = 21, 10mg/kg n = 22, 30mg/kg n = 23. Males: vehicle n = 18; 1mg/kg n = 11, 3mg/kg n = 11, 10mg/kg n = 11, 30mg/kg n = 12. Females: vehicle n = 20; 1mg/kg n = 9, 3mg/kg n = 10, 10mg/kg n = 11, 30mg/kg n = 11.Levels of significance for J-T test, and Mann Whitney U pair-wise comparisons: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

## 5.3. 3 Results summary

*To recap*: in the EPM, alterations in spatiotemporal measures, such as more open entries and, time spent in the open arms and less closed duration, represent an anxiolytic profile. Additionally, ethological measures, for example, more exploration (head-dipping) and, less risk-assessment (stretch-attending) are also indicators of an anxiolytic profile. The converse of these represents anxiogenesis.

EPM: In summary, the trend test results showed that diazepam caused dose-related increases in behaviours related to anxiolysis. For example, there were increased time and entries to the open arms and exploration, while time spent in the closed arms and risk-assessment behaviours decreased. In contrast, buspirone also had an anxiolytic profile, but it was more limited in the behaviours it affected and there were difference between males and females in ethological measures. Thus, spatiotemporal measures of time spent in, and entries to, the open arms increased in all gerbils. Ethological behaviours such as head-dipping, sedation, immobility and rearing differed between the sexes. In response to the anxiogenic drug caffeine, there was an anxiogenic dose-response profile: open-arm entries and exploration decreased, and these behaviours reached significance at the top dose in pair-wise testing, but there were some sex differences. FG7142 also had an anxiogenic profile, risk-assessment behaviour increased in all gerbils, and the effect was more pronounced in females, they entered the open arms significantly less than males and vehicle controls.

In the BWB, indicators of a decrease in anxiety include more time spent in the white side, an increased number of transitions between compartments, higher crossing frequency, a longer latency to initially enter the black compartment, and more exploration and locomotor activity in the box. Higher levels of anxiety-type behaviours are typified by more time spent in the black side, a shorter latency to enter the black compartment, less exploratory behaviours and, possibly, more immobility; although, these behaviours can represent sedation.

BWB: Diazepam's profile was anxiolytic. Results of the trend test indicated that exploration of the white side increased and this was most pronounced in males. Also, black duration significantly decreased in males but not females. Buspirone differed

between the sexes for all behaviours measured and only produced a truly anxiolytic profile in males at the lowest dose. In females, the dose-related increase in white duration provides evidence of anxiolysis. However, the decreases in crossing frequency and exploration and the increase in immobility indicate that sedation was predominant. Thus, the BWB was able only to detect anxiolysis in males in response to buspirone. There were sex differences in response to caffeine and the profile was not clearly anxiogenic. In males at lower doses, movement around the box and time in the white side was higher than vehicle-treated animals, whereas, at the top dose, movement was less than vehicle-treated animals. However, spatiotemporal measures in males were not in keeping with anxiogenesis, white time increased and black time decreased in a dose-relatedfashion. Similarly, females' activity in the white side was higher than vehicle-treated animals at the top two doses. However, at these two doses, crossing frequency was lower after reaching a peak at the middle dose. In other words, at these doses, caffeine failed to produce anxiogenesis in the BWB. In FG7142-treated gerbils, there was anxiolysis rather than anxiogenesis indicated by increased latency to enter the black compartment, increased activity in the white side, decreased black duration and immobility. Locomotor activity was lower in males but only at the middle doses, but freezing behaviour was lower at the top dose. The only indication of a potential increase in anxiety was in males at the top dose; movement around the white side was significantly lower.

## 5.4 DISCUSSION: THE EFFECTS ANXIOLYTIC AND ANXIOGENIC DRUGS IN THE GERBIL EPM AND BWB: Are both models valid models of anxiety?

### 5.4.1 Discussion of the effects of anxiolytic and anxiogenic drugs in the gerbil EPM.

Diazepam's anxiolytic profile, particularly at the top two doses, is consistent with other reported findings in female gerbils (Varty, Morgan et al., 2002). However, if closed entries are taken to be a measure of sedation, as reported in the EPM in mice (Rodgers & Johnson, 1995) and in rats (Cruz et al., 1994), then there was sedation at all but the lowest (0.05mg/kg) dose. Risk-assessment behaviours are reported to be more sensitive to anxiolytic drugs and to the type of anxiety found in general anxiety disorder in

humans (Blanchard & Blanchard, 1989b; Griebel, Blanchard, & Blanchard, 1997; Blanchard et al., 1993; Blanchard et al., 2001). Given that locomotor duration did not differ significantly between vehicle and any of the top two doses, nor were there any increases in immobile duration, as would be expected if sedation were a prevalent feature, it can be concluded that the decreasing trend in closed entries is not entirely related to locomotor activity, but was a reflection of fewer entries to, and, time spent in the closed arms, and therefore of less anxiety-like behaviours in the gerbils in general. Thus, it would appear that the gerbil EPM is more in line with the two-factor model proposed by Wall & Messier, (2000 & 2001). In this model they proposed that the EPM measures only two main factors: anxiety measured by open exploration (entries and duration) and unprotected head-dips, and protected exploration measured by rearing, closed exploration (entries and duration) and, stretch-attend behaviour. In diazepam treated females rearing was significantly less at the top dose, indicating a reduction in protected exploration and therefore less anxiety.

Diazepam's lack of difference between male and female gerbils on measures related to anxiety is similar to findings in other rodents. For example, the rat showed no differences between the sexes in its anxiolytic response to diazepam and the effective anxiolytic dose of 1mg/kg was similar to the gerbils tested here (Hagenbuch, Feldon, & Yee, 2006).

Additionally, fit results indicate that female gerbils are more sensitive to the sedative and anti-seizure properties of diazepam than male gerbils. Studies on untreated gerbils indicated no sex differences in susceptibility to seizures, indicating that this sex difference is because of an interaction of diazepam with female gerbils (Kaplan & Miezejeski, 1972). In rats, seizure susceptibility and its treatment with BDZs has been shown to be dependent on sex and strain (Brandt, Glien, Potschka, Volk, & Loscher, 2003).

Following buspirone administration, anxiolysis was evident, according to Wall & Messier's (2000) two-factor model, at all but the lowest dose when only total entries were higher. In addition, according to this model, anxiolysis was particularly apparent in males at the top dose; at this dose rearing significantly decreased. However, if less closed arm entries are to be taken as a measure of sedation, as in the factor analysis models of Rodgers and Johnson (1995) in mice and Cruz et al., (1994) in rats, then pure

anxiolysis was evident only at 10mg/kg. The model reported here differs slightly from Varty's validation in female gerbils (Varty et al., 2002) in which there was only pure anxiolysis at 3mg/kg. At higher doses, fewer closed entries led them to believe that there were some drug-related sedation effects taking place (Varty et al., 2002).

It is interesting that only spatiotemporal and not risk-assessment behaviours were affected by buspirone. Risk-assessment measures are reported to be more sensitive to factors related to anxiety, particularly GAD, for which buspirone is prescribed (Blanchard, 1991). However, avoidance of and escape from open entries, indicated by lower numbers of open entries and time spent in them, has been suggested to relate to panic and phobia, for which buspirone is not effective (Cole & Rodgers, 1995; Cheeta et al., 2000; Uchiyama, Toda, Hiranita, Watanabe, & Eyanagi, 2008). It is perhaps also important to bear in mind that in humans buspirone takes up to two weeks to have an anxiolytic effect and so acute tests do not necessarily have good construct and predictive validity for this drug.

The differences in response to buspirone between the sexes indicate that female gerbils might be less sensitive to the anxiolytic effects of buspirone at the lower doses. Interestingly, work in rats has shown differences between male and female rats in serotonergic neuron density in the dorsal and median raphé (Dominguez, Cruz-Morales, Carvalho, Xavier, & Brandao, 2003). This might provide an explanation for the differences in response to 5HT-1A antagonist and partial agonist buspirone

The anxiogenic drugs, caffeine and FG7142, gave the expected profiles. However, even though the overall ethograms were anxiogenic the behavioural profiles differed for each drug. In the case of caffeine, less open-arm exploration (open arm time) and head-dip frequency reflected anxiogenesis, but this was only at the top (30mg/kg) dose. This result is comparable with work in other species of rodent in which only high doses of caffeine cause anxiety and lower doses act as a stimulant (Kulkarni et al., 2007; Bhattacharya, Satyan, & Chakrabarti, 1997). The sex differences in response to caffeine are similar to those seen in humans (Botella & Parra, 2003) with males being more sensitive to the acute anxiogenic effects of caffeine in both species. When compared with females, open entries were less, and, closed entries more in male gerbils. Unsurprisingly, caffeine caused a dose-related increase in seizures in both male and

female gerbils. Caffeine is widely used in seizure research in rodents for its ability to cause seizures (Morgan & Durcan, 1990).

Similarly, FG7142 also had an anxiogenic profile at the top dose (30mg/kg) in both male and female gerbils, which confirmed results in other gerbil EPMs (Varty, Morgan et al., 2002). The decrease in locomotor duration at this dose, although reflective of sedation, might also be a sign of less exploration because of increased anxiety. Interestingly, spatiotemporal measures were affected only by FG7142 in females. The fact that anxiogenesis was reflected in behavioural measures alone and not in spatiotemporal measures, stretch-attend frequency significantly increased in all gerbils, could be likened to GAD type anxiety. Risk-assessment measures such as stretch-attends are reported to be more sensitive to factors related to anxiety, particularly GAD (Blanchard et al., 1990), whereas increased escape from open entries have been suggested as relating to panic (Cole & Rodgers, 1995; Cheeta et al., 2001).

Also, at the top dose, females were more anxious than males in that they entered the open arms less and the closed arms more. Interestingly, in Varty's female gerbil EPM, a similar behavioural profile emerged at 30mg/kg even though in their validation the baseline was altered for anxiogenic drug testing to prevent ceiling effects; lighting levels were altered to give a less aversive baseline (Varty, Morgan et al., 2002).

FG7142 also caused a dose-related increase in seizures in male gerbils, which is not unsurprising since FG7142 injections are often used to induce seizures in epilepsy research (Stanford et al., 1989).

### 5.4.2 Discussion of the effects of anxiolytic and anxiogenic drugs, in the gerbil BWB

The drug profiles in the BWB were more complex than those of the EPM.

The anxiolytic effects of diazepam are in keeping with previously reported findings in rats, mice and gerbils (Holmes, Iles, Mayell, & Rodgers, 2001; Chaouloff et al., 1997; Lapiz & Hogg, 2001; Costall et al., 1989; Crawley & Goodwin, 1980) and those obtained in the EPM. Like the EPM results, the profile at the lowest dose was weakly

anxiolytic as only exploratory behaviours were higher and there was no change in spatiotemporal measures at this dose. The anxiolytic profile was strengthened with increasing dose. Diazepam appeared to alter the spatiotemporal measures in males only, which highlights the need to examine both behaviour and location when studying gerbils in the BWB. It is common in other rodent models to report only spatiotemporal measures; if this had been the case here then the anxiolytic effect of diazepam would have only been reported in male gerbils at the top two doses, thus limiting the sensitivity and usefulness of the model. The lack of sex differences in response to diazepam concurs with those obtained in the gerbil EPM, discussed above, as well as those reported for rodents in other models, such as the rat EPM and the marble burying test (Wilson, Burghardt, Ford, Wilkinson, & Primeaux, 2004; Chadda & Devaud, 2004).

In contrast with results from diazepam administration, there were dose-related sex differences in response to buspirone. In particular, males showed greater immobility and less activity at lower doses than females. Sex differences in response to drugs acting on $5HT_{1A}$ have been reported in other models of anxiety and species of rodent. For example, females were much more sensitive to the effects of $5HT_{1A}$ agonist 8-OH-DPAT than males in the rodent anxiety defence battery of tests (Blanchard et al, 1991). Concurrent with this, only males responded to the anxiolytic effects of buspirone at the lowest dose; exploratory behaviour was higher and immobility and time in the black side were less than vehicle. These findings are similar to those in the gerbil EPM (above) and to those reported previously in male gerbils (Lapiz & Hogg, 2001). In contrast, similar doses in females led to more exploration in the light side of the box; this observation could be interpreted as a mild anxiolytic effect. However, at higher doses of buspirone, exploratory behaviour was lower and immobility higher, probably reflecting sedation; this was similar to reports by Varty and co-workers (2002) about buspirone's effects in their gerbil EPM. Immobility is not uncommon following acute administration of buspirone and is probably because of its alpha-2-adrenoceptor antagonist action, which affects glucocorticoid release. This, in turn, suppresses locomotor activity masking its anxiolytic effects (Lim et al., 2008). In rats, these effects wear off after the first hours following administration (Lim et al., 2008). Perhaps a longer uptake time is required before testing buspirone's effects in this model. On the other-hand, it is possible that testing buspirone at a lower dose in females might have proved more effective, since sedation was also a predominant feature at several of the higher doses tested in Varty et al.'s female gerbil EPM, whereas in Varty's model,

anxiolysis was detected at lower doses (Varty, Morgan et al., 2001). In a similar way to the EPM the differences between males and females in the density of serotonergic pathways in the parts of the brain associated with fear and anxiety, in particular the DR (Bouali et al., 2003; Birzniece et al., 2001; Dominguez et al., 2003), might help to explain this difference in response between the sexes. Taken together, these findings suggest that males appeared to be more sensitive to the effects of this drug in both models. Thus, the BWB appears to successfully detect the effects of the 5HT-1A agonist buspirone in male gerbils, but shows limited utility in female gerbils. These results also highlight the importance of construct and ecological validity, since in humans, buspirone only exerts its clinical effects in relieving anxiety (GAD) after at least two weeks of administration, which would argue the case for chronic, rather than acute, studies in these models having more validity.

With regard to the anxiogenic drugs, the effects of caffeine were somewhat ambiguous. Males showed lower black side duration but at lower doses caffeine appeared to have stimulant effects, increased activity and exploration. By contrast, at the highest dose caffeine produced an anxiogenic behavioural profile (reduced crossing frequency and exploration), although this profile could also be interpreted as sedation. In females, anxiogenic effects were apparent at the lowest dose. In other studies, caffeine appears to have an inverted U-shaped mode of action, in that activity decreases at intermediate doses compared with lower doses but increases again at higher doses (Hascoet & Bourin, 1998). Also, whether anxiety or activity is created is dependent on dose. The dose used here was possibly not high enough to induce anxiety, since, in other species of rodent, for example mice, higher doses than the ones used here are required to create anxiety (Baldwin, 1989; Hascoet & Bourin, 1998; Simmons, 1996). These rather ambiguous findings reflect earlier studies in mice, which have either reported that caffeine had an anxiogenic profile, a stimulant effect, or produced no change in behaviour in the BWB (Hascoet & Bourin, 1998; Hascoet, et al., 2001; Yacoubi et al., 2000). As with buspirone, sex differences were apparent in response to caffeine. Previous research has indicated that a link might exist between caffeine and oestrogen; oestrogen in female mice, but not male mice, blocks the neuroprotective effects of caffeine (Xu, et al., 2006). This might partially explain the sex differences described here.

The dose response profile of FG7142 was not convincingly anxiogenic, as the predominant effect was an increase in exploratory behaviour in the white compartment. Similar baseline effects on FG7142's ability to induce anxiety have been reported in other behavioural paradigms of anxiety (Carey et al., 1992). In males, the higher doses also decreased locomotor activity in the white side. These findings partly concur with previous studies which suggest that FG7142 typically reduces exploration of the aversive white compartment of the BWB (Costall et al., 1989). However, they differ from an earlier study, which suggested that FG7142 did not alter behaviour of male gerbils in the BWB (Lapiz & Hogg, 2001). Previous studies of the effects of FG7142 in male mice in the BWB have been contradictory (Bourin & Hascoet, 2003). Thus, the absence of anxiogenic effects in this case does not necessarily suggest that gerbils cannot be used in the BWB.

Female gerbils' lack of response to FG7142 might be partly explained by a neurosteroid interaction with this drug (Shansky, et al., 2004). In rats, the anxiogenic effects of FG7142 have been shown to be dependent on stage of oestrous. In conditions of low oestrogen, such as oestrous, FG7142 does not cause anxiety. When oestrogen is high, such as during proestrous, FG7142 is an effective anxiogenic agent (Carey, et al., 1992). In this study, stage of oestrous was not tested and female gerbils were selected regardless of their stage in the oestrous cycle, because earlier studies had suggested it did not alter their behaviour in the BWB (Bridges & Starkey, 2004).

The BWB used here is not alone in its inability to detect anxiogenesis caused by caffeine and FG7142; other workers have also found that the BWB is limited in its ability to detect anxiogenic drugs such as caffeine in mice (Bourin & Hascoet 2003; Hascoet & Bourin 1998). It is interesting to note that these results in the BWB are contrary to those of the EPM, where anxiety was evident at the top dose and this predominantly in female gerbils. However, the fact that the BWB model was not very responsive to drugs which are used to treat GAD might suggest that it is modelling some other aspect of anxiety. It has been suggested that altering the light intensity over the light compartment of the BWB might interfere with the expression of GAD related behaviours (Blanchard et al., 2008). It is also interesting to note that in the elevated T-maze FG7142 has been shown to be effective in creating the types of behaviours that reflect GAD, but not the types of behaviours that relate to panic; whereas, caffeine

failed to affect behaviours related to GAD or to panic in the same model (Graeff, Ferreira Netto, & Zangrossi, 1998).

The differences in behavioural profile between male and female gerbils highlights that the response of male and female gerbils to anxiolytic drugs should be accounted for when conducting studies of this type. These differences have been reported in other species too (Blanchard, Shepherd, et al., 1991; Shepherd, Flores, Rodgers, Blanchard, & Caroline Blanchard, 1992). They also suggest that current tests might need some adaptation to be used successfully in female rodents and that the effect of the stage of the oestrous cycle on drug action needs to be taken into account.

## 5.5 EPM AND BWB VALIDATION SUMMARY AND CONCLUSIONS.

Of the two models tested, the EPM, in particular, was bidirectionally sensitive to the effects of anxiolytic and anxiogenic drugs and showed predictive validity in both male and female gerbils in a manner comparable with Varty's female gerbil EPM (Varty, Morgan et al. 2002). Diazepam was anxiolytic in both male and female gerbils on all measures. In contrast, buspirone's anxiolytic profile was limited to spatiotemporal measures, while ethological behaviours differed between the sexes. In response to the anxiogenic drugs, caffeine gave an anxiogenic dose-response profile that reached significance at the top dose, but there were some sex differences, while, in response to FG7142, risk-assessment behaviour increased in all gerbils. In addition females were generally more anxious than males. By contrast, the BWB was more limited in its ability to detect the actions of anxiolytic drugs, and this seemed to be limited to diazepam in both sexes and only buspirone in males. However, the fact that buspirone's anxiolytic effects take a few weeks to become clinically effective in humans is possibly also the case in gerbils. This observation would argue against the validity of acute testing of this type of drug in animal models. Of the two models, the EPM seemed to validate more clearly, indicating that the BWB might need some adjustments before it can be widely used as a model of anxiety.

The differences in drug profile between the two models highlight the fact that the two models might not be modelling the same aspects of anxious behaviour or, at least, that

the EPM might be more sensitive to certain aspects of anxious behaviour and the BWB to others. Studies have shown that the BWB might reflect activity related to avoidance; whereas, the EPM might measure behaviours related to behavioural inhibition and scanning (see section 2.2.3 for a further discussion). These latter types of behaviours have been likened to worry in humans. Thus, the EPM is possibly more related to aspects of GAD; whereas, the BWB might be reflecting avoidance/escape behaviours more like phobia or panic, which are not responsive to the types of drugs used to treat GAD (Blanchard et al., 2008).

Bearing in mind these differences and that, in the odour studies, chronic effects are of interest as well as acute effects, it was decided to proceed with both models. Recognising that there are these differences between the BWB and the EPM might give some indication of whether the two odours have similar or different mechanisms of action if they do prove to be anxiolytic in these models. However, it is also recognised that any interpretation of results in the acutely tested gerbil BWB might be subject to limitation in its current form.

In the next section the use of these models to assess both acute and chronic effects of the two odours, lavender, *Lavandula angustifolia* Mill, and rose, *Rosa damascena* Mill, essential oils will be discussed. Since rose oil odour has also been shown to have anxiolytic effects possibly via a differing mechanism than lavender (see section 1.4.5), it was decided to compare its effects to lavender oil as a second odour study. Additionally, a third study was conducted examining the effects of chronic diazepam in both these models in order to compare the effects of a widely used anxiolytic drug with those of the EO odours. It was also an aim to compare chronic buspirone with the odours' effects and those of diazepam, but unfortunately in the end that was not possible because the project licence holder emigrated and thus any work carried out under this project had to cease consistent with the Animals (Scientific Procedures) Act 1986.

Validation results showed sex differences in both models in response to all of the drugs tested. Additionally, other workers have shown that stage of oestrous might influence the effects of anxiolytic drugs (see previous discussion). Therefore, it was decided to include stage of oestrous of female gerbils as a measure in the odour studies that follow.

Studies have shown that in order to detect anxiolysis it is better to use animals that are more anxious at baseline (Varty et al., 2002). There has been some evidence in this laboratory that individual housing of gerbils is one way achieving this higher anxiety baseline (Starkey, Normington & Bridges, 2007). Therefore, the gerbils in the next study were individually housed prior to and for the duration of the odour studies.

These three studies are presented in the next chapter (chapter 6).

# CHAPTER 6 AN EXAMINATION OF THE EFFECTS OF LAVENDER (*LAVANDULA ANGUSTIFOLIA*) AND ROSE (*ROSA DAMASCENA*) ESSENTIAL OILS AND DIAZEPAM FOLLOWING SHORT AND LONG-TERM ADMINISTRATION IN THE GERBIL ELEVATED PLUS-MAZE AND GERBIL BLACK-WHITE BOX [1]

## 6.1 INTRODUCTION

Following the validation studies of the gerbil EPM and BWB with two widely used anxiolytic drugs, as described in the previous chapter, this chapter describes three studies which were conducted in these models to examine the effects of lavender EO odour (*L. angustifolia* Mill.) on anxiety and to compare its effects with the effects of another reputedly anxiolytic odour, rose (*Rosa damascena* Mill.) essential oil odour, and the commonly used anxiolytic drug diazepam.

Initially two pilot studies, one for each odour, were conducted over one hour to assess any anxiolytic effects prior to conducting the main study (See appendix 2 for odour pilot studies). Results indicated that lavender caused mild anxiolysis and sedation particularly in male gerbils. These gerbils spent less time in the closed arms of the EPM and there were fewer percentage protected head-dips in male gerbils when compared with controls. In the BWB anxiolytic effects were milder and sedation was more prevalent, locomotor activity was less and, while not quite reaching statistical significance, latency black was longer than in controls. It is interesting to note that females appeared more anxious following acute lavender exposure; protected head-dips were higher in female gerbils. However, even though these effects were anxiolytic they were very different to diazepam's and buspirone's effects in both models (see Gerbil validation chapter, chapter 5). Nevertheless, lavender's effects could be compared to diazepam's, which, at the lowest concentration, had an effect on exploration (head-dips increased).

[1] Most of this chapter has been published in the following publications:
Bradley, B. F., Starkey, N. J., Brown, S. L., & Lea, R. W. (2007). Anxiolytic effects of Lavandula angustifolia odour on the Mongolian gerbil elevated plus-maze. J.Ethnopharmacol., 111, 517-525.
Bradley, B. F., Starkey, N. J., Brown, S. L., & Lea, R. W. (2007). The effects of prolonged rose odor inhalation in two animal models of anxiety. Physiol Behav., 92, 931-938.

In contrast to lavender, rose oil increased anxiety. It caused an increase in protected head-dips in both males and females in response to the EPM and a decrease in time spent in the white compartment in the BWB. Interestingly, in other studies acute rose oil has been reported to have anxiolytic effects (Umezu, et al., 1999; de Almeida, et al., 2004).

It has been suggested that the ability of essential oils to relieve anxiety are only transient (Cooke & Ernst, 2000). Therefore, if they have any value at all, it will be only in the short-term. There have been no properly controlled studies to address longer-term effects of lavender or rose essential oil and their effects on anxiety.

There is plenty of published evidence in the peer-reviewed scientific literature that lavender possesses acute anxiolytic properties. However, many of the studies are poorly controlled and provide only evidence that lavender might relieve anxiety temporarily (see section 1.4). Furthermore, there are even fewer studies that provide any evidence for rose oil's ability to relieve anxiety (see section 1.4.5). It has already been mentioned in the previous chapter that a true test of an anxiolytic is not an acute test, as is often conducted, but a chronic study. Often, prescribed anxiolytic drugs only begin to work after 10 days (Sinclair & Nutt, 2007). Thus, the aim here was to compare the effects of lavender EO with rose oil, which might also have anxiolytic properties but via differing neurochemical targets than those of lavender (Umezu, et al., 1999; Buchbauer, et al., 2004). If the anxiolytic effects were caused by pleasant odour effects then anxiolysis would be only expected to be short-term. The aims also included a comparison of these odours with diazepam. Diazepam was chosen because it gave a clear anxiolytic profile in both models of anxiety in the validation studies.

It was also of interest to compare whether any anxiolytic effects caused by the odours gave the same or differing behavioural fingerprints and whether one was more effective in one model or the other. This was the case with the anxiolytics discussed in the previous chapter; acute buspirone and acute diazepam both had anxiolytic effects, but gave differing behavioural fingerprints.

Thus, the study conducted here compared the effects of one-day or two-weeks of lavender or rose odour exposure, or injected diazepam after thirty minutes or two weeks administration.

# 6.2 MATERIALS AND METHODS

## 6.2.1 Animals

At testing all gerbils were mature and aged between 28 and 38 weeks (males 82.8±1.6 g and females 67±1g). In total, 291 gerbils were included in the study: 144 males and 147 females. All procedures were conducted in accordance with the Animals (Scientific Procedures) Act 1986. Ethical approval was obtained from the University's Animal Procedures Committee.

## 6.2.2 Chemicals

Both *Lavandula angustifolia* Mill. and *Rosa damascena* Mill. *(*botanic author Philip Miller, 1768 for both*)* EOs were purchased from Robert Tisserand Ltd. Robert Tisserand identified the species. Organic *Lavandula angustifolia,* obtained from steam distillation of flower heads grown in the foothills of the French Pyrenees, was purchased from Robert Tisserand Ltd. UK. The voucher specimen is stored at Tisserand Ltd. UK (Personal communication: Karl Watson, Tisserand Ltd. UK). Likewise, rose oil was also obtained by steam distillation of the petals of Rosa damascena flowers grown in Bulgaria and purchased from Robert Tisserand Ltd.

EO composition was determined by GC–MS, injection volume 1µL (Column: SGE, BPX5 (non-polar) bonded phase fused silica; 0.22mm i.d.; 25m length; 0.25m film thickness; splitter on, ratio 50:1 Carrier: Helium. Injector 250° C, detector 250° C. Column 50° C, 2 min; 5° C/min to 100° C; 20° C/min to 250° C for 0.5 min. GC/MS: Perkin-Elmer Turbo-Mass) using a modified version of the Adams (1995) method as described by Kim and Lee (2002).

Odour exposure was via an electronic vaporiser and aromastone (Robert Tisserand Ltd.) placed in the animal holding room and the experimental suite, but out of reach of the

animals during behavioural testing. The odour was refreshed three times daily (8:00, 12:00 and 16:00 h) with four drops of the EO to achieve the concentration commonly recommended by aromatherapists (Moss et al., 2003).

Diazepam (1 mg/kg) *(Sigma–Aldrich Fancy Rd., Poole, Dorset, UK)*, was dissolved in distilled water with 5% of Tween-20 and sonicated for 20 min. The dose used in this study was shown to be effective in the EPM studies (see Chapter 5) and in other gerbil validation studies (Varty, Morgan et al., 2002).

## 6.2.3 Procedure

The animals in the control (no odour) group were tested two weeks prior to the lavender oil group to ensure that these animals were not exposed to the EO odour. During the two week break between the control and lavender study the rooms used for the studies were thoroughly cleaned to minimise any odour carry over from the previous group. Again, following the lavender oil study the room was thoroughly cleaned and left empty for a further two weeks. This was to ensure that gerbils in the rose odour group were not exposed to any residual lavender odour.

In all of these odour studies, following behavioural testing, the gerbils were weighed and stage in the oestrous cycle of each female gerbil was determined using the method of Nishino and Totsukawa (1996); with stages II and III combined to make four stages of oestrous rather than five (proestrous, oestrous, metoestrous, dioestrous). After this, gerbils were returned to their home cages. A Kruskall-Wallace analysis of dependent variables from each stage of oestrous in the EPM and the BWB revealed no significant differences in behaviour between each stage of oestrous in each odour condition. Thus, all analyses were conducted regardless of stage of oestrous.

### 6.2.3.1 Lavender odour

After two weeks habituation to handling, the gerbils were moved to the odour room and exposed either to lavender odour (acute lavender: males n = 14, females n = 12; chronic lavender males n = 13, females n = 12) or no-odour control (water only: males n = 13, females n = 13). Daily handling of gerbils continued throughout this time. Gerbils exposed acutely were tested on the mazes the next day after 24h exposure to lavender

odour. Gerbils in the chronic lavender group were exposed daily for 14 days to lavender and tested on day 15.

#### 6.2.3.2 Rose odour

Following this, after 2 weeks habituation to handling, the gerbils in the rose group were moved to the odour room and exposed either to rose odour (acute rose: males n=12, females n=12; chronic rose: males n=12, females n=12) or control (water only: males n=14, females n=13). Daily handling of gerbils continued throughout this time. The same pattern of exposure to the rose oil odour was followed as for the lavender odour groups. Gerbils exposed acutely were tested on the mazes the next day after 24 h exposure to rose odour. Gerbils in the chronic rose group were tested on day 15 following 14 days exposure to rose oil.

#### 6.2.3.3 Diazepam

Diazepam, or vehicle alone, was administered by intraperitoneal injection (1 ml/100 g body weight) either once (vehicle control: males n =19, females n =23, or 1 mg/kg diazepam: males n =11, females n =10), or each morning for the 14 day duration of the study (vehicle control: males n =12, females n =8 or 1 mg/kg diazepam: males n =13, females n =10). In addition, gerbils were weighed and handled daily.

### 6.2.4 Data analysis

As with the validation studies, data from gerbils that had seizures were excluded from the main analysis. Seizure data were analysed separately for association between seizure occurrence and experimental condition.

Much of the data failed to meet the assumptions required for analysis by parametric methods, showing positive or negative skews of greater than 1.96 (Tabachnick & Fidell, 2001). As it was predicted that the chronic administration of lavender would create stronger effects than acute administration, results were analysed using the Jonckheere-Terpstra (J–T) test, which is designed to detect trends for ordered alternatives. This test is more powerful than the Kruskall-Wallis non-parametric ANOVA in this case of sequentially ordered data (Siegel & Castellan, 1988). Where a significant effect was

found ($p < 0.05$) one-tailed Mann–Whitney *U*-tests were used for pair-wise testing of no-odour control versus each odour condition

Diazepam data were analysed pair-wise (Chronic Diazepam vs. Vehicle Control) by Mann-Whitney U-tests. Significance was set at the conventional 0.05 level one tailed.

The J–T test does not perform interaction analyses. To identify any possible sex-based interactions in the data, pre-planned two tailed pair-wise testing of male versus female gerbils for each odour group and control were conducted for each DV. Any behaviour which showed significant differences in any condition between the sexes were analysed separately for the J–T tests and pair-wise comparisons.

## 6.2.5 GC/MS Analysis of EOs

### 6.2.5.1 Lavender oil

The components of the lavender oil used in this study were analysed by GC/MS. Identification of the main peaks was by comparison of the retention times of the peaks with those of known standards and then by confirmation with the NIST GC/MS library and an in-house monoterpene library. The main lavender components were identified as linalyl-acetate (43.98%), linalool (38.47 %), lavandulyl-acetate (4.81 %), ß-myrcene (1.44 %), terpinen-4-ol (1.25 %), ß- terpineol (1.05%), cis-linalool oxide (0.77%), trans-linalool oxide (0.66%) and ocimene (0.55 %) (see Figure 6.1). Lavender EO has previously been characterised (Shellie et al., 2002) and the data in the chromatogram from the present study compare well with the published data.

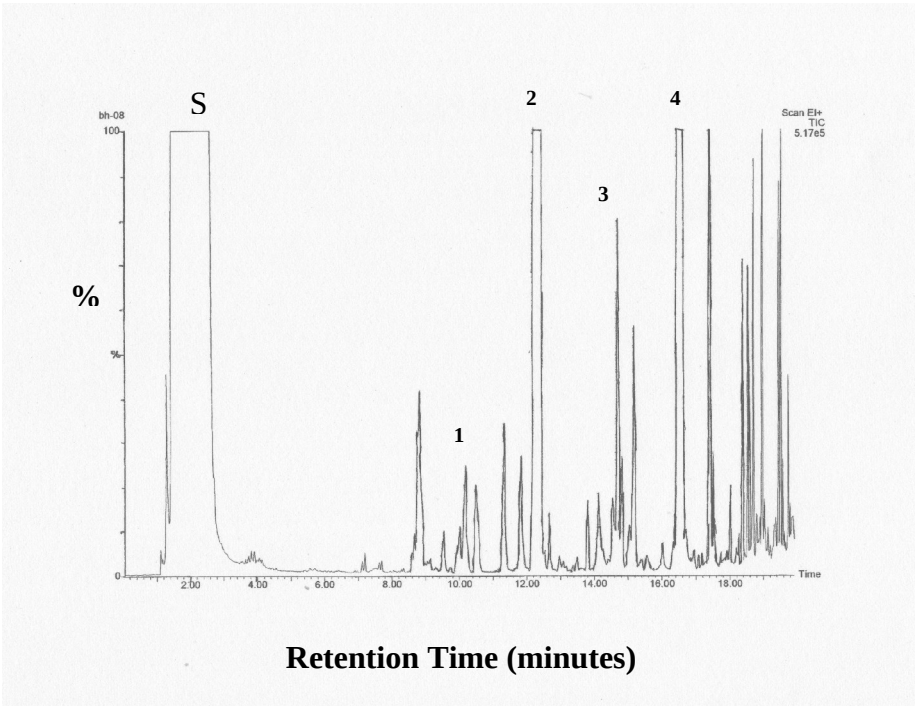



**Figure 6-1 GC/MS profile of *Lavandula angustifolia* Mill. EO.GC/MS conditions: Column: SGE, BPX5 (non polar) bonded phase fused silica. Injector 250$^{O}$ C, Detector 250$^{O}$ C. Column 50$^{O}$ C, 2 minutes; 5$^{O}$ C / minute to 100$^{O}$ C; 20$^{O}$ C/ minute to 250$^{O}$ C for 0.5 minutes. GC/MS: Perkin-Elmer Turbo-Mass. Peaks: S = solvent front, 1 = ocimene, 2 = linalool, 3 = terpinen – 4- ol, 4 = linalyl-acetate.**

### 6.2.5.2 Rose oil

The main components of rose oil were identified as myrcene (3.25%), benzyl alcohol (3.76%), rose oxide (0.57%), phenyl-ethyl alcohol (2.39%), geraniol (17.69%), ß citronellol (36.31%), nerol (8.12%), geranyl-acetate (1.73%) and methyl-eugenol (2.84%). Rose oil has been previously characterised and the data in the chromatogram (see Figure 6.2) compares well with other published data (Ozel, Gogus, & Lewis, 2006; Umezu et al., 2002; Jirovetz et al., 2005).

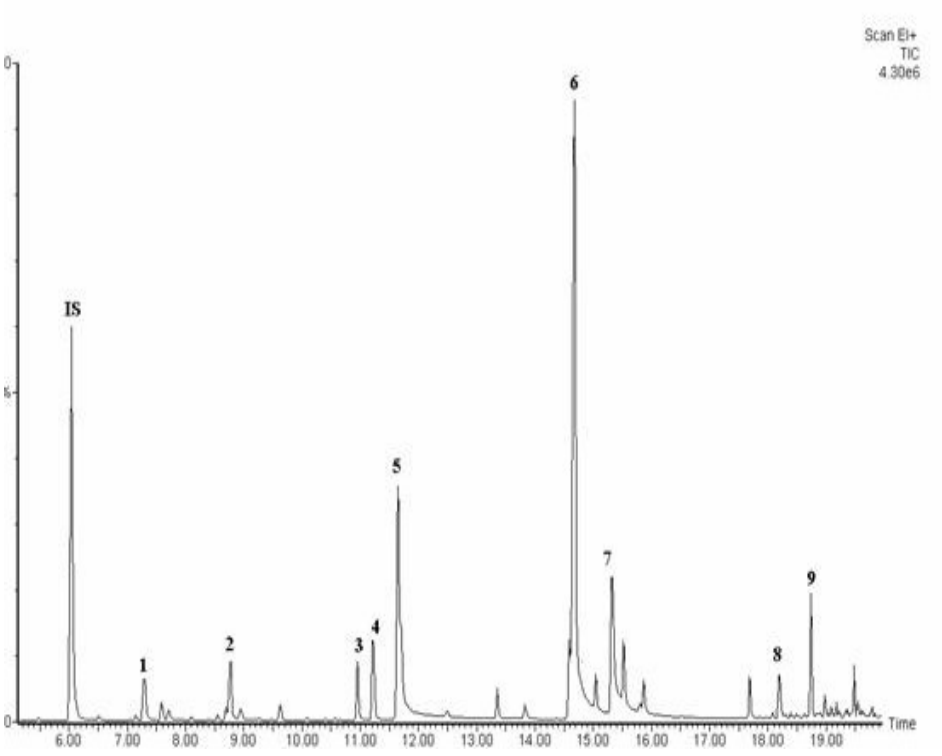



**Figure 6-2 GC/MS profile of *Rosa damascena* Mill. EO.GC/MS conditions: Column: SGE, BPX5 (non polar) bonded phase fused silica. Injector 250$^{O}$C, Detector 250$^{O}$C. Column 50$^{O}$C, 2 minutes; 5$^{O}$C / minute to 100$^{O}$C; 20 $^{O}$C/ minute to 250$^{O}$C for 0.5 minutes. GC/MS: Perkin-Elmer Turbo-Mass. Peaks: IS = internal standard, 1 = myrcene, 2 = benzyl alcohol, 3 = rose oxide, 4 = phenyl ethyl alcohol, 5 = geraniol, 6 = â-citronellol, 7 = nerol, 8 = geranyl acetate, 9 = methyl eugenol,**

# 6.3 RESULTS

## 6.3.1. Exclusions as a result of seizures

In the no-odour control group, two female gerbils had seizures on the EPM and one male in the BWB; their data were excluded from the study. For details of the number of gerbils excluded from the EPM because of seizures see Table 6.1. See Table 6.2 for the BWB seizure data. A chi-squared analysis revealed no significant associations between any odour or diazepam and the number of fits that occurred.

**Table 6.1 The number of gerbils excluded from the elevated plus-maze on the basis of seizures**

| Odour/drug | Number of fits in the elevated plus-maze | | | |
|---|---|---|---|---|
| | Acute | | Chronic | |
| | Male | Female | Male | Female |
| **Lavender** | 1 | 0 | 0 | 1 |
| **Rose** | 0 | 1 | 0 | 2 |
| **Diazepam vehicle** | 0 | 2 | 0 | 2 |
| **Diazepam (1mg/kg)** | 3 | 0 | 1 | 0 |

**Table 6.2 The number of gerbils excluded from the black-white box on the basis of seizures**

| Odour/drug | Number of fits in the black white box | | | |
|---|---|---|---|---|
| | Acute | | Chronic | |
| | Male | Female | Male | Female |
| **Lavender** | 0 | 0 | 0 | 0 |
| **Rose** | 0 | 0 | 0 | 1 |
| **Diazepam vehicle** | 1 | 3 | 0 | 0 |
| **Diazepam (1mg/kg)** | 0 | 0 | 0 | 0 |

## 6.3.2 Lavender odour studies

### 6.3.2.1 Lavender odour results in the EPM

There were significant differences between males (n =12) and females (n =7) in percentage protected head-dip, when exposed to chronic lavender odour (Mann-Whitney U = 39, $p < 0.05$) (see Table 6.3).

Therefore, for this variable the two sexes were analysed separately. In the J-T analysis, only females showed a significantly decreasing trend of protected head-dip ($t= 2.11$, $p < 0.05$). Pair-wise comparisons revealed fewer protected head-dips ($U = 10$, $p < 0.01$) in females when the chronic lavender gerbils were compared to controls.

Prolonged exposure to lavender odour in all gerbils revealed significantly increasing trends for percentage open entries ($t = 1.74$, $p < 0.05$) and head-dip frequency ($t = 5.29$, $p < 0.001$), while there were decreasing trends for closed entries ($t = -1.72$, $p < 0.05$) and stetch-attend frequency ($t = 5.87$, $p < 0.001$).

Pair-wise comparisons of the acute lavender odour-condition compared to control revealed higher head-dip frequency ($U= 35$ $p < 0.001$), and lower stetch-attend ($U= 71$, $p < 0.001$) and percentage protected head-dip ($U = 165$, $p < 0.05$) frequencies. See Table 6.3 for median and inter-quartile range results.

All gerbils (both male and female) exposed to chronic lavender odour compared to control gerbils exhibited a greater percentage of open entries ($U = 166$, $p < 0.05$) and head-dip frequency ($U = 28$, $p < 0.001$); while, their percentage closed entries ($U = 166$, $p < 0.05$) and stetch-attend frequenies ($U = 25$, $p < 0.001$) were less than controls (See Table 6.3).

Comparisons of results of gerbils exposed to acute lavender with those exposed to chronic lavender revealed less stetch-attends in the chronic condition than in the acute condition ($U = 210$, $p < 0.05$) (Table 6.3).

**Table 6.3 Effects of prolonged (two weeks) vs. 24 hour lavender odour or no-odour control on male and female gerbil behaviour on the elevated plus-maze.**

| Dependent variable | No odour | Acute lavender | Chronic lavender |
|---|---|---|---|
| **Total entry** | 21.00**,25.50,** 31.00 | 23.00**, 27.50,** 33.00 | 22.00**, 26.00*,** 28.00 |
| **% Open entry** | 45.95, **50.42,** 58.00 | 45.45**, 52.33,** 60.86 | 50.00**, 58.33,** 63.33 |
| **% Closed entry** | 44.22, **50.00,** 60.51 | 40.90**, 49.13,** 55.88 | 40.00**, 46.66 *,** 52.38 |
| **Open duration** | 94.94, **130.03,** 159.38 | 114.00**, 140.37,** 170.2 | 136.80**, 150.18,** 165.72 |
| **Closed duration** | 82.95, **101.78,** 108.35 | 75.10**, 105.65**, 124.85 | 83.10**, 96.78,** 107.56 |
| **Immobile duration** | 4.13, **11.47,** 22.48 | 0.00**, 0.00,** 3.79 | 0.00**, 1.30,** 3.28 |
| **Locomotor duration** | 130.74**, 141.53,** 162.89 | 135.66**, 142.57,** 152.50 | 151.50**, 160.87,** 171.91 |
| **Head-dip frequency** | 13.00**, 16.39, 23.50** | 33.00**, 42.00***,** 52.00 | 33.00**, 46.00***,** 56.00 |
| **Stretch-attend frequency** | 23.00, **27.00,** 30.68 | 15.00**, 18.00***,** 24.00 | 11.00, **16.00***,** 18.00 |
| **Male % Protected Head-dip** | 8.51**, 24.24,** 33.33 | 4.69**, 11.79,** 13.16 | 10.2**, 13.16,** 17.14 |
| **Female % Protected Head-dip** | 11.11**, 31.03,** 45.83 | 4.46**, 9.29,** 20.45 | 4.16**, 5.52**,** 10.17 |

Gerbils were exposed to lavender odour 4 drops three times per day in an 'aroma stream' vaporiser for 24 hours per day, one day for acute and 14 days for prolonged exposure. (See text for further details). Data are presented as ($25^{th}$ percentile) **median** ($75^{th}$ percentile). * Levels of significance Mann Whitney U one tailed: * $p < 0.05$, **$p< 0.01$, ***$p< 0.001$. Sample sizes: No Odour n = 24; Acute Lavender n = 24; Chronic Lavender n = 19. Male gerbils: control n = 13; acute lavender n = 12; chronic lavender n = 12.Female gerbils: control n = 11; acute lavender n = 12; chronic lavender n = 7.

#### 6.3.2.2 Lavender odour results in the BWB

There was a significant difference between males (n= 12) and females (n = 13) in the no-odour control condition in the time spent in the white compartment. Females spent longer in the compartment than males ($U = 32$, $p < 0.05$). As a result, time spent in the white compartment was examined separately for both males and females in the J-T trend-test and no significant trends were identified across the control and treatment groups.

Lavender odour caused an increasing trend in latency black (J-T = 3.04, $p < 0.05$) and immobile duration (J-T = 2.08, $p < 0.05$). Pair-wise comparisons of acute lavender versus control indicate that latency black ($U = 231.5$, $p < 0.05$) was significantly longer in the acute condition than controls and that it increased even more after chronic exposure compared to controls ($U = 166.5$, $p < 0.01$). However, prolonged exposure to lavender odour also caused significantly more sedation; after two weeks exposure immobile duration significantly increased ($U = 250$, $p < 0.05$). For median and inter-quartile range results see, Table 6.4.

**Table 6.4 The effects of prolonged (two weeks) vs. 24 hour lavender odour or no-odour control on gerbil behaviour on the black-white box**.

| Dependent variable | Odour group | Median and inter-quartile ranges | | |
|---|---|---|---|---|
| | | **All gerbils** | **Males** | **Females** |
| **Latency black** | **control** | 1.21, **1.49,** 8.41 | 1.35, **1.87,** 5.63 | 1.21, **1.49**, 8.84 |
| | **acute lavender** | 1.54, **3.02*,** 8.90 | 1.81, **3.41,** 8.90 | 1.48, **2.45,** 9.75 |
| | **Chronic lavender** | 2.47, **6.65*,** 12.24 | 2.80, **6.65,** 8.52 | 2.36, **5.55,** 16.12 |
| **Crossing frequency** | **control** | 30.00, **35.00,** 38.00 | 31.00, **34.50**, 39.00 | 27.00, **35.00,** 38.00 |
| | **acute lavender** | 32.00, **35.50,** 40.00 | 32.00, **36.00,** 44.00 | 31.50, **34.50,** 39.50 |
| | **chronic lavender** | 29.00, **34.00,** 36.00 | 33.00, **36.00,** 36.00 | 28.00, **31.00,** 34.50 |
| **% duration white** | **control** | 48.15, **52.03,** 55.40 | 46.75, **48.21,** 52.29 | 52.03, **54.47,** 55.95 |
| | **acute lavender** | 48.69, **52.79**, 56.25 | 48.99, **52.81,** 56.11 | 47.10, **52.25,** 59.63 |
| | **chronic lavender** | 49.47, **54.36,** 60.03 | 47.55, **53.59,** 58.73 | 51.54, **59.07,** 64.65 |
| **% duration black** | **control** | 35.05, **39.62,** 41.66 | 38.62, **40.58,** 42.17 | 34.62, **35.76,** 39.80 |
| | **acute lavender** | 34.32, **37.23,** 40.82 | 34.32, **37.23,** 40.42 | 33.72, **37.84,** 40.93 |
| | **chronic lavender** | 31.46, **38.43,** 43.38 | 33.00, **39.56,** 43.38 | 29.03, **35.97,** 41.59 |
| **Locomotor duration** | **control** | 62.60, **72.30,** 77.02 | 54.85, **67.07,** 73.45 | 67.63, **73.79,** 81.49 |
| | **acute lavender** | 64.16, **72.10,** 78.00 | 62.12, **71.64,** 77.48 | 65.93, **72.10,** 89.02 |
| | **chronic lavender** | 62.58, **70.14,** 75.29 | 62.58, **69.74,** 73.27 | 62.32, **71.86,** 87.22 |
| **Exploration frequency white** | **control** | 94.00,**106.00,**111.0 | 101.50,**105.50,**108.00 | 92.00, **106.00,** 116.00 |
| | **acute lavender** | 84.00,**98.00,** 104.00 | 86.00, **95.00,** 103.00 | 77.50, **102.50,** 107.00 |
| | **chronic lavender** | 87.00,**99.00,** 115.00 | 82.00, **99.00,** 107.00 | 88.50, **114.50**, 139.50 |
| **Exploration Duration white** | **control** | 93.90,**106.02,**114.2 | 98.08, **108.84,** 114.49 | 90.59, **103.73**, 108.47 |
| | **acute lavender** | 93.28,**106.30,**114.5 | 96.64, **108.11,** 114.85 | 86.60, **102.87**, 112.73 |
| | **chronic lavender** | **92.52,103.56,114.66** | **96.84, 103.56, 107.45** | **89.82, 103.89, 120.50** |
| **Immobile duration** | **Control** | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 | 0.00, **0.00**, 0.00 |
| | **acute lavender** | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 | 0.00, **0.00**, 0.74 |
| | **chronic lavender** | 0.00, **0.00*,** 0.99 | 0.00, **0.00,** 0.99 | 1.21, **1.49**, 8.84 |

Gerbils were exposed to lavender odour 4 drops three times per day in an 'aroma stream' vaporiser for 24 hours per day, one day for acute and 14 days for prolonged exposure. (See text for further details). Data are presented as ($25^{th}$ percentile) **median** ($75^{th}$ percentile). * Levels of significance Mann Whitney U one tailed: * $p < 0.05$, **$p< 0.01$, ***$p< 0.001$.Sample sizes: No Odour n = 25; Acute Lavender n = 26; Chronic Lavender n = 25.Males No Odour n = 12; Acute Lavender n = 14; Chronic Lavender n = 13. Females No Odour n = 13; Acute Lavender n = 12; Chronic Lavender n = 1

## 6.3.3 Rose odour

### 6.3.3.1 Rose odour results in the EPM

A summary of the behavioural effects of rose oil are presented in Table 6.4. In the acute rose odour condition there were significant differences between males and females in percentage open duration ($U = 25$, $p < 0.05$), which was significantly higher in males compared with females. In contrast, percentage protected head-dips ($U = 30$, $p < 0.05$) were significantly higher in females than males. Male and female data for these behaviours were examined separately in the J-T test; no significant trends were identified across the control and treatment groups for either sex (see Table 6.5 for medians and inter-quartile ranges). In the chronic rose condition, there were no significant differences between males and females.

For the remaining variables there were increasing trends in total entries ($J\text{-}T = 2.24$, $p < 0.05$), rear frequency ($J\text{-}T = 2.42$, $p < 0.05$) and head-dip frequency ($J\text{-}T = 5.83$, $p < 0.001$), and decreasing trends in immobile duration ($J\text{-}T = 4.25$, $p < 0.001$) and stetch-attend frequency ($J\text{-}T = 2.35$, $p < 0.01$). These variables were analysed pair-wise for each odour condition versus the no-odour condition.

Acute exposure to rose oil led to significant higher rear frequency ($U = 178.5$, $p < 0.05$) and total head-dip frequency ($U = 100$, $p < 0.001$) accompanied by a significantly lower immobile duration ($U = 88$, $p < 0.001$) compared to the no-odour condition.

Fourteen days exposure to rose oil odour resulted in significantly more total entries ($U = 124.5$, $p < 0.05$), higher rear frequency ($U = 121$, $p < 0.05$), more total head-dips ($U = 13$, $p < 0.001$) and a significantly lower immobile duration ($U = 60$, $p < 0.001$) compared to controls.

Comparing acute versus chronic rose odour to look for true trends, there was a significantly increasing trend in head-dip frequency ($U = 129.5$, $p < 0.05$) following chronic exposure.

**Table 6.5 The effects of acute and chronic rose oil odour exposure on male and female gerbil behaviour on the elevated plus-maze.**

| Dependent variable | Odour/drug | Median and inter-quartile ranges | | |
|---|---|---|---|---|
| | | All Gerbils | Male | Female |
| Total entries | control | 22.00,**26.00**,31.00 | 19.00, **25.00,** 29.00 | 24.00, **27.00,** 32.00 |
| | acute rose | 25.00,**29.50,**33.00 | 25.00, **27.50,** 30.00 | 26.00, **30.00,** 33.00 |
| | chronic rose | 26.00, **31.5**, 36.00 | 26.00, **30.00,** 34.00 | 25.50, **29.00,** 35.00 |
| % Open entry | control | 45.95,**50.00**, 55.77 | 41.38, **50.00,** 50.00 | 48.38, **52.94,** 55.78 |
| | acute rose | 42.5**, 54.92** ,63.63 | 53.33, **56.85,** 62.07 | 38.89, **42.50,** 46.67 |
| | chronic rose | 44.44**, 52.66,** 55.88 | 42.86,**48.98,**55.56 | 45.19, **53.31,** 57.94 |
| % Closed entry | control | 44.80**, 50.0,** 60.51 | 48.39, **50.00,** 53.85 | 44.22, **47.06,** 51.62 |
| | acute rose | 40.62, **50.00,**65.05 | 37.93, **43.15,** 46.67 | 53.33, **57.50,** 61.11 |
| | chronic rose | 44.44, **51.0**, 57.14 | 44.44, **51.03,** 57.14 | 42.06, **46.69,** 54.80 |
| % Open duration | control | 31.65, **43.34,** 53.13 | 33.02, **43.80,** 52.80 | 33.52, **44.13,** 53.12 |
| | acute rose | 28.68, **41.77,** 51.62 | 41.36, **43.36,** 51.61 | 21.85, **26.09,** 30.13 |
| | chronic rose | 31.87, **40.49,** 52.68 | 36.62, **41.70,** 52.74 | 36.89, **43.46,** 47.75 |
| % Closed duration | control | 28.51, **34.4**4, 36.11 | 10.78, **32.58,** 35.33 | 28.72, **32.84,** 35.93 |
| | acute rose | 28.10, **37.85,** 47.77 | 28.10, **32.31,** 35.66 | 45.52, **47.46,** 48.46 |
| | chronic rose | 31.38**, 35.06,** 38.49 | 27.11, **33.44,** 38.21 | 31.68, **34.03,** 40.14 |
| Rear frequency | control | 17.00, **23.00**, 27.00 | 6.00, **24.00,** 27.00 | 17.50, **21.00,** 25.00 |
| | acute rose | 21.51**, 27.50**, 37.00 | 22.00, **25.00,** 29.00 | 29.00, **36.00,** 42.00 |
| | chronic rose | 23.00**, 29.00**, 32.00 | 21.00, **29.00,** 32.00 | 22.50, **25.50,** 32.50 |
| Immobile duration | control | 4.13, **11.18**, 19.24 | 1.20, **12.97,** 28.21 | 1.20, **12.97,** 28.21 |
| | acute rose | 0.00, **0.00,** 3.41 | 0.00, **0.00,** 4.78 | 0.00, **0.00,** 4.78 |
| | chronic rose | 0.00, **0.00,** 6.04 | 0.00, **0.00,** 0.66 | 0.00, **0.00,** 0.66 |
| Head-dip frequency | control | 13.00, **16.78,** 23.50 | 6.00, **13.00,** 15.00 | 6.00, **13.00,** 15.00 |
| | acute rose | 23.00**, 29.00,** 43.02 | 29.00, **31.50,** 46.00 | 29.00, **31.50,** 46.00 |
| | chronic rose | 38.00, **44.50,** 52.19 | 39.00, **45.00,** 56.00 | 39.00, **45.00,** 56.00 |
| Stretch-attend frequency | control | 24.50, **27.00,** 30.68 | 19.00, **27.00,** 30.00 | 19.00, **27.00,** 30.00 |
| | acute rose | 20.00, **24.50,** 28.01 | 19.00, **20.00,** 30.00 | 19.00, **20.00,** 30.00 |
| | chronic rose | 19.00, **22.50,** 25.00 | 22.00, **25.00,** 28.00 | 22.00, **25.00,** 28.00 |
| % Protected Head-dip | control | 9.02, **24.62,** 40.03 | 8.50, **24.24,** 33.33 | 9.40, **26.62,** 43.50 |
| | acute rose | 8.51, **14.58,** 22.18 | 6.65, **9.09,** 15.28 | 15.13, **25.00**, 34.96 |
| | chronic rose | 11.54, **14.61,** 23.40 | 10.95, **17.84,** 25.71 | 13.70, **16.24,** 22.10 |

J-T statistic is based on Monte Carlo assumptions using 10000 sampled tables with starting seed 2000000. Sample size: All gerbils control n= 25, acute rose odour n = 22, chronic rose odour n = 25. Males: control n = 14, acute rose odour n = 12, chronic rose odour n = 12. Females: control n = 11, acute rose odour n = 11, chronic rose odour n =10.

* Levels of significance one tailed: * p < 0.05, **p< 0.01, ***p< 0.001. J-T results all gerbils [i], male [ii;] female [iii]

#### 6.3.3.2 Rose odour results in the BWB

A comparison of males and females for each odour group revealed no significant difference between the sexes for any behaviour in the BWB. Thus, data were analysed for the group as a whole, irrespective of sex, and are summarised in Table 6.6.

Prolonged exposure to rose oil led to significantly increasing trends in crossing frequency (J-T = 2.22, $p < 0.05$), latency black (J-T = 2.77, $p < 0.01$), percentage duration white (J-T = 3.43, $p < 0.001$), exploration frequency white (J-T = 4.03, $p < 0.001$) and locomotor duration (J-T = 2.78, $p < 0.001$), while there were significantly decreasing trends in percentage black duration (J-T = -3.08, $p < 0.05$).

Pair-wise comparisons were conducted when the J-T test revealed a significant trend. Short-term exposure to rose oil odour resulted in a significantly longer latency to enter the black compartment (U = 182, $p < 0.01$), percentage white duration (U = 168, $p < 0.01$) and a higher exploratory frequency in the white area (U = 100, $p < 0.001$) compared to the no-odour controls. Percentage time spent in the black compartment (U = 160, $p < 0.01$) was significantly less than controls.

Similarly, comparisons of no odour versus chronic exposure to rose oil odour for these variables revealed a higher crossing frequency between the compartments (U = 167, $p < 0.01$), a longer latency to enter black compartment (U = 179, $p < 0.05$) and a longer percentage time spent in the white area (U = 149, $p < 0.01$), more exploratory frequency white (U = 99, $p <0.001$) and higher locomotor duration (U = 154, $p < 0.001$) were also apparent. In addition, the percentage time spent in the black compartment (U = 178, $p < 0.05$) was significantly lower. Furthermore, a comparison of acute versus chronic rose odour exposure revealed a significantly longer time spent in locomotor duration (U = 185, $p < 0.05$).

**Table 6.6 The effects of acute and chronic exposure to rose oil odour on gerbil behaviour in the black-white box.**

| Dependent variable | Odour group | Median and inter-quartile ranges |
|---|---|---|
| **Crossing frequency** | control | 29.00, **34.00,** 38.00 |
| | acute rose | 30.00, **34.50,** 41.00 |
| | chronic rose | 33.00, **40.00,** 44.00 |
| **Latency black** | control | 1.21,**1.49,**8.41 |
| | acute rose | 1.84, **5.73,**12.58 |
| | chronic rose | 1.95, **5.05,** 14.55 |
| **% Duration white** | control | 47.08, **51.82,** 55.40 |
| | acute rose | 53.29, **58.86,** 61.21 |
| | chronic rose | 53.36, **60.80,** 66.42 |
| **% Duration black** | control | 35.05, **39.71,** 41.82 |
| | acute rose | 30.17, **32.33,** 38.01 |
| | chronic rose | 27.64, **31.51,** 38.65 |
| **Exploratory Frequency white** | control | 93.00, **105.00,** 111.00 |
| | acute rose | 115.50, **123.00,** 136.00 |
| | chronic rose | 111.50, **125.00,** 143.50 |
| **Exploratory Duration white** | control | 91.53, **105.00,** 114.42 |
| | acute rose | 105.85, **116.60,** 122.48 |
| | chronic rose | 91.12, **110.22,** 118.84 |
| **Locomotor duration** | control | 58.63, **71.70,** 77.02 |
| | acute rose | 63.17, **72.66,** 78.95 |
| | chronic rose | 68.34, **82.56,** 89.76 |
| **Immobile duration** | control | 0.00, **0.00,** 0.00 |
| | acute rose | 0.00, **0.00,** 0.00 |
| | chronic rose | 0.00, **0.00,** 0.00 |

Sample size: control n= 26, acute rose odour n = 24, chronic rose odour n = 24.

* Levels of significance one tailed: * $p < 0.05$, **$p< 0.01$, ***$p< 0.001$

## 6.3.4 Diazepam (i.p)

### 6.3.4.1: Diazepam EPM

Data are summarised in Table 6.7 below. There were no sex differences following acute treatment. Overall, acute treatment with diazepam led to significantly more total entries ($U = 118$, $p < 0.05$), percentage open entries ($U = 89.5$, $p < 0.05$) and total head-dips ($U = 53.5$, $p < 0.001$) and a longer open duration ($U = 65$, $p < 0.01$) compared to vehicle-treated controls. Accompanying this, percentage closed entries ($U = 104$, $p < 0.01$) and stretch-attends ($U = 131.5$, $p < 0.05$) were significantly less and closed duration shorter ($U = 98$, $p < 0.01$) than in vehicle treated gerbils (See Table 6.7).

Pair-wise comparisons between males and females in the chronic diazepam and chronic vehicle control groups revealed a significantly higher stetch-attend frequency in the chronic vehicle-control condition in females compared to males ($U = 1.5$, $p < 0.01$) see Table 6.5. In addition, females treated with chronic diazepam showed significantly fewer stretch-attends than males ($U = 34$, $p < 0.05$).

Pair-wise comparisons of vehicle control and diazepam for each sex for these variables revealed that in chronically-treated female gerbils the frequency of stretch-attending was significantly less ($U = 14.5$, $p < 0.01$) when compared to same sex vehicle-treated controls. There were no significant differences in males.

In all gerbils, percentage open entries ($U = 122.5$, $p < 0.05$), open duration ($U = 130$, $p < 0.05$) and head-dip frequency ($U = 66.5$, $p < 0.001$) were significantly higher when treated chronically with diazepam compared with vehicle control. In contrast, immobile duration was significantly lower ($U = 111$, $p < 0.01$) (See Table 6.7).

**Table 6.7 The effects of acute (30 minute) diazepam (1mg/kg), versus acute saline vehicle (i.p), and chronic (two weeks) diazepam (1mg/kg), versus saline vehicle (daily (i.p.) injection, on gerbil behaviours on the elevated plus-maze. Table of median and inter-quartile ranges.**

| Dependent variable | Acute vehicle control | Acute diazepam | Chronic vehicle control | Chronic diazepam |
|---|---|---|---|---|
| **Total entries** | 17.09,**28.50** 35.00 | 47.00, **50.50*,**53.00 | 24.00, **28.00,** 33.00 | 29.00, **37.00***,42.00 |
| **% open entries** | 36.36, **44.44,**56.52 | 50.00, **59.55**,65.96 | 45.74, **54.67,** 61.65 | 45.94, **60.64,** 71.43 |
| **%closed entries** | 40.00, **52.77,** 60.00 | 34.04, **40.45,**50.00 | 38.34, **45.33,** 54.26 | 28.57, **39.36,** 54.05 |
| **Open duration** | 52.79, **96.25,** 141.20 | 167.76,**181.91****207.95 | 115.56,**144.36,**177.40 | 150.61, **171.55*,** 199.80 |
| **Closed duration** | 70.10,**96.66,**110.46 | 41.93,**55.90**,**,**69.59 | 86.35,**100.11,**132.47 | 34.24,**55.73,**81.49 |
| **Rear Frequency** | 2.00,**8.50,**16.00 | 9.00,**10.50*,** 11.00 | 24.50, **26.50,** 28.00 | 10.00,**22.00,**30.00 |
| **Locomotor duration** | 56.79, **105.14,**125.26 | 90.34,**102.28,**112.28 | 141.66,**146.38**, 154.92 | 135.35, **155.58,**165.42 |
| **Locomotor duration males** | 33.40,**104.12,**115.43 | 15.37**, 46.31,** 108.46 | 140.69,**146.07,** 153.69 | 135.69,**153.82,**165.28 |
| **Locomotor duration females** | 73.45,**96.46,** 108.02 | 47.79, **82.86,**95.28 | 115.82,**145.46,** 151.98 | 162.90,**170.75*,**181.40 |
| **Immobile duration** | 3.58, **20.42,** 48.88 | 0.00, **5.25,*****13.90 | 2.61, **8.35,** 17.08 | 0.00, **0.00**,** 11.36 |
| **Head-dip Frequency** | 13.00, **27.00,** 43.77 | 51.00, **59.50***** **,** 86.00 | 25.50, **29.50,**45.50 | 43.00, **51.00***,73.00 |
| **Stretch-attend frequency (males)** | 1.00, **3.00,** 4.00 | 0.00, **2.00,** 3.00 | 8.00,**15.00,**17.00 | 15.00, **18.00,** 22.00 |
| **Stretch-attend frequency (females)** | 1.50,**5.00,**12.00 | 1.00,**2.00,**5.00 | 26.00, **27.50,** 29.00 | 8.50,**13.50*,** 22.00 |

Acute vehicle n = 40, acute diazepam n= 18.Chronic vehicle control n= 20, chronic diazepam n= 22. Males acute vehicle n= 19, acute diazepam n = 8; chronic vehicle control n = 12, chronic diazepam n = 12. Females acute vehicle control n= 23, acute diazepam n = 10; chronic vehicle control n = 8, chronic diazepam n= 10. * Levels of significance one tailed: * $p < 0.05$, **$p< 0.01$, ***$p< 0.001$.

### 6.3.4.2 Diazepam BWB

Data are summarised in Table 6.8 below. A comparison of male and female gerbils in the acute diazepam group revealed that males spent significantly less time in the black compartment than females (percent duration black $U = 26$, $p < 0.05$). In addition, males spent significantly more time exploring the white side compared to females (exploratory frequency white $U = 26.5$, $p < 0.05$) and more time moving around in the box than females (locomotor duration $U = 15$, $p < 0.01$). Thus, analyses of these variables were conducted separately for each sex.

When compared with male vehicle controls, male gerbils treated acutely with diazepam spent significantly less time in the black compartment (percent duration black $U = 35$, $p < 0.01$), and significantly more time exploring the white compartment (exploration frequency white $U = 31$, $p < 0.01$) than vehicle-treated male gerbils, whereas, female gerbils spent less time than vehicle controls moving around the BWB ($U = 44$, $p < 0.01$).

Acute treatment with diazepam also led to some non sex-specific effects, including significantly higher frequency of crossing between compartments ($U = 204.5$, $p < 0.001$), longer duration of exploratory behaviour in the white compartment ($U = 233$, $p < 0.01$) and shorter immobile duration ($U = 285$, $p < 0.05$) compared to vehicle-treated controls. These data are summarised in Table 6.8 below.

The chronic diazepam condition analysis revealed that females explored the BWB significantly more than male gerbils (crossing frequency $U = 29.5$, $p < 0.05$). Therefore, subsequent analyses involving this measure were conducted separately for males and females. Follow-up tests revealed that frequency of crossing between compartments was significantly higher in male gerbils after two weeks of diazepam administration compared with same sex vehicle controls ($U = 11.5$, $p<0.001$). Similarly, in females, crossing frequency was significantly higher ($U = 0$, $p < 0.001$) compared with same sex vehicle controls (See Table 6.8 below).

With regard to non-sex specific effects, chronic treatment with diazepam led to a significantly higher frequency of exploratory behaviour in the white compartment compared to vehicle-treated gerbils ($U=115$, $p < 0.01$) (See Table 6.8).

**Table 6.8 The effects of acute (30 minute) diazepam (1mg/kg) versus acute saline vehicle i.p, and chronic (two weeks) diazepam (1mg/kg) versus saline vehicle (daily i.p. injection) on gerbil behaviours on the black-white box. Median and inter-quartile ranges.**

| Dependent variable | Acute Vehicle | Acute Diazepam | Chronic Vehicle | Chronic Diazepam |
|---|---|---|---|---|
| **Crossing frequency (All gerbils)** | 37.00**,43.50,** 51.00 | 48.00**,56.00***,** 68.00 | 34.50**, 39.00, 41.**00 | 50.00**, 53.00,** 58.00 |
| **Crossing frequency (Males)** | 38.00, **42.50,** 50.00 | 49.00, **58.00,** 66.00 | 34.00, **38.50,** 43.00 | 46.00, **50.00***,** 53.00 |
| **Crossing frequency (Females)** | 35.00, **46.50,** 54.00 | 47.00, **54.50,** 73.00 | 35.00, **39.00,** 40.00 | 53.00, **57.00***,** 59.00 |
| **Latency black** | 4.56, **8.24,** 22.08 | 4.66, **6.75,** 13.46 | 1.79, **5.85,** 9.72 | 2.03, **3.85,** 7.17 |
| **% White entries** | 48.00, **52.06,** 54.55 | 46.81, **51.14,** 54.72 | 44.44, **46.29,** 48.27 | 43.00, **45.28,** 47.11 |
| **% Black entries** | 45.45, **47.94,** 52.00 | 45.28, **48.86,** 53.19 | 51.73, **53.71,** 55.56 | 52.89, **54.72,** 57.00 |
| **% Duration white** | 41.13, **46.82,** 57.89 | 44.63, **47.22,** 52.73 | 43.85, **47.42,** 53.32 | 40.23, **46.68,** 49.83 |
| **% Duration black** | 32.4, **39.98,** 44.78 | 31.45, **33.63,** 36.37 | 36.03, **42.03,** 46.36 | 36.53, **41.65,** 44.76 |
| **% Duration black (Males)** | 36.67, **40.60,** 46.15 | 29.58, **32.22**,** 33.86 | 37.11, **42.03,** 48.19 | 35.48, **40.71,** 42.71 |
| **% Duration black (Females)** | 30.43, **35.81,** 42.65 | 33.63, **36.37,** 44.14 | 36.03, **42.30,** 46.36 | 41.65, **43.26,** 46.78 |
| **Exploratory Frequency White** | 56.00, **73.50,** 84.00 | 80.00, **94.00,** 104.00 | 99.00, **110.00,** 126.50 | 125.50, **138.00**,** 152.50 |
| **Exploratory Frequency White (Males)** | 56.50, **74.00,** 83.00 | 87.00, **101.00**,** 109.50 | 91.50, **120.00,** 133.00 | 123.00, **138.00,** 153.00 |
| **Exploratory Frequency White (Females)** | 57.50, **67.00**, 86.00 | 76.00, **86.00,** 98.00 | 105.00, **108.00,** 110.00 | 128.00, **138.00,** 152.00 |
| **Exploratory Duration White** | 41.06, **50.42,** 57.85 | 53.72, **61.43***,** 73.64 | 80.07, **91.06,** 105.57 | 88.13, **95.31,** 102.98 |
| **Locomotor Duration** | 56.10, **64.25,** 73.30 | 52.33, **62.07,** 64.82 | 61.28, **71.09,** 83.14 | 59.53, **68.82,** 73.74 |
| **Locomotor Duration (Males)** | 56.76, **66.27,** 72.14 | 62.74, **63.97,** 69.76 | 55.44, **71.95,** 79.12 | 65.29, **69.91,** 76.16 |
| **Locomotor Duration (Females)** | 56.92**, 65.16,** 79.99 | 45.88**, 52.44***,** 55.82 | 66.11**, 70.46,** 85.81 | 56.74**, 59.93,** 68.82 |
| **Immobile Duration** | 0.00, **2.25,** 7.92 | 0.00, **0.00***,** 0.38 | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 |

Sample size: All gerbils, acute vehicle control n = 42, acute diazepam n= 21; chronic vehicle control n= 20, chronic diazepam n= 23. Males, acute vehicle N = 19, acute diazepam n = 11; chronic vehicle control n =12, Chronic diazepam n= 13. Females acute vehicle n = 23, diazepam n = 13; Chronic vehicle control n =10, Chronic diazepam n= 10 * Levels of significance one tailed: * $p < 0.05$, **$p< 0.01$, ***$p< 0.001$.

### 6.3.5 Summary of results.

GC/MS profiles of both oils indicate that the oils used in these studies compare well with those used in other studies and meet the recommended and required standards for *L. angustifolia* and *R. damascena* oils.

Results indicate that prolonged exposure to either lavender or rose oil odour has anxiolytic effects in the models tested here and these profiles differed for each oil and both were unaffected by stage of oestrous.

Lavender

In the EPM, acute exposure to lavender odour caused mild anxiolysis in both male and female gerbils. This is indicated by a higher frequency of exploratory behaviour (total head-dips) and less risk assessment behaviour (protected head-dipping and stetch-attending). In the BWB effects were less marked; latency to enter the aversive black compartment was longer in both acute and chronic (two-week exposure) conditions. Lavender also appeared to cause mild sedation after prolonged exposure in the BWB. An inspection of the data revealed that this immobility was very slight and that the immobile duration median remained at zero. Chronic exposure (2 weeks) to lavender odour in the EPM potentiated the anxiolytic effects, percentage open entries and head-dip frequency were higher, while closed entry frequency, stretch-attend frequency and percentage protected head-dips (in females) were less than no-odour controls. Fewer closed entries indicate that lavender odour caused slight sedation in the gerbils. However, relatively more open-entries and head-dips in the chronic lavender condition and fewer stretch-attends in both acute and chronic groups lend support to lavender having genuine anxiolytic effects, particularly in this model, and not simply locomotor effects.

Rose

Acute rose odour had an anxiolytic effect on only ethological measures in the EPM and caused more exploration around the EPM and less freezing behaviour. However, traditional measures of anxiolysis, such as the frequency and duration of open entries and the duration of closed entries, were unaffected. Chronic rose gave a similar profile

to that of acute rose in the EPM, but with the addition of more total entries. Again, there was no significant change in the percentage of closed entries, an indication of drug locomotor effects, indicating that this represents exploration of both arms of the maze and possibly anxiolysis. In contrast to the EPM, rose odour's effects in the BWB were very markedly that of an anxiolytic drug. It was characterised by a longer latency for gerbils to move from the white compartment to the safety of the black compartment, more exploration of the white compartment, and less time in the black compartment. This profile was further strengthened in the chronic condition with more exploration of the whole box (crossing frequency and percentage locomotor duration); these behaviours have been shown in previous studies to represent anxiolysis (Hascoet & Bourin, 2003; Lapiz & Hogg, 2001).

Diazepam

Diazepam gave the traditionally expected anxiolytic profile on both spatiotemporal and ethological measures in the EPM. Similar to lavender's profile, but contrasting with rose oil's profile, acute diazepam caused less closed entries; this might be explained as either sedation or as anxiolysis (see Section 5.4). In the BWB, acute diazepam caused more exploratory behaviour (crossing frequency and the duration of exploration in the white area of the box) and some sedation as evidenced by an increase in time spent immobile. Chronic diazepam had a similar profile to acute diazepam but was lacking in sedation and with more exploration. However, even the profile of chronic diazepam appears to be somewhat less anxiolytic than that of rose oil odour for gerbils in the BWB.

# 6.4 DISCUSSION AND CONCLUSION

## 6.4.1 Does prolonged exposure to lavender odour act as an anxiolytic in the gerbil?

Previously, studies have reported acutely administered lavender EO to cause sedation; the odour decreased motility in male and female mice in locomotor tests (Buchbauer, Jirovetz, Jager, 1991) and orally-administered diluted lavender decreased movement in the EPM (Guillemain et al., 1989). These results are similar to lavender odour's effects on gerbils in the pilot studies reported here (appendix 2) where acute, one-hour's,

exposure to lavender had mildly anxiolytic effects and caused some sedation. It has been suggested, in humans though not rodents specifically, that this initial mild anxiolytic response is only transient (Cooke & Ernst, 2000). Contrary to these suggestions, results indicate that, rather than being a transient effect that dissipates, prolonged lavender odour exposure has a greater anxiolytic effect than acute lavender exposure. These results provide evidence for a pharmacological mode of action for lavender. Additionally, pair-wise testing of acute and chronic lavender data revealed a significant trend and, although this was only on one behavioural measure: stretch-attend frequency, which decreased, this again indicates a decreasing anxiolytic trend with increased odour exposure (Blanchard, et al., 1993).

Although differing in some detail, the chronic EPM lavender profile appears to be broadly similar to the diazepam profile. Gerbils treated with chronic diazepam exhibited lower levels of anxiety-related behaviour. This was reflected in more open-entries and less stretch-attends and after 2-weeks exposure to lavender odour the decrease in anxiety is almost identical to chronic diazepam. However, after only 24 h exposure, acute lavender only affected risk-assessment and exploration measures; whereas, acute diazepam also affected spatiotemporal measures (more total entries, open entries, open duration and decreased closed duration and entries). The decreased closed entries in the diazepam condition indicate possible sedation. These results indicate, perhaps, that although lavender is having an anxiolytic effect it is achieving its results via differing pathways to those of diazepam. It is of interest that studies looking at the acute and chronic effects of antidepressant Gepirone, a 5HT-1A agonist, in rats, yielded results similar to those obtained with lavender odour in the gerbil EPM (Silva & Brandao, 2000). Acute administration of Gepirone affected only ethological measures reflecting anxiolysis, while after 15-day, chronic, treatment with Gepirone traditional arm entry measures were also significantly anxiolytic. These results more closely mirror those seen in this study when gerbils were exposed acutely and chronically to lavender odour. Consistent with this are other reports, which have suggested that lavender's mode of action, although similar to diazepam, might act upon multiple pathways by both BDZ-related and non-BDZ related mechanisms (Umezu, 2000). Since lavender EO odour is a complex mixture of chemicals (Shellie et al., 2002) it might possess the potential to work upon multiple pathways. Furthermore, Elizabetsky et al. (1995) suggested that linalool prevents glutamate (the main excitatory neurotransmitter) from binding to its receptors in the neocortex of rats. Therefore, rather than potentiating the action of

GABA, the main inhibitory neurotransmitter, it is possible that lavender odour is working by inhibiting the opposing excitatory neurotransmitter system. In addition, studies have shown that lavender odour and its components raise the levels of cAMP in smooth muscle (Lis-Balchin & Hart, 1999). There is a class of glutamate receptors, metabotropic G protein coupled glutamate-receptors, which modify neuronal and glial excitability through G protein subunits acting on membrane ion-channels and second messengers such as cAMP (Lis-Balchin & Hart, 1999). Additionally, the terpenes in EOs have been shown to have an influence on mitochondrial membranes and oxidative phosphorylative processes (Bakkali, et al., 2008). Mitochondrial control of energy levels in the cell as well as calcium levels have been shown to influence anxiety in mice tested in the EPM and BWB (Einat, Yuan, & Manji, 2005).

The observation that the anxiolytic actions of both lavender and diazepam when administered in chronic form were more pronounced in females is interesting. Previous studies in rats report sex differences depending on stage of oestrous, or no sex differences on testing in the EPM, after administration of acute diazepam (Fernandes et al., 1999; Wilson, et al., 2004). There has been no report of sex differences in gerbils treated with diazepam and tested on the EPM. Although sex-differences have been reported in gerbils that have not received prior drug treatment but have been tested on the EPM. Females were found to have a more anxious baseline when compared with male gerbils (Bridges & Starkey, 2004). Given the similarities of the lavender results with the Gepirone results, and the fact that in other rodents there are sex differences in the serotonin receptor system in the DR nucleus (Dominguez, et al., 2003), the sex differences in the gerbils exposed to chronic lavender in this study might also be because of differences in the serotonin receptor systems in the DR nucleus. It is possible that chronic lavender odour is interacting with the serotonergic system in females to produce the enhanced anxiolytic effects after chronic lavender odour exposure. There is some evidence in humans for lavender having estrogenic effects (Henley, et al., 2007) and oestrogen receptor ß has been shown to interact with the serotonergic system in the DR (Weiser, et al., 2008).

Compared with the EPM results, lavender had very mildly anxiolytic effects in the BWB (latency to enter the black side increased following both acute and chronic administration). As mentioned in the previous chapter, it is possible that these two models are measuring different aspects of anxiety. If this is the case then it is possible

that lavender is having more of an effect on the cognitive forward-thinking aspects of anxiety, related to worry rather than to avoidance, particularly in females. However, since there are limitations to the validation of the BWB in its current form, this needs further future investigation.

In comparison, diazepam had an anxiolytic profile in both acute and chronic conditions, although there was some sedation following acute administration; exploration (crossing frequency and white exploration) increased both acutely and chronically and the sedation seen following acute administration (longer immobile duration) was no longer present following chronic administration. Interestingly, and again in contrast with lavender, there were sex differences in response to diazepam in the BWB. Males were less anxious following acutely administered diazepam than female gerbils. However, following chronic administration, anxiolytic profiles were broadly the same for both males and females providing further evidence for a differing mechanism of action of lavender when compared to that of diazepam.

### 6.4.2 Does prolonged exposure to rose odour also act as an anxiolytic in the gerbil?

Similar to the lavender results, habituation to rose odour did not take place. In contrast to the pilot studies, where one hour's exposure to rose oil failed to produce anxiolysis, rose oil appeared to have anxiolytic properties in both the EPM and BWB, which were unaffected by stage of oestrous. Furthermore, the behavioural fingerprints obtained are not identical to those of diazepam or lavender in either model. Unlike lavender in the EPM, acute rose odour showed an anxiolytic effect only on ethological measures, while causing more exploration around the EPM and less freezing behaviours. However, traditional measures of anxiolysis, such as the frequency and duration of open entries and the duration of closed entries, were unaffected. Chronic rose gave a similar profile to that of acute rose in the EPM, but with the addition of more total entries. Again, there was no significant change in percentage-closed entries, which are an indication of drug locomotor affects (Rodgers & Johnson, 1995). Therefore, this represents increased general exploration of both arms of the maze and possibly anxiolysis by increasing the approach component of the anxiety approach-avoidance conflict. Although rose's EPM profile is one of mild anxiolysis it is very different from the anxiolytic profile of diazepam, which, as already mentioned, gave the traditionally expected anxiolytic

profile on both spatiotemporal and ethological measures. Acute diazepam also caused some sedation, as shown by less closed entries, contrasting with the rose oil profile, where there was no sign of sedation. It also differs from that of lavender, which, although its profile was more one of risk-assessment and exploration than diazepam, lavender did, also, have some effects on spatiotemporal measures.

In contrast to the EPM, and lavender in the BWB, rose odour's effects in the BWB were what would be expected of an anxiolytic drug. It was characterised by a longer latency for gerbils to move from the white compartment to the safety of the black compartment as well as more exploration of the white compartment and less time in the black side. This profile was further strengthened in the chronic condition, with more exploration of the whole box (crossing frequency and percentage locomotor duration). This has been shown in previous studies to represent anxiolysis (Lapiz & Hogg, 2001; Rodgers & Johnson, 1995). In comparison, acute diazepam caused more exploratory behaviour (crossing frequency and the duration of exploration in the white area of the box) as well as some sedation as evidenced by less time spent immobile. Chronic diazepam had a similar profile to acute diazepam, but without the sedation and with more exploration. However, even the profile of chronic diazepam appears to be somewhat less anxiolytic than that of rose oil odour for gerbils in the BWB. Likewise, lavender had only very mildly anxiolytic effects in the BWB. It affected only the time taken for gerbils to escape from the aversive white compartment to the relative safety of the black compartment. Thus, in contrast to lavender, rose oil’s results in the BWB suggest that rose oil has an effect on the behaviours that are linked to the avoidance aspects of anxiety. However, given the problems in validating the BWB, further work is needed in order to substantiate this claim.

Similar to lavender, these differing profiles suggest that rose oil odour and diazepam are not acting via the same mechanisms. Indeed, it has already been suggested that rose oil's mechanism of action is probably not via the benzodiazepine route (Umezu, 1999) and these EPM findings appear to be supportive of this. As already mentioned, in the EPM, spatiotemporal measures have been identified as good indicators of benzodiazepine activity. In contrast, risk-assessment behaviours, without alterations to spatiotemporal measures, might be more typical of non-benzodiazepine anxiolytic actions, for example 5HT-1A agonists such as buspirone (Cole & Rodgers, 1994; Griebel et al., 1997) and ipsapirone (Setem et al., 1999). These behaviours are thought to be more reflective of

the worry components of anxiety (Wall & Messier, 2001). Further evidence for the action of rose oil, potentially via a biogenic amine-type pathway, is provided by inhibition of immobile effects by reserpine when rose oil was tested in the murine-forced swimming test (Khalaj & Farzin, 2006). Reserpine acts presynaptically to block monoamine neurotransmitter release from the vesicles in which they are encapsulated in the synapse. However, studies using the calcium channel-blocker Verapamil indicate that it has weak effects in the murine EPM and strongly anxiolytic effects in the murine BWB (Kozlovskii & Prakh'e IV, 1995), which is remarkably similar to the effects of rose oil found here. Furthermore, *in-vitro* experiments looking at the effects of rose oil on the guinea pig trachealis muscle provide evidence that rose oil might exert some of its effects via the action of calcium channels (Boskabady, Kiani, Rakhshandah, 2006). Rose oil is a complex mixture of chemicals, which might act on multiple pathways (Ozel, et al., 2006). In Vogel's and the Geller–Seifter anti-conflict tests, citronellol and 2-phenethyl-alcohol were shown to be the components mainly responsible for anti-conflict effects (Umezu et al., 2002). Geraniol has also been identified as a component of rose oil (Umezu et al., 2002). High concentrations of geraniol have estrogenic activity (Howes et al., 2002), and estrogens have been shown to reduce anxiety in female rats (Hill, Karacabeyli, Gorzalka, 2007). This is one possible explanation for the differences between male and female gerbils in the EPM. However, sex differences in response to rose oil odour were minor and only observed in the EPM following acute exposure. Female gerbils exhibited higher levels of anxiety, less open entries and more protected head-dip behaviour compared to males. However, after 2 weeks exposure to rose odour any sex differences disappeared. These results contrast with the sex differences observed in diazepam treated gerbils in both the EPM and BWB. This also suggests that rose oil might reduce differences in anxiety-like behaviour between males and females.

### 6.4.3 Conclusions

These studies provide evidence for lavender and rose EOs having longer-term effects, which potentiate over time, rather than just having limited temporary effects as suggested by one meta-analysis of their anxiolytic effects in humans (Cooke & Ernst, 2000).

The fact that these two odours appear to have differing mechanisms of action adds weight to the argument for specific pharmacological effects for each odour. Broadly speaking, lavender had more potent anxiolytic effects in the EPM; whereas, rose oil's anxiolytic effects appear to be more prevalent in the BWB. As mentioned in chapter 5 (see summary Chapter 5.5), the BWB did not validate as well as the EPM, but was included here as it might be measuring aspects of avoidance-type anxiety rather than general anxiety. Studies by other workers indicate that behaviour in the BWB might be mediated by amygdala function, whereas, in the EPM it might be hippocampal function that predominates (Do-Rego et al., 2006; McHugh, et al., 2004). Thus, rose oil might have more of an effect on the avoidance and arousal aspects of anxiety, which are thought to be mediated by the amygdala. In contrast, lavender's effects might be mediated by the behavioural inhibition system and therefore have an influence on the cognitive aspects of anxiety, such as risk-assessment, which has been likened to human worry (Gray & McNaughton, 2003; Blanchard et al., 1993).

The fact that animals rapidly habituate to the presence of odours would lend support to lavender and rose possessing distinct and specific longer-term pharmacological effects. Prolonged odour administration causes olfactory receptor adaptation and reductions in neuronal responses to that odorant (Wilson, in press). This being the case, any longer-term effects probably have nothing to do with odour preference at all, since the gerbils will no longer detect the presence of the odour.

However, one limitation of these studies pertains to the possible role of odour preference in gerbils. It is very difficult, and perhaps even impossible, to account for odour preferences in gerbils. Perhaps the differing behavioural profiles between the two odours could be accounted for by some innate preference for either odour. Without actually controlling for the odour, it is impossible to say. The use of animals that have not previously been exposed to lavender or rose odour eliminates confounding due to learning mechanisms. However, the gerbils' natural preferences for odour might have some confounding effect. Future researchers might consider the use of treatments, such as zinc sulphate ($ZnSO_4$), that impair the nasal mucosa. Although it should be noted that such treatments might carry some methodological issues in that in some species they do not always cause complete anosmia, for example the rat (Slotnick, Glover, & Bodyak, 2000). Furthermore, impairments to the olfactory senses have been shown to occur in other mental illnesses, such as depression (Atanasova et al., 2008), and as such might

confound studies attempting to examine the effects of odours on anxiety. In this vein, olfactory bulbectomy of the rat has been used as a model of depression (Song & Leonard, 2005).

Another approach to overcome this preference problem and to establish any true anxiolytic properties, which do not require the odour for anxiolytic effects, would be to feed encapsulated essential oil to participants. This method of delivery has been used in studies examining the cognitive and mood enhancing effects of other EOs, such as lemon balm (M. officinalis) and Spanish sage (S. lavandulaefolia), in humans (Kennedy, et al., 2002; Tildesley et al., 2003). Similarly, oral gavauge has been used in mice to deliver acute doses of lavender prior to testing on the EPM (Guilleman et al., 1989). However, in this study, the lavender was not encapsulated and so it could be argued that any effects are mediated by odour preference or some other mechanism that involves odour. Although lavender EO has not been fed to humans in encapsulated form prior to the studies reported here, linalool, the major component of lavender, has been massaged into the abdomens of participants who were wearing oxygen masks, and therefore not exposed to its odour effects (Heuberger et al., 2004). In Heuberger's study (2004), removal of either the odour or the other components of lavender also seem to have removed any cognitive anxiolytic effects, while retaining linalool's physiological and autonomic anxiolytic effects. These results indicate that the odour might be required for the cognitive, mood-enhancing, anxiolytic properties to be effective. Alternatively, perhaps the whole oil, rather than one component, is required for anxiolysis. However, since the olfactory system, including the phylogenetically old and conserved entorhinal cortices, and the limbic system (the seat of anxiety) are very closely linked, perhaps removing the odour might also remove lavender's propensity to mediate anxiolysis. Likewise, feeding lavender to participants could also cause any active components of the oil to be rendered inactive during digestion. With these limitations in mind, it was decided to proceed with phase two of the thesis and use orally-administered encapsulated lavender as the route of administration in a randomised, placebo-controlled and double-blind study.

Thus, in part one of this thesis the problem of whether lavender EO odour has only transient effects, or whether it has properties more akin to a drug, which like many drugs is potentiated over time, has been addressed. It has been confirmed that lavender's

anxiolytic effects, in the rodent models tested here, potentiate over time. However, these studies do not address the question of whether odour preferences play a part in lavender (or rose) oil's effects. Hence, the second part of this thesis attempts to begin to answer the question of whether the odour of lavender is required for its mood enhancing anxiolytic effects. Because of the difficulties of feeding gerbils (and rodents in general) encapsulated EOs without them chewing it and detecting its odour and the fact that it is not possible to assess the cognitive components of anxiety, such as worry and mood in gerbils, then the second part of this thesis will be conducted using human participants.

In addition, a further longer-term aim of the second part of the thesis is to conduct longer-term studies in humans too. However, this is dependent on establishing a suitable test and it would only be worth conducting chronic studies if results of acute testing in humans provided reason to believe that lavender's longer-term effects are worthy of investigation.

Lavender was chosen for this next phase of studies, rather than rose oil, because it was lavender that had more potent effects in the EPM. The EPM was the model that gave more reliable validity in terms of expected responses to known anxiolytic and anxiogenic drugs. In addition, there has been very little research on rose oil compared to lavender. More is known about lavender's potential mechanisms of action. However, in the future it would also be of interest to investigate rose oils effects in a similar human study.

# CHAPTER 7 ORAL ADMINISTRATION OF LAVENDER IN HUMANS: A LABORATORY TEST OF ANXIOLYTIC EFECTS.

## 7.1 INTRODUCTION

In the previous chapter it was shown that in a widely used preclinical model of anxiety, the EPM, lavender odour has anxiolytic effects. As discussed, one limitation to the previous work is that it was not possible to take into account any natural preferences for the odour that the gerbils might have had.

Oral administration of lavender oil by sealed capsule presents an opportunity to test double-blind placebo-controlled studies of putative pharmacological effects in humans. Oral administration of EOs is common in Europe (Schnaubelt, 1998) and studies have shown that capsule administrations of lemon balm, *Melissa officinalis* (Kennedy, et al., 2002) and Spanish sage, *Salvia lavandulaefolia* (Tildesley et al., 2003) EOs have beneficial effects on human cognitive performance. However, there has been no published study examining the anxiolytic effects of lavender in humans in a double-blind randomised placebo-controlled trial.

Although there have been no placebo-controlled randomised double-blind studies of lavender odour, there is some evidence for the effectiveness of lavender oil in the treatment of anxiety in humans (see Introduction, section 1.4). However, as discussed in section 1.4, the locus of anxiolytic effects in humans is unclear. Pharmacological effects cannot easily be differentiated from either innate or learned preferences for lavender odour. Moreover, it has been shown that pleasant odours can positively modulate mood (Weber & Heuberger, 2008; Burnett et al., 2004) (see section 1.4).

Suspenseful film-clips have been used to elicit anxiety (Santagostino et al., 1996; Tull & Roemer, 2007; Aftanas, Reva, Savotina, Makhnev, 2004). They have also been shown to increase levels of physiological arousal typical of the somatic components of anxiety, such as cardiovascular measures, electrodermal skin response (GSR) and

respiration (Totten & France, 1995; Kreibig, et al., 2007). Heart-rate variation (HRV) is vagally mediated and is an index of the central peripheral feedback capacity, it is lower in anxious participants (Thayer & Lane, 2007; Brosschot, Van Dijk, & Thayer, 2007; Pieper, Brosschot, van der, & Thayer, 2007; Friedman & Thayer, 1998; Gorman & Sloan, 2000; Johnsen et al., 2003; Watkins, Grossman, Krishnan, & Sherwood, 1998; McCraty, Atkinson, Tiller, Rein, & Watkins, 1995; Thayer & Friedman, 2002). Thus, anxiolytic effects are associated with HRV increases. Since HRV is vagally mediated, it might be a sensitive measure of the effects of anxiolytics on the parasympathetic nervous system (McCraty et al., 1995). There is also some evidence that parasympathetic measures might be more sensitive to lavender's effects than the more often tested GSR and HR (Duan et al., 2007; Saeki, 2000).

Other measures to be included in the study are measures of facial muscle response. There is a high correlation between activity in the corrugator muscle in the face, the muscle that causes the frown lines between the eyebrows, and higher levels of stress and anxiety (Cacioppo & Tassinary, 2001). Salivary cortisol is widely used as a measure of HPA axis activity and is expected to rise in response to stress in a significant proportion of the population (Höferl, et al., 2006). Measures of blood pressure are also widely used as indicators of autonomic activation and hence they too were included.

The aim of this study was to provide a randomised double-blind and placebo-controlled test of the effects of capsule administration of lavender in humans. It was expected that oral administration of lavender oil capsules (100 µl and 200µl) would have dose-dependent anxiolytic properties, measured using self-report measures of anxiety and mood, and physiological measures of respiration, electrodermal response, heart-rate and HRV. However, if orally-administered lavender failed to have anxiolytic effects then this might argue that the odour is required for any potential anxiolytic effect or could be because the active constituents of the lavender are being eliminated or inactivated during digestion.

# 7.2 MATERIALS AND METHODS

## 7.2.1 Design

A randomised placebo-controlled double-blind study was conducted to compare the effects of three lavender doses (0 µl, 100 µl, 200µl) and sex on participants in response to a neutral film, an anxiety-provoking film, and a light-hearted recovery film. Ethical approval was granted by the departmental ethics committee (see appendix three, section one) and was in accordance with the British Psychological Society Code of Conduct (2006) and the Declaration of Helsinki (1964).

## 7.2.2 Participants

Ninety seven healthy adult non-smoking participants (58 females, 39 males) aged 18-74 years (mean 35.56 +/- 1.19 SEM); BMI ranging from 17.5- 40.8 (mean 22.91+/- 1.88), were recruited from the general population. Recruitment was via poster, flier, newspaper articles (see appendix3 section 1) and two local radio appearances (BBC Radio Lancashire's 'Tony Livesey's Breakfast Show' and Carole Turner's chat show 'Later with Carole'). Group sizes were as follows: placebo, males n= 12, females n= 19; 100µl lavender, dose males n= 13, females n=19; 200µl lavender, dose males n= 14, females n= 18.

### 7.2.2.1 Participant screening

Following advice from Mr Venkat Iyer (Neurosurgeon, Royal Preston Hospital) all potential participants were screened for allergies to lavender by means of a patch test: a drop of the capsule contents was applied to the inside forearm of participants and massaged in; any itching or swelling in this area over the next 24 hours was reported. Only one person reacted to the lavender capsule contents and did not take part in the study. Of the remaining participants, one female vomited during the baseline phase and therefore data from this participant were excluded from the study (final n=96).

On recruitment, participants were given a participation pack to complete, which contained an overview of the experimental protocol and a consent form enquiring about the fitness of participants. Anyone who was not in good health or was taking prescribed

medication was screened out at this stage of recruitment. Information about height and weight was also sought; this part of the form was completed on arrival at the laboratory prior to taking part in the study. The pack also included dietary instructions; for 24 hours prior to the study participants abstained from a list of food, beverages and toiletries containing the components of lavender as well as from alcohol, tea, and coffee (see appendix 3, section 3, for participation pack). Participants were free to withdraw from the study at any time.

### 7.2.3 Film-clips

Suspenseful film-clips are effective and ethically acceptable tools for the creation of anxiety (Kreibig et al., 2007). Gross and Levenson, (1995) have created a library of films demonstrated to influence discrete moods (Rottenberg, Ray, & Gross, 2007). 'Silence of the Lambs' was selected from this library (Clip length: 3'29") and edited following Gross and Levenson's recommendations. The clip depicts a lone female detective who is searching in a dark cellar for a psychopathic killer; it has been shown to elicit mild fear/ anxiety (Gross & Levenson, 1995). Anxiety effects from films are additive (Rottenberg et al., 2007). This clip was followed immediately by a nine minute edited version of 'Open Water', featuring a couple being stalked by sharks. This was shown in pilot studies to be effective in changing moods by the positive and negative affect schedule (PANAS) (Watson et al.,1988) and by Gross and Levenson's film questionnaire (Rottenberg et al., 2007) (see appendix 4). Any scenes of violence or injury were edited out to prevent elicitation of emotions related to disgust rather than anxiety.

In addition 'Nature Watch' a 30 minutes TV documentary program, was validated as a neutral film and shown to cause no positive or negative affect, or anxiety. Finally a recovery film was selected from Laurel and Hardy's 'Towed in a Hole', and was screened for ten minutes to prevent participants leaving the laboratory feeling anxious.

All film-clips were viewed on a Panasonic NV-DS (TY – SP42P8W-K) 106.7 cm screen width TV, which was linked to a Panasonic DVD player (S29 D).

## 7.2.4 Lavender Capsules

Gelatine lavender capsules, were manufactured by Power Health Ltd., Pocklington, York, YO42 1NR (Tildesley et al., 2005), and contained either organic *Lavandula angustifolia* oil (100µl or 200µl), which was purchased from Tisserand Ltd., (www.Tisserand.com), and combined with sunflower oil, or just sunflower oil (placebo). GC/MS studies showed that lavender reached the bloodstream 15 minutes following ingestion of the capsules and reached a maximum at 30 minutes following the ingestion of 200µl of lavender (data presented in appendix 5). In addition, the manufacturers indicated that the capsule would disrupt at 30 minutes following ingestion (personal communication email Mandy Sharp).

## 7.2.5 Measurements

### 7.2.5.1 Self-report measures

Spielberger's state-trait anxiety questionnaire (STAI- Y) was used to measure state and trait anxiety (Spielberger, Gorusch, Lushene, Vagg, & Jacobs, 1983)**.** The STAI-Y is a well-validated and widely used measure of state and trait anxiety in laboratory studies and in clinical practice (Spielberger et al., 1983; Grös, Antony, Simms, & McCabe, 2007). It is reported to have been used in over 3000 studies and has averaged internal consistencies of above 0.89, Chronbach's alpha, and test –retest reliability of r= 0.88 for trait anxiety and r = 0.77 for state anxiety, as would be expected (Grös et al., 2007).

State anxiety is transitory; it is present at a specific moment in time. In contrast, trait anxiety is a more permanent feature of personality and represents a predisposition to experience state anxiety (Grös, et al., 2007). People with high trait anxiety are more likely to perceive a stressful situation as dangerous or threatening and are more likely to experience higher state anxiety (Spielberger, 1983).

A general measure of normal mood was also sought. Emotional experience has been shown to have two general dimensions: positive and negative affect. These emerge

consistently in research across a broad range of experiences (Watson et al., 1988). The positive and negative affect scales (PANAS), used in this study, have been developed and validated to measure these factors and are very reliable, giving a Chronbach's alpha of 0.83 (Watson et al., 1988). Negative affect is strongly associated with measures of general neuroticism and anxiety and is often used as a measure of normal anxiety (Gray & Watson, 2007).

#### 7.2.5.2 Physiological measures

Physiological parameters were recorded simultaneously and continuously during each phase of the study using the Biopac MP100 Hardware (Biopac systems Inc.) and *AcqKnowledge* software version 3.8.1(11.03.2004, Biopac systems Inc. Santa Barbara, California, USA). Sampling rate was at 500Hz.

Heart-rate: ECG100B amplifier set to R-wave; gain 1000; upper frequency response 35Hz; lower frequency response 0.05 Hz. ECG electrodes: 3M electrodes (Southern Syringe Ltd., Manchester). Electrode placement was in a three lead unipolar modified chest configuration following the modified I lead version (Stern, Ray, & Quigley, 2001). Galvanic skin response: GSR100B amplifier: gain 50 μmho; upper frequency response 10 Hz; lower frequency response 0.5Hz. Ag/AgCl finger electrodes (TSD103A) were used via the constant voltage (0.05V) technique. Electrodes filled with a high impedance electrolyte paste Gel 100 (Biopac Inc.) were placed on the second phalanx of the middle and index finger of the non-dominant hand (Cacioppo & Tassinary 1999).

Respiration rate: A strain gauge respiration transducer, TSD201 and the RSP100C amplifier measured breathing-rate. The transducer was placed around participant's thorax, just below the breast line and on the sternum. Gain settings: low pass filter; 10Hz; 0.5Hz filter set to DC and 0.5 Hz filter set to DC.

Facial EMG: The left corrugator muscle signal was measured using an EMG100B amplifier set at: gain 5000; lower frequency response 500Hz; HDN 100Hz; upper frequency response 1.0 Hz and surface electrodes Ag/AgCl (EL 204S), 4mm contact area. EMG was measured by placing two electrodes filled with conductive gel (Gel 100 Biopac inc.) over the eye, one over the brow bone on the *corrugator supercilii* muscle

and one on the forehead (ground) following the placement described by Cacioppo and Tassinary (1999) these were secured with surgical tape.

Salivary Cortisol: Saliva samples were taken after the neutral film, after the anxiety film after debriefing, and just prior to participants leaving the laboratory, 20minutes after the end of the anxiety film, when it is expected that, on average, there will be a rise in salivary cortisol in response to stress (Höferl, et al., 2006). Salivary cortisol was analysed using the DRG salivary cortisol kit (IDS Ltd., Boldon, Tyne and Wear). This kit is a standard competitive Enzyme Linked Immuno-Sorbent Assay (ELISA). In this kit, the wells of a standard 96 well micro-titre plate are coated with anti-cortisol antibody. Standard volumes (100 µl), of known and unknown concentrations of cortisol in the standards, controls, and samples being tested, are added to separate wells; in addition, a standard volume of cortisol linked to an enzyme (conjugate) is added to each of the wells. These compete for binding sites on the antibody molecules. Following a specified incubation time (one hour) any unbound material is washed from the wells and a substrate for the enzyme, 3, 3, 5, 5, tetra-methyl-benzidine, is added to each of the wells of the plate. The plate is incubated for a further thirty minutes. The reaction is stopped by adding acid, such as sulphuric or hydrocholoric and the concentration of the product, produced by the enzyme-substrate reaction, is measured using optical density readings at 450nm wavelength. Finally, a standard curve is constructed from the known amounts of cortisol in the standards and the absorbance values from the unknown samples are extrapolated from the standard curve.

The lower detection limit of the assay was 1.14ng/ml. The intra- and inter-assay coefficients of variation were 4.83%, 1.37%, 1.84% over the standard curve and 6.4% respectively.

Blood pressure readings, systole and diastole, were taken every three minutes by a Critikon Dinamap vital-signs monitor 8100, following the procedure reported in Cacioppo and Tassinary (1999).

## 7.2.6 Procedure

The study took place in the Department of Psychology's purpose built health suite (see Figure 7.1 below for suite layout)

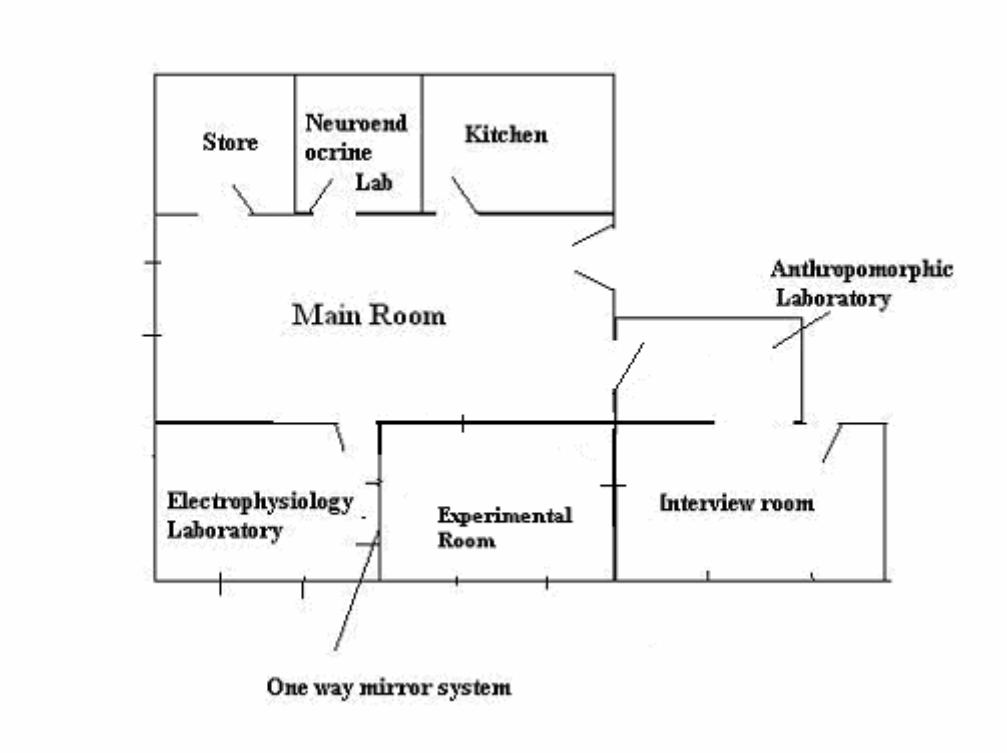



**Figure 7-1 Schematic plan of the Health Psychology Research Suite**

On arrival at the waiting room, compliance with dietary restrictions was confirmed. Participants were asked to complete general health questionnaires, a consent form and initial self-report measures. Next, participants were seated in a comfortable chair in the experimental room and the physiological transducers were attached (see Figure 7.2 of a mock participant below).

**Figure 7-2 The stress laboratory, a 'mock' participant about to watch the film-clips**

The transducers were also attached to the Biopac and computer situated on the other side of a one-way mirror in an adjacent electrophysiological laboratory, from where the experimenter could observe proceedings. During the study, any communication between participant and experimenter took place via an intercom system linking the two rooms. Participants were facing away from the one-way mirror system to minimise any reactivity bias. The Biopac physiological system was situated on the other side of a one-way mirror in an adjacent room, and transducer leads were fed through two conduits to this room and attached to the transducers. Once the study had begun, the experimenter observed proceedings via the one-way mirror system. Participants watched the film-clips in the dark. Lights were turned on at the end of each film-clip to allow participants to complete the self-report measures.

Baseline measures were taken for approximately ten minutes. During this time participants were asked to relax and imagine a pleasant situation, such as lying on a warm beach. Once baseline measures were relatively stable, participants were asked to complete self-report measures. Following this, the participants took their allocated dose

of lavender capsules with a small cup of water. Viewing of film-clips proceeded with the neutral film and then questionnaires followed by the anxiety clip, questionnaires and finally the recovery clip. Timings of film-clips were: neutral clip immediately after taking the capsules, anxiety-provoking clip 35 minutes after capsule administration, and recovery clip 50-minutes after capsule administration. During viewing time, physiological measures were taken continuously. Participants were asked to engross themselves in the clips as much as possible. They were asked to complete the self-report measures with regard to how they felt while watching the respective clips. Timing of the clips was based on the time taken for lavender's major components to peak in the bloodstream following oral administration (see appendix 5) so that the average peak concentration of lavender in the bloodstream coincided with the anxiety film-clip.

### 7.2.7 Data reduction

All data were screened manually prior to data reduction and any movement artefacts or noise were either removed or filtered out using the appropriate filter for the signal being reduced.

Heart-rate (bpm) data were transformed into 30 second mean rates for each phase except baseline. For baseline phase, the rate of the last minute was taken. Each of the data points for each phase was subtracted from the baseline to adjust for baseline values, as recommended by Stern, Ray, and Quigley (2001), Howell, (2002), Tabachnik and Fidell, (2001). The means of each of these baseline adjusted change scores were calculated for each phase of the study.

Measures of HRV in the time domain were derived from HR following recommended guidelines (Malik et al., 1996; Berntson et al., 1997; Niskanen, Tarvainen, Ranta-aho, & Karjalainen, 2004). This measure of HRV has been shown to be more robust to changes in breathing-rate than the alternative power spectral density methods (Penttila et al., 2001). Measures taken were mean and standard deviation of inter-beat-interval (RR) data (SDRR )( measured in seconds); root mean square of differences of successive RR intervals (RMSSD); and the percentage value of consecutive RR intervals that differ more than 50ms (NN50%). These measures are considered indices of cardiac parasympathetic activity (Penttilä et al., 2001).

For GSR data the overall tonic skin level (GSL) was calculated for each 30 second phase and treated as for HR data. The mean number of galvanic skin responses (GSR frequency) was extracted by removing the drifting baseline using a high pass filter (IIR) set to 0.05Hz. Only peaks above 0.02 μmho were counted as responses.

For breathing-rate data peaks were counted manually and expressed as breaths per minute.

### 7.2.8 Data analysis

All data were adjusted relative to baseline by subtracting baseline measures from those obtained during each phase of the study (Howell, 2002; Heuberger et al., 2004). These data were screened for normality for each group following the recommended procedures (Tabachnick & Fidell, 2001).

Self-report data met the assumptions of normality and were analysed using a mixed design MANCOVA (SPSS version15): 2 (*sex*) x 3 (*lavender dose*: 0 μl, 100 μl, 200μl)x 2 (*time*: neutral, anxiety) with sex and dose as between-group variables and time as a within-group variable.

Physiological data were analysed by first comparing male and female data for sex differences using the Mann-Whitney U test. Wherever there was a sex difference subsequent analyses were performed separately for males and females; otherwise, all data were combined. To test for the effects of lavender dose, the Jonckheere-Terpstra test for ordered alternatives, which is more powerful than the Kruskall-Wallace nonparametric ANOVA (Siegel & Castellan, 1998) was employed for each phase of the study; followed by pair-wise comparisons of each dose with placebo using the Mann-Whitney U-test where appropriate. Alpha levels were set at 0.05.

## 7.3 RESULTS

See Table 7.1 below for participant characteristics

**Table 7.1 Mean and standard deviations of age, height, weight and trait anxiety scores on male and female participants.**

| Lavender dose | Males | | | | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | age | height | weight | Percent Body fat | STAI TRAIT Anxiety | N | age | height | weight | Percent Body fat | STAI TRAIT Anxiety |
| **placebo** | 12 | 31.17 (9.94) | 176.58 (8.84) | 81.30 (15.86) | 20.49 (6.75) | 39.36 (9.62) | 19 | 32.32 ( 8.31) | 162.22 (6.03) | 62.90 (10.36) | 29.43 (6.94) | 38.58 (8.81) |
| **100** | 13 | 38.85 (11.25) | 178.19 (4.95) | 85.65 (15.58) | 20.14 (6.13) | 37.46 (7.43) | 20 | 43.05, (14.66) | 164.97 (7.66) | 72.82 (15.95) | 33.77 (8.81) | 37.65 (9.31) |
| **200** | 14 | 33.50 (10.83) | 181.21 (3.44) | 85.89 (15.04) | 19.71 (6.03) | 32.57 (5.12) | 19 | 33.47 (9.95) | 164.75 (6.74) | 65.48 (11.26) | 30.04 (7.16) | 37.63 (10.18) |
| **Total** | 39 | 34.56 (10.91) | 178.78 (6.04) | 84.40 (15.21) | 20.10 (6.22) | 36.29 (7.86) | 58 | 36.40 (12.20) | 163.97 (6.84) | 67.10 (13.26) | 31.10 (7.80) | 37.95 (9.29) |

Group sizes: Males placebo n= 12, 100μl n= 13, 200μl n= 14. Females placebo n= 19, 100μl n= 19, 200μl n= 18. Significance level * $p < 0.05$.

## 7.3.1 Self-report measures

Mean levels of change in anxiety, positive affect and negative affect at different doses of lavender are shown in Table 7.2 (below).

**Table 7.2 Effects of orally-administered lavender capsules on state anxiety scores after the neutral film. Data presented are baseline adjusted means and SEM. Statistical significance: * p < 0.05. Group sizes placebo n= 31, 100 µl n = 32, 200 µ l = 32.**

| Difference score | Lavender dose | All participants Mean (S.D) | Males Mean (S.D) | Females Mean (S.D) |
|---|---|---|---|---|
| **State anxiety (neutral – baseline)** | **placebo** | -3.50 (6.34) | -2.71 (5.95) | -4.00 (6.68) |
| | **100** | -4.28 (7.54) | -2.69 (7.73) | -5.37 (7.42) |
| | **200*** | **-7.32*** (6.92) | -4.50 (5.96) | -9.52 (6.96) |
| **State anxiety (anxiety film– baseline)** | **placebo** | 2.89 (10.50) | 1.62 (6.92) | 3.68 (12.36) |
| | **100** | 5.59 (12.80) | 3.62 (7.91) | 6.95 (15.35) |
| | **200** | 6.54 (11.44) | 5.93 (8.23) | 7.01 (13.65) |
| **Negative affect (neutral – baseline)** | **placebo** | -0.97 (3.60) | -0.58 (4.08) | -1.21 (3.36) |
| | **100** | -2.59 (4.56) | -3.15 (5.11) | -2.21 (4.25) |
| | **200** | -0.72 (3.27) | 0.43 (4.15) | -1.61 (2.09) |
| **Negative affect (anxiety film– baseline)** | **placebo** | 3.61 (7.25) | 1.83 (2.59) | 4.74 (8.94) |
| | **100** | 5.44 (7.38) | 2.85 (4.98) | 7.21 (8.32) |
| | **200** | 5.94 (6.70) | 6.21 (7.00) | 5.72 (6.66) |
| **Positive affect (neutral – baseline)** | **placebo** | -7.99 (7.69) | -6.90 (6.07) | -8.68 (8.65) |
| | **100** | -5.44 (8.89) | -7.46 (7.38) | -4.05 (9.74) |
| | **200** | -8.84 (8.40) | -7.21 (7.26) | -10.10 (9.19) |
| **Positive affect (anxiety film– baseline)** | **placebo** | -4.14 (5.86) | -2.70 (5.39) | -5.05 (6.10) |
| | **100** | -3.94 (7.19) | -2.69 (5.91) | -4.79 (7.99) |
| | **200** | -3.69 (7.64) | -3.21 (8.26) | -4.06 (7.34) |

Group sizes: Males placebo n= 12, 100µl n= 13, 200µl n= 14. Females placebo n= 19, 100µl n= 19, 200µl n= 18. Significance level * p < 0.05.

Mixed MANCOVAs for each measure: state anxiety, negative affect and positive affect for the two phase of the study (neutral film and anxiety film each corrected for baseline) revealed a significant main interaction for state anxiety for each phase of the study and lavender dose ($F(2, 86) = 4.67$, $p < 0.05$ eta $^2$ =0.1). Adjustment was made for one covariate (trait anxiety), which did not contribute significantly to the model. Thus, separate ANCOVAs were conducted to examine the effects of lavender dose on state anxiety following the neutral and anxiety phases of the study. Alpha levels were set at $p< 0.025$ for each ANCOVA, and because of uneven group sizes, the conservative Pillai's trace criterion used. There was a significant effect for lavender dose on state anxiety following the neutral film ($F(2, 93) =4.10$, $p < 0.05$; eta $^2$ = 0.08). Adjustment was made for trait anxiety which contributed significantly to the model, ($F(1, 93) = 7.10$, $p < 0.01$, eta $^2$= 0.07). Post-hoc comparisons using the LSD test (for 3 means) revealed a significantly greater decrease in state anxiety after the neutral film following administration of the 200 µl dose of lavender when compared with placebo: mean difference 5.50, $p < 0.05$. The 100µl group had a mean difference of 2.27, $p < 0.05$ (see Figure 7.3).

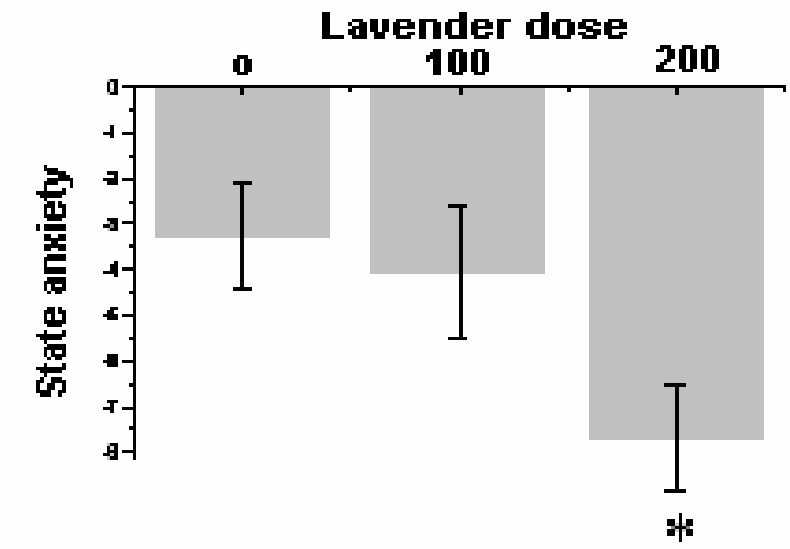



**Figure 7-3 The effects of orally-administered lavender capsules on state anxiety scores after the neutral film. Data presented are baseline adjusted means and SEM. Statistical significance: * p < 0.05. Group sizes placebo n= 31, 100 µl n = 32, 200 µl = 32.**

During the anxiety film-clip phase of the study lavender dose did not have a significant effect on state anxiety ($F (2, 93) = 0.68$, $p= 0.51$, partial eta $^2 =0.01$). There were no significant effects of lavender on either negative or positive affect scores.

### 7.3.2 Physiological measures

For medians and inter-quartile ranges see Table 7.3. All data were adjusted for baseline (see Methods Section 7.2.8).

**Table 7.3 Baseline adjusted group medians and inter-quartile ranges of different physiological measures during a neutral, anxiety and light hearted recovery film following orally-administered encapsulated lavender (100µl, 200 µl) or placebo (sun flower oil).**

| Physiological measure | Lavender dose (µl) | All participants | Males | Females |
|---|---|---|---|---|
| **Heart-rate (BPM) neutral** | placebo | -18.91, **1.78**, 10.55 | | |
| | 100 | -13.71, **1.18,** 13.98 | | |
| | 200 | -14.56, **-11.30*,** 27.08 | | |
| **Heart-rate (BPM) anxiety** | placebo | -93.07, **20.09,** 31.12 | | |
| | 100 | -63.91, **6.28,** 18.85 | | |
| | 200 | -74.26, **3.06,** 23.84 | | |
| **Heart-rate (BPM) recovery** | placebo | | -23.59, **-20.42,** 3.96 | -113.99,**-73.19,**13.20 |
| | 100 | | -75.52,**-42.68,** 11.23 | -55.80, **-11.97,** 4.12 |
| | 200 | | -73.59,**-37.69,** 21.07 | -86.02, **-52.52,** 6.11 |
| **Diastole neutral** | placebo | -2.96, **-0.58,** 1.46 | | |
| | 100 | -2.63, **-0.35,** 1.79 | | |
| | 200 | -4.08, **-1.42,** 2.36 | | |
| **Diastole anxiety** | placebo | -2.21, **0.00,** 3.88 | | |
| | 100 | -0.73, **0.97,** 3.54 | | |
| | 200 | -2.95, **0.67,** 3.67 | | |
| **Diastole recovery** | placebo | -4.40, **-1.67,** 1.89 | | |
| | 100 | -2.63, **0.56,** 3.79 | | |
| | 200 | -1.83, **0.00,**2.83 | | |
| **Systole neutral** | placebo | -4.15,**-2.00,**0.00 | | |
| | 100 | -4.29, **-2.36,** 0.58 | | |
| | 200 | -4.73, **-1.33,** 2.25 | | |
| **Systole anxiety** | placebo | -1.50, **1.89,** 3.90 | | |
| | 100 | -0.75, **1.71,** 5.18 | | |
| | 200 | -0.92, **2.50,** 5.34 | | |
| **Systole recovery** | placebo | -3.34, **0.67,** 2.81 | | |
| | 100 | -3.25, **1.09,** 4.50 | | |
| | 200 | -1.80, **0.67,** 6.11 | | |
| **Cortisol neutral (unadjusted for baseline)** | placebo | 5.18, **5.22,** 6.97 | | |
| | 100 | 3.96, **5.25,** 5.71 | | |
| | 200 | 4.10, **4.99,** 5.82 | | |
| **Cortisol anxiety (unadjusted for baseline)** | placebo | 4.65, **4.75,** 6.12 | | |
| | 100 | 3.36, **4.64,** 5.68 | | |
| | 200 | 4.15, **4.74,** 5.61 | | |
| **Cortisol recovery (unadjusted for baseline)** | placebo | 4.91, **5.10,** 6.65 | | |
| | 100 | 4.41, **5.20,** 6.06 | | |
| | 200 | 4.37, **5.04,** 5.69 | | |
| **Galvanic skin level (µS) neutral** | placebo | -0.16, **-0.60,** 0.70 | | |
| | 100 | 0.24, **-0.57,** 1.21 | | |
| | 200 | 0.62, **-0.87*,** 1.11 | | |
| **Galvanic skin level (µS) anxiety** | placebo | -0.14, **0.36,** 0.83 | | |
| | 100 | 0.36, **0.82,** 1.08 | | |
| | 200 | 0.00, **0.66,** 0.91 | | |
| **Galvanic skin level (µS) recovery** | placebo | 0.04, **0.65,** 1.22 | | |
| | 100 | 0.40, **0.78,** 1.11 | | |
| | 200 | 0.54, **0.79,** 1.26 | | |

| | | | | |
|---|---|---|---|---|
| **Galvanic skin frequency (μS) neutral** | placebo | -0.54, **-0.32,** 0.00 | | |
| | 100 | -0.07, **-0.00**, 0.33 | | |
| | 200 | -0.19, **-0.00,** 0.03 | | |
| **Galvanic skin frequency (μS) anxiety males** | placebo | | 0.00, **-0.0260,** 0.07 | -0.10, **0.03,** 0.03 |
| | 100 | | -0.05, **0.0000,** 0.01 | -0.07, **0.05,** -0.02 |
| | 200 | | 0.02, **0.0013*,** 0.01 | -0.03, **0.02,** -0.00 |
| **Galvanic skin frequency (μS) recovery** | placebo | -0.02, **0.00,** -0.04 | | |
| | 100 | -0.00, **0.02,** 0.08 | | |
| | 200 | -0.00, **0.02,** 0.04 | | |
| **Corrugator EMG neutral** | placebo | 0.0000, **0.0002,** 0.0016 | | |
| | 100 | 0.0000, **0.0002,** 0.0008 | | |
| | 200 | 0.0000, **0.0002,** 0.0013 | | |
| **Corrugator EMG anxiety** | placebo | 0.0000, **0.0003,** 0.0016 | | |
| | 100 | 0.0001, **0.0004,** 0.0013 | | |
| | 200 | 0.0000, **0.0003,** 0.0016 | | |
| **Corrugator EMG recovery** | placebo | 0.0000, **0.0005,** 0.0019 | | |
| | 100 | 0.0000, **0.0005,** 0.0018 | | |
| | 200 | 0.0000, **0.0005,** 0.0022 | | |
| **Respiration rate Breaths per min) neutral** | placebo | 0.06, **1.38,** 2.89 | | |
| | 100 | 0.37, **1.89,** 3.90 | | |
| | 200 | 0.75, **2.00,** 2.75 | | |
| **Respiration rate Breaths per min) Anxiety** | placebo | -0.10, **0.03,** 0.03 | | |
| | 100 | -0.07, **0.05,** 0.01 | | |
| | 200 | -0.03, **0.02,** 0.00 | | |
| **Respiration rate Breaths per min) recovery** | placebo | -2.50, **0.00,** 1.00 | | |
| | 100 | -1.00, **1.50,** 2.50 | | |
| | 200 | -1.00, **0.50,** 2.00 | | |

Group sizes Placebo n= 31, 100μl = 33, 200μl = 33, significance levels * p< 0.05, ** p < 0.01.

**Heart-rate**

No sex differences in HR were detected in the neutral phase of the study and male and female data were combined. These data were analysed separately for males and females during the anxiety film but not the neutral film or the recovery film.

Analysis revealed a main effect for lavender dose on heart-rate during the neutral film (J-T = 1.77, $p < 0.05$) but not the anxiety (J-T =0.33) or recovery clips (J-T = 0.25). Pair-wise comparisons showed that, at 200µl lavender, heart-rate was significantly less than placebo ( U = 402, $p < 0.05$) see Figure 7.4.

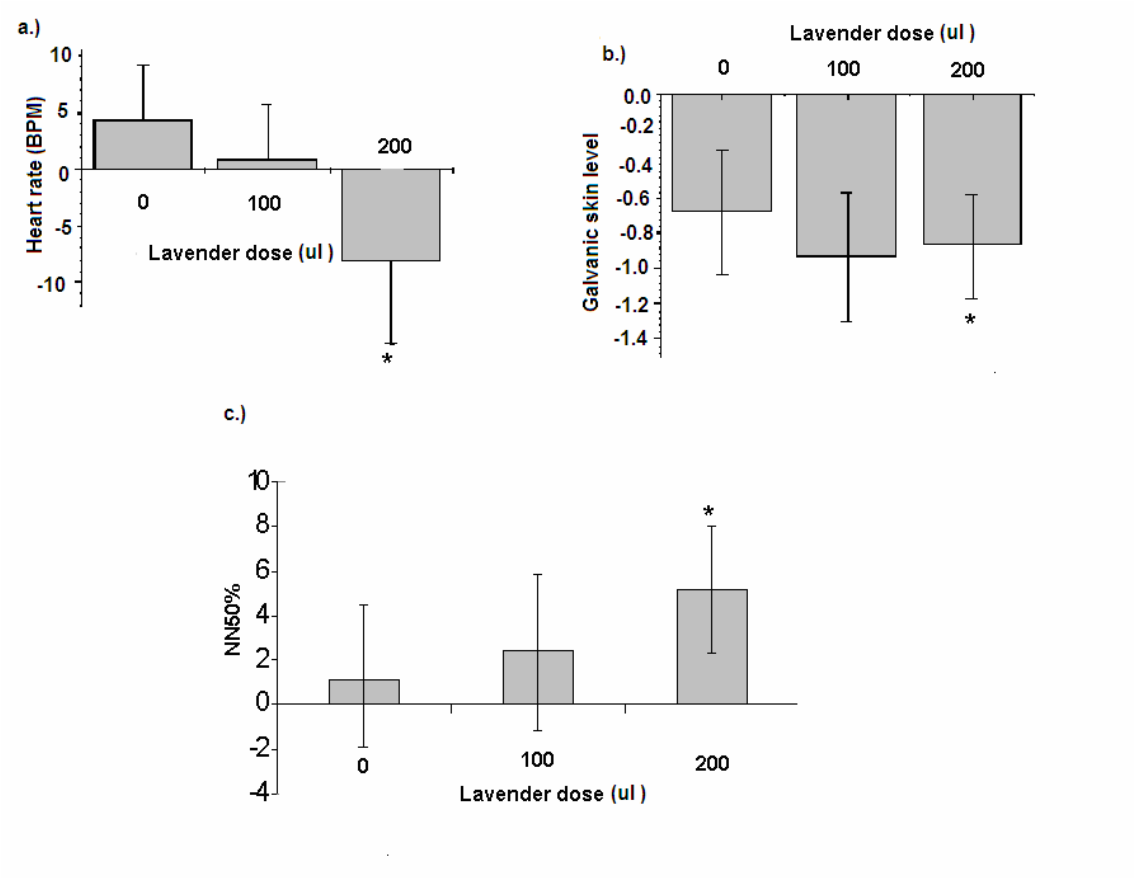



**Figure 7-4 The effects of orally-administered lavender capsules on (a), heart-rate, (b), galvanic skin level and (c), heart-rate variation (NN50%) during the neutral film-clip. Data presented are baseline adjusted means and SEM. Statistical significance:* $p < 0.05$. Group sizes placebo n= 32, 100 µl n= 32, 200 µl = 33.**

**Galvanic skin response**

There were sex differences in GSR frequency (U = 63.5, p < 0.05) during the anxiety but not the neutral film. These data were analysed separately for males and females during the anxiety film but not the neutral film.

Analysis of neutral film data revealed a decreasing trend in galvanic skin level with increasing lavender dose (J-T = 1.73, p < 0.05) in all participants. Pair-wise follow up tests showed this trend reached statistical significance at 200μl lavender compared to placebo (U = 372, p < 0.05); see Figure 7.6. During the anxiety-provoking film there was a dose-relatedincrease in galvanic skin response in males (J-T = 1.90, p < 0.05). Further pair-wise analysis indicated that this reached significance at the top dose of lavender (U = 41.5, p < 0.05) see Figure 7.5.

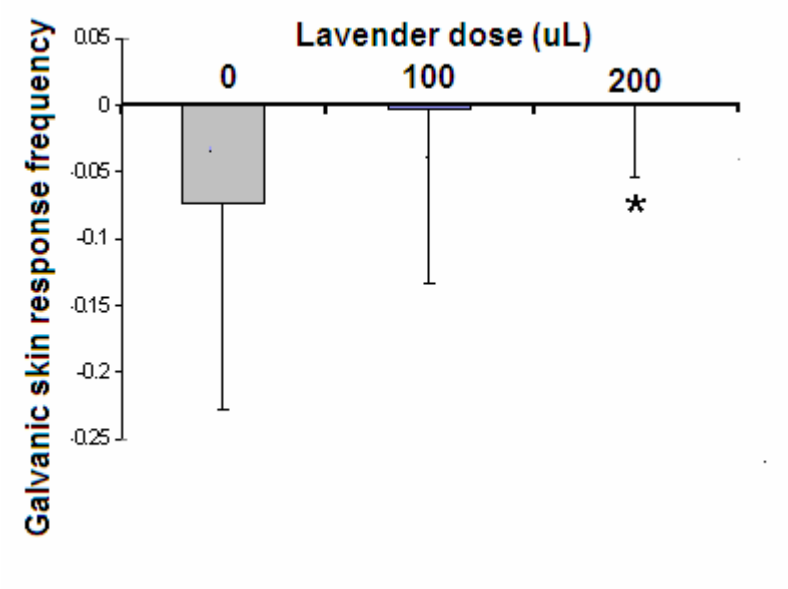



**Figure 7-5 The effects of orally-administered lavender capsules on galvanic skin response frequency during the anxiety film-clip in males. Data presented are baseline adjusted means and SEM. Statistical significance: * p < 0.05. Group sizes placebo n= 13, 100 μl n = 13, 200 μl = 14.**

Lavender failed to have any significant effects on respiration (breaths per minute) systolic or diastolic blood pressure, cortisol or corrugator EMG during any of the film-clips (see Table7.2).

**Table 7.4 Baseline adjusted median and inter-quartile ranges of heart-rate variation while watching a neutral, anxiety inducing and light hearted recovery film-clip film following orally-administered encapsulated lavender (100µl, 200 µl) or placebo (sun flower**

| Dependent variable | Lavender dose (µl) | All participants | males | females |
|---|---|---|---|---|
| **Neutral SDRR** | **Placebo** | -0.01, **0.02,** 0.11 | | |
| | **100** | -0.02, **0.01,** 0.07 | | |
| | **200** | 0.00, **0.02,** 0.09 | | |
| **Anxiety SDRR** | **placebo** | | -0.03, **0.02,** 0.07 | -0.07, **-0.01,** 0.01 |
| | **100** | | -0.03, **0.00,** 0.01 | -0.05, **0.01,** 0.03 |
| | **200** | | -0.04, **-0.02,** 0.00 | -0.02, **0.01,** 0.08 |
| **Recovery SDRR** | **placebo** | -0.02, **0.01,** 0.10 | | |
| | **100** | -0.03, **-0.004**, 0.06 | | |
| | **200** | -0.01, **0.00,** 0.02 | | |
| **Neutral RMSSD** | **placebo** | -15.05, **11.90,** 106.37 | | |
| | **100** | -43.66, **-0.41,** 67.36 | | |
| | **200** | -10.64, **23.22,** 128.44 | | |
| **Anxiety RMSSD** | **placebo** | | -49.49, **15.77,** 99.16 | -121.78, **18.13,**10.43 |
| | **100** | | -46.46, **2.75,** 16.85 | 68.25,**12.49,**64.44 |
| | **200** | | -72.22, **-23.49,** -0.24 | -17.04, **22.69*,**168.01 |
| **Recovery RMSSD** | **placebo** | | -42.17, **6.23,** 75.15 | -62.09, **14.97,**108.76 |
| | **100** | | -57.09, **5.67,** 86.76 | -45.91, **-3.48,**78.64 |
| | **200** | | -24.66, **-5.98,** 0.05 | 0.52, **27.49,** 61.68 |
| **Neutral NN50%** | **placebo** | -6.14, **-0.01,** 4.51 | | |
| | **100** | -4.30, **0.53,** 4.93 | | |
| | **200** | -0.98, **4.09***, 7.04 | | |
| **Anxiety NN50%** | **placebo** | -12.01, **-2.68,** 1.51 | | |
| | **100** | -6.77, **-0.76,** 3.24 | | |
| | **200** | -9.76, **-2.89,** 3.04 | | |
| **Recovery NN50%** | **placebo** | | -2.05, **0.26,** 8.04 | -5.84, **-0.07,** 5.07 |
| | **100** | | 0.75, **4.14,** 9.23 | -3.91, **-0.94,** 9.90 |
| | **200** | | -14.35, **-2.77**, -0.42 | 1.67, **7.19*,** 9.00 |

Group sizes Placebo n= 31, 100µl = 33, 200µl = 33, significance levels * p< 0.05, ** p < 0.0

**Heart-rate variation**

For median and inter-quartile range measures of HRV, see Table 7.4. All data were adjusted for baseline (see Methods section 7.2.8).

A number of HRV parameters differed according to sex. These were SDRR at 200μl ($U = 78$, $p < 0.05$) and RMSSD at 200μl ($U = 71$, $p < 0.05$) during the anxiety film, and RMSSD at 200μl ($U = 76$, $p < 0.05$) and NN50% at 200μl ($U= 59$, $p <0.01$) during the recovery film-clip. These were analysed separately by sex.

Regardless of sex, during the neutral film there was a significant lavender dose-related increase in HRV (NN50%) in all participants ($J\text{-}T = 1.74$, $p < 0.05$). When each dose was compared with placebo this reached significance at 200μl ($U = 395$, $p < 0.05$) (see Figure 7.4).

In females, there were significant lavender dose-related trends in RMSSD during the anxiety film-clip ($J\text{-}T = 1.87$, $p < 0.05$) and in NN50% ($J\text{-}T = 1.94$, $p < 0.05$) during the recovery film-clip. Further pair-wise analysis of each lavender dose, compared with placebo, during the anxiety film showed that RMSSD was higher at the 200μl dose of lavender ($U = 115$, $p < 0.05$). Likewise, during the recovery film NN50% was significantly higher ($U = 114$, $p < 0.05$) at top dose (see Figures 7.6 and Table 7.4).

There were no trends in any of these measures in males; however, there was a non-parametric multivariate effect, in males, as measured by the Kruskall-Wallace test. There were significant group differences between the doses in the NN50% measure during the recovery film ($H = 6.10$, $p < 0.05$). However, pair-wise testing in males of each dose, compared with placebo for this measure, revealed no significant effects.

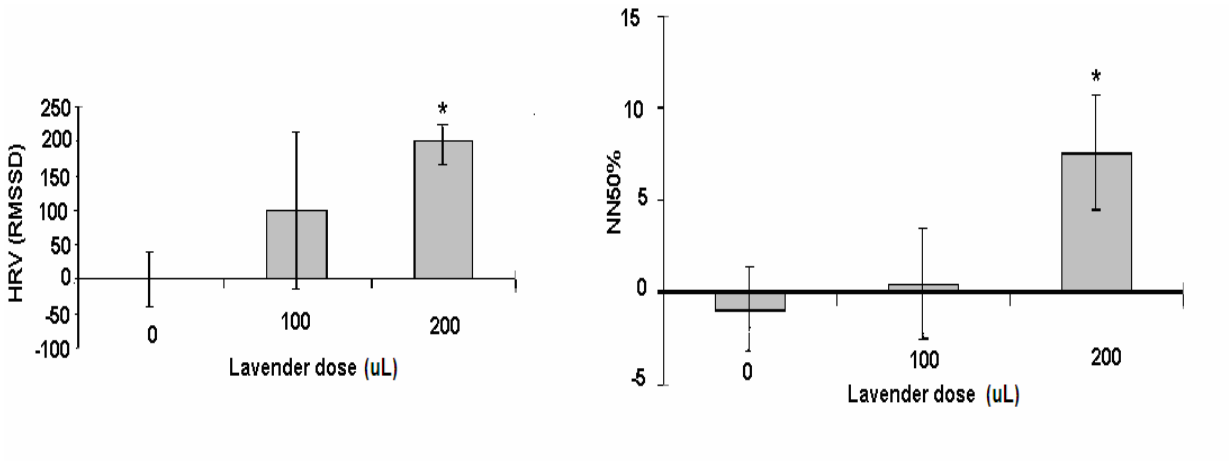



**Figure 7-6 The effects of oral lavender administration on heart-rate variation in females while watching an anxiety eliciting film-clip (RMSSD) and a recovery film-clip (NN50%). Data presented are baseline adjusted means and SEM.Statistical significance: * p < 0.05. Group sizes placebo n= 19, 100μl= 19, 200μl= 19; significance * p< 0.05.**

## 7.4 DISCUSSION

Using a placebo-controlled double-blind design, the effects of orally-administered lavender capsule were tested on self-report and physiological indicators of anxiety. Compared to the placebo, a 200μl dose caused a greater self-reported state anxiety reduction from baseline after the neutral film. There were no between-group differences after the anxiety-producing clips. Similar effects were noted for the physiological measures, with 200μl lavender causing lower heart-rate and GSR, and higher HRV, compared to placebo, after the neutral clip. Fewer between-dose differences were observed after the anxiety-provoking film-clip. 200μl administration was associated with a higher GSR in males after the anxiety clips suggesting an anxiogenic effect. In contrast, a higher HRV suggests a parasympathetically mediated anxiolytic effect in females. Again, HRV was higher during the recovery clip in females who had taken the 200μl lavender dose.

The most striking feature of these findings is that, after the neutral film-clip, lavender administration showed anxiolytic effects on self-report, HR, GSR and HRV measures, but that this effect was largely not sustained over the anxiety clips. There are a number of possible explanations for this. Firstly, it is possible that lavender's effects

might be of a short duration and could therefore have elapsed by the time that the anxiety-provoking film was shown. However, the fact that lavender was still active in the recovery phase in females weakens this explanation.

Secondly, lavender might be insufficiently strong to modulate highly anxiety-provoking stimuli. Acute, capsule-administered lavender might show effectiveness during resting states, but does not greatly moderate experimentally induced anxiety. The effect of lavender on anxious states could be dependent on chronic rather than acute administration. Studies examining effects in animals indicate that, like other anxiolytic drugs, lavender's anxiolytic effects potentiate over time (Bradley et al., 2007a). Thus, acute administration might not be sufficient to moderate the levels of anxiety experienced in this study. Indeed, many drugs prescribed to treat anxiety do not have anxiolytic effects when taken acutely; the SSRIs and buspirone often increase anxiety in the short-term, but are efficacious after a few weeks of administration (Sinclair & Nutt, 2007).

Alternatively, perhaps the dose of lavender was not high enough to elicit an anxiolytic response to the anxiety-provoking films in the human study. However, in studies looking at other EOs the converse has been found to be true, with lower doses being more effective in improving cognition and mood, particularly when participants were tested a few hours after capsule administration rather than immediately following digestion (Tildesley et al, 2005). These studies showed that low doses of Spanish sage EO (50μl) continued to have effects on mood for up to 6 hours after administration.

One possible explanation is that qualitatively different aspects of anxiety might have been elicited with apprehensive anxiety elicited during the neutral film, and panic anxiety, characterised by an inability to escape, elicited during the anxiety-provoking clips. In males, lavender increased GSR, an indication of increased sympathetic arousal. This increased sympathetic activity is similar to results found in response to diazepam administration in males (Teixeira-Silva et al., 2004). It is possible that the anxiety-eliciting clips produced anxiety more related to panic than mild apprehension since participants could not easily escape from watching the film-clips. Thus, the film-clips might represent proximal threat as described by Blanchard and Blanchard (1988) from which there is no easy escape. Hence, lavender could have reduced

anxiety related to apprehension and approach during the neutral film, but had less effect on panic/avoidance type anxiety in males. It might be significant that even prescribed anxiolytics, such as diazepam, and 5HT drugs, such as chlormipramine, have often lacked anxiolytic effects against experimentally induced anxiety (Teixeira-Silva et al., 2004); sometimes these drugs have even increased experimental anxiety (Guimaraes, Zuardi, & Graeff, 1987; Zuardi, 1990; Fell et al., 1985 in (Geddes, Gray, Millar, & Asbury, 1993)). Additionally the lack of cortisol response to the anxiety-eliciting film-clip lends weight to this, since there have been many reports that, during panic attacks, plasma (and thus salivary) cortisol does not increase (Garcia-Leal et al., 2005). The lack of cortisol response was not unexpected, since this has been found to be the case in other research using short film-clips (Hubert & Jong-Meyer, 1991). However, the fact that males showed an increased sympathetic response would indicate that the film elicited the rapid locus coeruleus, sympathetic arousal response, possibly mediated via the amygdala (Gorman & Sloan, 2000). In other film research, salivary cortisol has been shown to correlate with reports of mood. In this study, although there were trends of increasing negative affect, the mood measures failed to reach significance (Hubert & Jong-Meyer, 1989).

The increases in HRV in female participants, although only one measure, might be an indication that lavender interacted with the parasympathetic nervous system in females to modulate anxiety. However, similar HRV results in females subjected to the odour of lavender, following ten minutes inhalation (Saeki & Shiohara, 2001), provide evidence that lavender does increase HRV in females and that this is a true effect in this study too. Moderation of HRV by sex might reflect an interaction of lavender with neurosteroids. Estrogens also have a beneficial effect on HRV (Mercuro et al., 2000; Rosa Brito-Zurita et al., 2003) and there is evidence that lavender interacts with steroids (Henley, Lipson, Korach, & Bloch, 2007). Furthermore, estrogens have been shown to interact with the cholinergic system, which mediates vagal nerve activity, and also the 5HT system (McEwen & Alves, 1999). This might suggest alternative mechanisms of action for lavender. It has been reported that lavender lessened the anti-cholinergic side-effects of the antidepressant imipramine (Akhondzadeh et al., 2003). Thus, lavender could modulate the cholinergic system positively, which might explain its effects in increasing HRV here. There is evidence that linalool, a major component of lavender, exerts some of its

effects via modulation of muscarinic transmission as well as opiodergic, dopaminergic and $K^+$ channels (Peana et al., 2004).

Studies on the *in-vitro* neurotropic effects of lavender would suggest that any cholinergic effects are not because of direct interactions of lavender with the cholinergic system (Atanassova-Shopova & Roussinov, 1970). Furthermore, animal studies provide evidence for lavender having similar effects on behaviour to drugs that mediate their effects via 5HT pathways (Bradley et al, 2007a; chapter 6, Section 6.4). Interestingly, in both the 5HT and cholinergic systems there are differences between the male and female brain (McEwen & Alves, 1999). Other studies in animals provide evidence for a modulatory role of 5HT on cholinergic systems particularly in brain regions involved in anxiety, for example the dorsal hippocampus (File et al., 2000). SSRIs, which also mediate their effects via 5HT pathways, also have beneficial effects in increasing HRV in anxiety sufferers (Gorman & Sloan, 2000). Although there is a paucity of research on sex differences in response to anxiolytic drugs such as SSRIs, young (< 44) rather than older (>44), females have been shown to be more sensitive to the effects of 5HT drugs than males (Kornstein et al., 2000; Martenyi, Dossenbach, Mraz, & Metcalfe, 2001). Similarly, in this study, lavender seemed to have more effects in females than males. It would be interesting to investigate whether lavender has the same HRV-increasing effects in postmenopausal women, since the mean age of the females here was 35. Also, similar to drugs which exert their effects by direct interaction with the 5HT system, lavender's anxiolytic effects seem to potentiate over time (see chapter 6). Similar to this study lavender, seemed to have more potent anxiolytic effects in female rodents than male rodents (Bradley et al., 2007a). Thus, lavender's effects might be mediated via an interaction with the 5HT system.

Treatment of anxiety with BDZs is also influenced by sex as well as stage of oestrous (Kinrys & Wygant, 2005). Furthermore, lavender has been shown to potentiate GABA both *in-vitro* (Aoshima & Hamamoto, 1999) and *in-vivo* (Delaveau et al., 1989). In this study, females were tested regardless of their stage in the oestrous cycle and further investigation is required to ascertain whether lavender's beneficial effects are optimum at any particular stage of oestrous.

*In-vivo* and *in-vitro* studies on lavender and its components have shown it to have effects at a number of levels, such as on hormone, neurotransmitter, and second messenger systems. In addition to the neurotransmitter systems already mentioned, other examples of where lavender might interact include the glutamatergic system, responsible for excitation in the CNS (Elisabetsky et al., 1995), and histaminergic effects in the suprachiasmic nucleus, which is responsible for regulation of autonomic nervous system output (Tanida, Niijima, Shen, Nakamura, & Nagai, 2006). Lavender's interactions with second messenger systems are also wide ranging and include cAMP (Lis-Balchin & Hart, 1999), $K^+$ (Peana et al., 2004), $Na^+$, and $Ca^+$ channels (Ghelardini, Galeotti, Salvatore, & Mazzanti, 1999), and nitric oxide/ cGMP pathways (Koto et al., 2006). Lavender and its components have also been shown to interact directly with membranes (Teuscher et al., 1989; Teuscher et al., 1990). In addition, there is evidence that the whole oil is required for lavender to exert its beneficial anxiolytic effects (Barocelli et al., 2004) and since whole lavender oil is a complex mixture its pharmacological mode of action is also likely to be complex (Bradley et al., 2007a; Shellie et al., 2002).

It is therapeutically promising that lavender facilitated physiological recovery (by increasing HRV in a dose-response way during the recovery clip) since it is often after exposure to an acute stressor that anxiety develops (Matuszewich et al., 2007). Resilience and speed to recover from a stressor might be of importance in countering the onset of anxiety disorders, such as PTSD (Haglund, Nestadt, Cooper, Southwick, & Charney, 2007). These results could therefore, provide a role for the therapeutic properties of lavender in this important area of research. However, further investigation is needed, since there are no studies that examine the prolonged effects of lavender in humans. Although, in another study it was reported to still have positive effects on HRV for up to ten minutes following a lavender footbath (Saeki 2000). Furthermore, these results indicate that lavender still exerted an effect on the parasympathetic nervous system even though linalool was no longer present in the bloodstream: GC/MS pilot study results indicate that the linalool component of lavender was no longer present in the circulatory system during the recovery film (see appendix 5). However, studies examining the clearance rate of intragastrically administered linalool from mice indicate that a significant proportion of the linalool remained in body tissues for up to 72 hours, or longer, following ingestion and this might also be the case in humans (Parke, Rahman & Walker, 1974).

Importantly, these results provide support for a pharmacological effect of lavender aside from any effects because of its hedonic quality or conditioning. By contrast, in a study where participants were blinded to the dermal administration of linalool, thought to be one of the active components of lavender, it failed to have any effect on self-reported mood and only affected some physiological measures related to activation (Heuberger et al., 2004). However, since lavender did not have the predicted anxiolytic effect during the anxiety film it might be the case that the odour of the whole oil, or one or more of the components of lavender, are required for it to have anxiolytic effects during very stressful situations. It is also the case in treating anxiety that different drugs are required to treat immediate symptoms of panic than the more pervasive type of anxiety seen in general anxiety disorder (Gray & McNaughton, 2003; Sinclair & Nutt, 2007).

These results indicate a need for further work to examine whether the odour is required for lavender to exert its anxiolytic effects during conditions of high and immediate anxiety. Also, whether the failure of lavender to relieve anxiety in response to the anxiety film-clips was because of too low a dose being used, needs to be examined. Alternatively, a different type of test could be used to elicit anxiety more similar to GAD, such as the conditioned skin response test (Garcia-Leal et al., 2005).

In conclusion, acute orally-administered lavender appears to have beneficial effects in relieving mild anxiety and speeding recovery time. Proclivity to anxiety is a poor predictor of cardiac health (Gorman & Sloan, 2000) and twice as many women are reported to suffer from anxiety than men (Kinrys & Wigant, 2005). Given its beneficial effects on HRV in females, these results indicate that lavender's anxiolytic and stress-relieving effects in the longer term, particularly in females, are worthy of further investigation. Animal studies indicate that, similar to 5HT type drugs, lavender's effects potentiate over time (see Chapter 6). Future studies should also examine the effects of longer-term oral administration of lavender capsules on anxiety and wellbeing in both males and females.

# CHAPTER 8 SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

## 8.1 SIGNIFICANT CONTRIBUTIONS TO THE KNOWLEDGE BASE

There are three significant contributions to the knowledge base in this thesis:

First, these studies provide a validation of the gerbil EPM in males and females; this model has been validated previously only in female gerbils (Varty et al., 2002).

Second, the studies in gerbils have shown that both lavender and rose essential oil have anxiolytic effects which potentiate over time, rather than disappearing following acute odour administration (Cooke & Ernst, 2000). Lavender's effects were particularly apparent in females on measures related to risk-assessment.

Third, lavender had a clear dose-response effect in reducing mild anxiety in humans at rest, when tested acutely via oral administration (although it had little effect on induced anxiety). The route of administration, and the fact that lavender had dose-response effects, indicates that lavender's effects are not due to its odour properties but, more likely, are because of direct pharmacological effects. Again, and similar to results in gerbils, lavender's anxiolytic effects in human females were more noticeable particularly during the anxiety task and in the recovery phase of the study.

## 8.2 PARALLELS BETWEEN ANIMAL AND HUMAN STUDIES

Lavender had anxiolytic effects when tested acutely and chronically in the gerbil EPM and acutely in the study using human participants during the neutral film-clip. In contrast, lavender had only very mildly anxiolytic effects following chronic exposure to the odour when gerbils were tested in the BWB. It is interesting that the BWB might be modelling avoidance rather than the more cognitive, risk-assessment type, components of anxiety (see Chapter 5 section 5.4.2 and section 5.5; Gray & McNaughton, 2003). Anxiety is complex and, as discussed in the introduction, involves many different brain regions, depending on the type of anxiety experienced. Some examples of these brain regions include the amygdala, involved in avoidance and the phobic components of anxiety, and the higher cognitive regions, such as the septum, hippocampus, and frontal cortices, which are thought to be responsible for the conflicts that lead to excess worry and apprehension. In humans, during the anxiety film-clips, acute lavender had no anxiolytic effects in males and only very mildly anxiolytic effects in females. Similar to the BWB, it is possible that the anxiety film-clips used here also represent a more proximal type of threat leading to a desire to escape/avoid the films rather than the apprehension, which might have been experienced at the beginning of the study when watching the neutral film-clip. Further work is needed to examine whether lavender, both acutely and chronically, is more efficacious when worry-type anxiety is experienced rather than avoidance-type anxiety.

Interestingly, in both humans and gerbils, lavender's effects appeared to be more pronounced in females. Following both acute and prolonged testing in the gerbil EPM lavender's effects were more pronounced in females, particularly on risk-assessment type behaviours (protected head-dips). As mentioned, risk-assessment has been likened to human worry. In the human study, lavender had beneficial effects on HRV in females but not males; HRV has been related to decreased anxiety and faster recovery time. Lavender might be interacting with steroid hormones such as oestrogen. However, there are a number of differences between male and female brains, such as in the serotonin, glutamatergic, and cholinergic systems, just to mention three systems, and there is evidence that lavender might interact with all of

these systems (see earlier discussions). Since more females are reported to suffer from anxiety than males in the human population, then lavender is certainly worthy of further investigation.

## 8.3 POTENTIAL MECHANISMS OF ACTION

These results indicate that lavender's role in mediating anxiolysis might be via the more rostral brain structures, such as the prefrontal cortex and the septo-hippocampal system, involved in dealing with the approach-avoidance conflict created by the tasks used here (Degroot & Treit 2003). For example, in the EPM, lavender decreased risk-assessment behaviours; these types of behaviours have been linked to the cognitive, forward-oriented components of anxiety, such as worry (Carobrez & Bertoglio, 2005). Likewise, and also arguing in favour of this hypothesis, is the fact that, in the human study, lavender had anxiolytic effects during the neutral film when participants might have been apprehensive about what would happen in the next phase. In contrast, during the anxiety-provoking film-clips, lavender failed to have an anxiolytic effect on almost all measures tested. As discussed in section 7.4, it is possible that these clips caused avoidance-type anxiety rather than general anxiety. Interestingly, although the BWB did not validate in these studies, and therefore any interpretations that can be made from results using this model are limited, lavender only had very mild effects in the BWB in contrast to the EPM. It has been suggested that the BWB is a model of avoidance, which is controlled more by limbic structures, such as the amygdala, rather than the higher limbic/prefrontal regions, which are implicated in apprehension and general anxiety (see sections 2.2.3; 6.4.3).

Further evidence for lavender having an effect on the parts of the brain related to worry, i.e. the more frontal parts of the limbic system, comes from studies which show that lavender has an effect on working memory in humans (Moss et al., 2002). Although a wide network of brain regions are thought to be involved in working memory it is mainly the frontal cortex that is implicated. Injections of anxiolytic drugs into, and lesions to, the hippocampus have also been shown to impair working memory in a similar way to lavender's effects on working memory (McHugh, Niewoehner, Rawlins, & Bannerman, 2008; Gray & McNaughton, 2003). Furthermore, following pain, lavender enabled replacement of negative memories of

the pain with more positive memories i.e. that the pain was not so bad (Gedney et al., 2004). As mentioned in the introduction (section 1.2.4.2), drugs that have an effect on memory are being used in trials to replace bad memories with more positive ones. For example, drugs such as D-cycloserine, which is a partial NMDA agonist, have been shown to be of use in both rodents and humans in the unlearning of old negative memories and in replacing them with new positive memories (Davis, Ressler et al., 2006). At least one of lavender's components, linalool, has a mechanism of action which might be via ionotropic glutamate receptors, such as NMDA (Batista et al., 2008).

However, when compared with the results of other studies in humans (Heuberger et al.,2004), and likewise in rats (Cline et al., 2008), the results presented here in both humans and gerbils suggest effects for whole lavender oil or at least the contribution of other components, in addition to linalool, to lavender's mode of action. In both the Heuberger study and the Cline study, linalool alone failed to exert anxiolysis, whereas, in the studies here, whole lavender did have an anxiolytic effect. Perhaps lavender's complex mixture of chemicals might work synergistically to exert their effects overall, rather than on just one pathway, and it could be that the whole oil rather than one component is required. Interestingly, many prescribed anxiolytic drugs often work on more than one pathway in addition to their target pathway. For example, buspirone works on dopamine as well as 5HT-1A receptors and its metabolites also interact with alpha-2-adrenergic receptors (Leonard, 2003). Likewise, SSRIs are thought to work on a number of sites other than the serotonin reuptake transporter (Bianchi, 2008). Interestingly, these drugs also take up to two weeks to exert their anxiolytic effects in sufferers. This could be the case with lavender, since its effects in gerbils were very mild at first and potentiated over time. Similarly, in humans, acute effects were very mild and, in males, lavender actually increased sympathetic arousal in response to anxiety. Interestingly, the anxiolytic effects of SSRIs and buspirone take a few weeks before they become efficacious in relieving anxiety (Sinclair & Nutt, 2007). The fact that lavender has been shown to cause increases in cAMP (see section 1.4.4; Lis-Balchin & Hart, 1997, 1999) would lend weight to a mechanism of action via second messengers. Paradoxically, lavender had more pronounced anxiolytic effects in females in both humans and gerbils, arguing for an interaction with neurosteroids, such as oestrogens (Henley et al., 2007), or neurotransmitter systems that differ between males and females. Oestrogens in

conjunction with NMDA receptors have also been implicated with a role in hippocampal synaptic plasticity and memory (Mukai et al., 2006). Thus, whatever lavender's mechanism of action it is probably a complex one and via numerous different pathways. Hence, there is plenty of scope for future work.

## 8.4 FUTURE DIRECTIONS

Before investigating potential mechanisms of action, it is important to determine whether, and under what circumstances, lavender and its components actually relieve anxiety. For example, is lavender effective in providing relief in more cognitive forms of anxiety, such as worry, rather than the avoidance-type of anxiety? If lavender is more effective in relieving anxiety in situations of apprehension and GAD, rather than avoidance, then using film-clips might not be the best choice of test. Future studies might be better advised to look at the effects of lavender on the conditioned skin-conductance response test, which is thought to generate anxiety that is more like GAD-type anxiety (Garcia-Leal et al., 2005).

Testing lavender odour in gerbils indicated that lavender's effects potentiated over time. Do lavender's effects also potentiate over time in humans when lavender is administered orally? Longer-term studies need to be conducted to answer this question. The fact that it was the effects of the odour, and not oral administration, in gerbils might need to be taken into account in future studies in humans. However, that oral lavender had mildly anxiolytic effects in the human study would argue for lavender's odour not being required for its anxiolytic effects, at least in a relaxing situation. This is also confirmed by other studies examining the effects of EOs administered by routes other than odour in rodents (e.g. oral rosemary, Kovar et al., 1987; injected lavender, Umezu 2006) and humans (massage with linalool Heuberger et al., 2004; oral Spanish sage, and lemon balm, Tildesley et al., 2003; Tildesley et al., 2005).

However, in contrast to a relaxing low stress situation, is the odour required for lavender to have anxiolytic effects in more avoidance-type situations? The fact that

anxiolysis was not present on self-report measures during the anxiety-provoking task and only very weakly present in females, on HRV measures, does not really answer whether the odour needs to be present to relieve anxiety in an anxiety-provoking situation. In the human study, the odour of lavender was controlled to prevent confounding influences due to odour. However, looking at real world practicalities, it is of some use to speculate whether the odour potentiates the pharmacological effect. For example if lavender has a pharmacological effect and this effect is potentiated by its odour, then it would make more sense to use this route of administration and utilise both pharmacological and psychological routes to relieve anxiety in one administration, unless of course the person receiving the lavender does not like its odour!

Is lavender more effective in females than males? Results of both the gerbil and the human studies reported here indicate that lavender's effects are more beneficial in females than males. Lavender's beneficial effects on HRV, particularly in females, are interesting. As mentioned in the previous chapter, increased HRV is reflective of enhanced cardiovascular and psychological wellbeing and females are reported to suffer more from anxiety than are males. Interestingly facial expressions have been shown to be closely linked to cardiovascular reactivity (Lerner, Hariri, Dahl & Taylor, 2007). Future work will involve an examination of the video footage obtained from the human study reported here to look for any for sex effects related to lavender dose on facial expressions of anxiety.

Is there one component, or a group of components of lavender oil, that together might be more effective than the whole oil in relieving anxiety? A repeat study with some of lavender's components is warranted to ascertain whether it is the whole oil, or just one, two, or a few components that are responsible for its effects. Perhaps one or two of lavender's components that have not been so widely tested might be more effective than the whole oil, for example terpinene-4-ol, which is one of the breakdown products of linalool (Bickers et al., 2003).

## 8.5 LIMITATIONS

In the human study, the fact that lavender did not relieve anxiety during the anxiety film might have been because of the type of task; perhaps future work could involve other tasks which assess different aspects of anxiety, such as worry. In addition, perhaps showing the anxiety film nearer to the administration time of the capsules might have yielded different results. However, the study was designed to test the effects of lavender on anxiety when it was likely to be at its maximum in the bloodstream of all participants; GC/MS results indicated that this was after thirty minutes on average, which was when the anxiety film-clips were shown.

A comparison of the capsules with the odour would also have been of interest as well as a comparison of lavender with a positive control, such as diazepam. Additionally, use of the competitive $GABA_A$ receptor antagonist flumazenil would have been useful in identifying whether the films did elicit anxiety more akin to panic than apprehension.

It is also recognized that the gerbil studies were not without limitation as the problem of odour preference was not addressed. However, the fact that the gerbils were naïve to the odours tested means that some of the problems that occur when testing odours in humans, such as association and expectation effects, were circumvented. Likewise, the use of buspirone as a positive control in the prolonged studies would have been desirable, but for reasons beyond the control of the author this was not possible, although it was the intention to include buspirone as well as diazepam.

## 8.6 A FINAL WORD ABOUT ROSE OIL

The ability of rose oil to lessen anxiety without causing sedation and to reverse the trend for increased anxiety in females, would suggest that it and its components are worthy of further testing as a potential anxiolytic medication. Future work should involve a full investigation of the anxiolytic properties of rose EO and its components, in both animal models, such as the ones used here, and in human trials, particularly the type used here. Especially since the BWB could be a model of avoidance rather

than apprehensive anxiety and the anxiety film-clips might model the former rather than the latter type of anxiety.

## 8.7 CONCLUSIONS

In conclusion, it has been shown that prolonged exposure to lavender EO odour has anxiolytic effects in the models tested here, particularly in the EPM, and these effects are not merely because of its pleasant odour. Results in gerbils indicate that lavender's effects potentiate over time, and, since anxiety and stress are generally chronic conditions, studies examining the chronic effects of lavender in humans should be conducted. In humans, lavender had relaxing and anxiolytic effects in a low-anxiety situation, while, in both the gerbil and human studies described here, lavender's effects were more pronounced in the females of each species rather than in the males. This finding might be of particular importance, since anxiety is more prevalent in females (Kinrys & Wigant, 2005) and warrants further investigation.

Work is planned to continue on this valuable plant extract and perhaps in the future it, or its components, might prove to be a valuable and safe alternative in the search for drugs with fewer side-effects in the relief of mild anxiety and stress.

# REFERENCES


Abrams, J. K., Johnson, P. L., Hay-Schmidt, A., Mikkelsen, J. D., Shekhar, A., & Lowry, C. A. (2005). Serotonergic systems associated with arousal and vigilance behaviors following administration of anxiogenic drugs. *Neuroscience, 133,* 983-997.

Acosta, G. B. (1998). Administration of Cholecystokinin Sulphated Octapeptide (CCK-8S) Induces Changes on Rat Amino Acid Tissue Levels and on a Behavioral Test for Anxiety. *General Pharmacology, 31,* 637-641.

Adams, R. P. (1995). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy.* Carol Stream, IL, USA: Allured Publishing Corporation.

Aftanas, L. I., Reva, N. V., Savotina, L. N., & Makhnev, V. P. 2004, Neurophysiological correlates of induced discrete emotions in humans: an individual analysis, *Ross.Fiziol.Zh.Im I M.Sechenova*, vol. 90, no. 12, pp. 1457-1471

Agren, G. (1984). Pair Formation in the Mongolian Gerbil. *Animal Behaviour, 32,* 528-535.

Ågren, G., Zhou, Q., & Zhong, W. (1989). Ecology and Social Behaviour of Mongolian Gerbils, *Meriones unguiculatus,* at Xilinhot, Inner Mongolia, China. *Animal Behaviour, 37,* 11-27.

Akhondzadeh, S., Kashani, L., Fotouhi, A., Jarvandi, S., Mobaseri, M., Moin, M. et al. (2003). Comparison of Lavandula angustifolia Mill. tincture and imipramine in the treatment of mild to moderate depression: a double-blind, randomized trial. *Progress in Neuropsychopharmacoogy, Biology and Psychiatry, 27,* 123-127.

Albrechet-Souza, L., Borelli, K. G., & Brandao, M. L. (2008). Activity of the medial prefrontal cortex and amygdala underlies one-trial tolerance of rats in the elevated plus-maze. *Journal of Neuroscence Methods, 169,* 109-118.

Albrechet-Souza, L., Oliveira, A. R., De Luca, M. C., Tomazini, F. M., Santos, N. R., & Brandao, M. L. (2005). A comparative study with two types of elevated plus-maze (transparent vs. opaque walls) on the anxiolytic effects of midazolam, one-trial tolerance and fear-induced analgesia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29,* 571-579.

Alvaro, G. & Di Fabio, R. (2007). Neurokinin 1 receptor antagonists--current prospects. *Current Opinions in Drug Discovovery and Development 10,* 613-621.

Andlin-Sobocki, P. & Wittchen, H. U. (2005). Cost of anxiety disorders in Europe. *Euopean .Journal of Neurology., 12 Suppl 1,* 39-44.

Andreatini, R. & Bacellar, L. F. (2000). Animal models: trait or state measure? The test-retest reliability of the elevated plus-maze and behavioral despair. *Progress in Neuropsychopharmacology Biological Psychiatry, 24,* 549-560.

Antony, M. M., Orsillo, S. M., & Roemer, L. (2001). *Practitioners Guide to Empirically Based Measures of Anxiety.* AABT.

Aoshima, H. & Hamamoto, K. (1999). Potentiation of GABA(A) receptors expressed in Xenopus oocytes by perfume and phytoncid. *Bioscience Biotechnology and Biochemistry, 63,* 743-748.

APA (2000). *Diagnostic and Statistical Manual of Mental Disorders*. (4th Edition Text Revision ed.) Washington DC: American Psychiatric Association.

Aqil, M., Ahad, A., Sultana, Y., & Ali, A. (2007). Status of terpenes as skin penetration enhancers. *Drug Discovery Today, 12,* 1061-1067.

Archer, J. (1973). Tests for emotionality in rats and mice: A review. *Animal Behaviour, 21,* 205-235.

Archer, J. (1975). Rodent sex differences in emotional and related behavior. *Behavioral Biology, 14,* 451-479.

Argyropoulos, S. V., Sandford, J. J., & Nutt, D. (2000). The psychobiology of anxiolytic drugs. Part 2: pharmacological treatments of anxiety. *Pharmacology and Therapeutics, 88,* 213-227.

Assad, Y. O. H., Torto, B., Hassanali, A., Njagi, P. G. N., Bashir, N. H. H., & Mahamat, H. (1997). Seasonal variation in the essential oil composition of Commiphora quadricincta and its effect on the maturation of immature adults of the desert locust, Schistocerca gregaria. *Phytochemistry, 44,* 833-841.

Atanasova, B., Graux, J., El Hage, W., Hommet, C., Camus, V., & Belzung, C. (2008). Olfaction: A potential cognitive marker of psychiatric disorders. *Neuroscience & Biobehavioral Reviews, 32,* 1315-1325.

Atanassova – Shopova, S. & Roussinov, K. S. (1970). On certain central neurotropic effects of lavender Essential Oil. 13, 69-76. *Bulgarian Academic Science Bulletin Institute of Physiology, 13,* 69-76.

Atsumi, T. & Tonosaki, K. (2007). Smelling lavender and rosemary increases free radical scavenging activity and decreases cortisol level in saliva. *Psychiatry Reearch., 150,* 89-96.

Axel, R. (1995). The molecular logic of smell. *Scientific American, 273,* 154-159.

Badger, F. & Nolan, P. (2007). Use of self-chosen therapies by depressed people in primary care. *Journal of Clinical Nursing, 16,* 1343-1352.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils - A review. *Food and Chemical Toxicology, 46,* 446-475.

Baldwin, D. S., Anderson, I. M., Nutt, D. J., Bandelow, B., Bond, A., Davidson, J. R. et al. (2005). Evidence-based guidelines for the pharmacological treatment of

anxiety disorders: recommendations from the British Association for Psychopharmacology. *Journal Psychopharmaclogy, 19,* 567-596.

Ballantine, J., Bouckoms, A. J., Thomas, E. K., & Giriunas, I. E. (1987). Treatment of psychiatric illness by stereotactic cingulotomy. *Biological Psychiatry, 22,* 807-819.

Bangasser, D. A., Santollo, J., & Shors, T. J. (2005). The Bed Nucleus of the Stria Terminalis Is Critically Involved in Enhancing Associative Learning After Stressful Experience. *Behavioral Neuroscience, 119,* 1459-1466.

Baraza, E., Villalba, J. J., & Provenza, F. D. (2005). Nutritional context influences preferences of lambs for foods with plant secondary metabolites. *Applied Animal Behaviour Science, 92,* 293-305.

Barnea, A., Harborne, J. B., & Pannell, C. (1993). What parts of fleshy fruits contain secondary compounds toxic to birds and why? *Biochemical Systematics and Ecology, 21,* 421-429.

Barocelli, E., Calcina, F., Chiavarini, M., Impicciatore, M., Bruni, R., Bianchi, A. et al. (2004). Antinociceptive and gastroprotective effects of inhaled and orally-administered Lavandula hybrida Reverchon "Grosso" essential oil. *Life Sciences, 76,* 213-223.

Barry, J. M., Costall, B., Kelly, M. E., & Naylor, R. J. (1987). Withdrawal syndrome following subchronic treatment with anxiolytic agents. *Pharmacology Biochemistry and Behavior, 27,* 239-245.

Bastard, J. & Tiran, D. (2006). Aromatherapy and massage for antenatal anxiety: Its effect on the fetus. *Complementary Therapies in Clinical Practice, 12,* 48-54.

Basim, E. & Basim, H. (2003). Antibacterial activity of Rosa damascena essential oil. *Fitoterapia, 74,* 394-396.

Bateson, A. N. (2006). Further potential of the GABA receptor in the treatment of insomnia. *Sleep Medicine, 7,* S3-S9.

Batista, P. A., Werner, M. F., Oliveira, E. C., Burgos, L., Pereira, P., Brum, L. F. et al. (2008). Evidence for the involvement of ionotropic glutamatergic receptors on the antinociceptive effect of (-)-linalool in mice. *Neuroscience Letters, 440 (3),*299-303.

BBC, 2007. Warning on workers mental health. http://news.bbc.co.uk/1/hi/health/7680788.stm

Beck, A. T. & Clark, D. A. (1997). An information processing model of anxiety: Automatic and strategic processes. *Behaviour Research and Therapy, 35,* 49-58.

Bell, I. R., Schwartz, G. E., Amend, D., Peterson, J. M., & Stini, W. A. (1994). Sensitization to early life stress and response to chemical odors in older adults. *Biological Psychiatry, 35,* 857-863.

Bell, R. & Hobson, H. (1994). 5HT-1A receptor influences on rodent social and agonistic behavior: A review and empirical study. *Neuroscience & Biobehavioral Reviews, 18,* 325-338.

Belzung, C. & Philippot, P. (2007). Anxiety from a phylogenetic perspective: is there a qualitative difference between human and animal anxiety? *Neural Plasticity, 2007,* 59676.

Belzung, C. & Le Pape, G. (1994). Comparison of different behavioral test situations used in psychopharmacology for measurement of anxiety. *Physiology and Behavior, 56,* 623-628.

Bensafi, M., Rouby, C., Farget, V., Bertrand, B., Vigouroux, M., & Holley, A. (2002). Psychophysiological correlates of affects in human olfaction. *Clinical Neurophysiology., 32,* 326-332.

Beresford, I. J. M., Birch, P. J., Hagan, R. M., & Ireland, S. J. (1991). Investigation into species variants in tachykinin NK1 receptors by use of the non-peptide antagonist, CP-96,345. .*British Journal of Pharmacology,, 104,* 292-293.

Berliner, D. (2003). Natural Attraction. www.erox.com [On-line]. www.erox.com

Berliner, D. L. (1996). Steroidal Substances Active in the human Vomeronasal Organ Affect Hypothalamic Function. *Journal of Steroid Biochemistry and Molecular Biology., 58,* 1-2.

Berntson, G., Bigger, T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M. et al. (1997). Heart-rate variability: Origins, methods, and interpretive caveats. *Psychophysiology, 34,* 623-648.

Bhandawat, V., Reisert, J., & Yau, K. W. (2005). Elementary Response of Olfactory Receptor Neurons to Odorants. *Science, 308,* 1931-1934.

Bhattacharya, S. K., Satyan, K. S., & Chakrabarti, A. (1997). Anxiogenic action of caffeine: an experimental study in rats. *Journal of Psychopharmacology, 11,* 219-224.

Bianchi, M. T. (2008). Non-serotonin anti-depressant actions: Direct ion channel modulation by SSRIs and the concept of single agent poly-pharmacy. *Medical Hypotheses, 70,* 951-956.

Bickers, D., Calow, P., Greim, H., Hanifin, J. M., Rogers, A. E., Saurat, J. H. et al. (2003). A toxicologic and dermatologic assessment of linalool and related esters when used as fragrance ingredients. *Food and Chemical Toxicology, 41,* 919-942.

Birnbaum, S., Gobeske, K. T., Auerbach, J., Taylor, J. R., & Arnsten, A. F. T. (1999). A role for norepinephrine in stress-induced cognitive deficits: [alpha]-1-adrenoceptor mediation in the prefrontal cortex. *Biological Psychiatry, 46,* 1266-1274.

Birzniece, V., Johansson, I. M., Wang, M. D., Seckl, J. R., Backstrom, T., & Olsson, T. (2001). Serotonin 5HT-1A receptor mRNA expression in dorsal

hippocampus and raphé nuclei after gonadal hormone manipulation in female rats. *Neuroendocrinology, 74,* 135-142.

Bjering, S., Deinboll, I. I., & Maehlen, J. (2000). The olfactory sense. *Tidsskrift for Den Norske Laegeforening, 120,* 3719-3725.

Blanchard, D. C., Blanchard, R. J., Griebel, G., & Nutt, D. (2008). Chapter 2.4 Defensive behaviors, fear, and anxiety. In J.B.Robert (Ed.), *Handbook of Behavioral Neuroscience Handbook of Anxiety and Fear* (Volume 17 ed., pp. 63-79). Elsevier.

Blanchard, R. J. & Blanchard, D. C. (1989a). Antipredator Defensive Behaviors in a Visible Burrow System. *Journal of Comparative Psychology, 103,* 70-82.

Blanchard, R. J. & Blanchard, D. C. (1989b). Attack and defence in rodents as ethoexperimental models for the study of emotion. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 13,* S3-S14.

Blanchard, R. J., Blanchard, D. C., Griebel, G., & Nutt, D. (2008). Chapter 1.1 Introduction to the handbook on fear and anxiety. In J.B.Robert (Ed.), *Handbook of Behavioral Neuroscience Handbook of Anxiety and Fear* (Volume 17 ed., pp. *3-7). Elsevier.*

Blanchard, R. J., Blanchard, D. C., Rodgers, J., & Weiss, S. M. (1990). The characterization and modelling of antipredator defensive behavior. *Neuroscience and Biobehavioral Reviews, 14,* 463-472.

Blanchard, C. D., Hynd, A. L., Minke, K. A., Minemoto, T., & Blanchard, R. J. (2001). Human defensive behaviors to threat scenarios show parallels to fear- and anxiety-related defence patterns of non-human mammals. *Neuroscience and Biobehavioral Reviews, 25,* 761-770.

Blanchard, D. C., Shepherd, J. K., Carobrez, A. D., & Blanchard, R. J. (1991). Sex Effects in Defensive Behavior - Baseline Differences and Drug-Interactions. *Neuroscience and Biobehavioral Reviews, 15,* 461-468.

Blanchard, R. J., Yudko, E. B., Rodgers, R. J., & Blanchard, D. C. (1993). Defence system psychopharmacology: An ethological approach to the pharmacology of fear and anxiety. *Behavioural Brain Research, 58,* 155-165.

Blechert J., Lajtman M, Michael T, Margraf J, & Wilhelm F.H (2006). Identifying anxiety states using broad sampling and advanced processing of peripheral physiological information. *Biomedical Sciences Instrumentation., 42,* 136-141.

Blunt, E. M. (2003). Putting Aromatherapy in Practice. [Miscellaneous]. *Pharmacoeconomics, 17,* 329.

Bolmont, B. & Abraini, J. H. (2001). State-anxiety and low moods: Evidence for a single concept. *Physiology & Behavior, 74,* 421-424.

Bonasera, S. & Tecott, L. (2000). Mouse models of serotonin receptor function: toward a genetic dissection of serotonin systems. *Pharmacology and Therapeutics, 88,* 133-142.

Borg-Karlson, A. K. & Groth, I. (1986). Volatiles from the flowers of four species in the sections arachnitiformes and araneiferae of the genus Ophrys as insect mimetic attractants. *Phytochemistry, 25,* 1297-1299.

Boskabady, M. H., Kiani, S., & Rakhshandah, H. (2006). Relaxant effects of Rosa damascena on guinea pig tracheal chains and its possible mechanism(s). *Journal of Ethnopharmacology, 106,* 377-382.

Botella, P. & Parra, A. (2003). Coffee increases state anxiety in males but not in females. *Human Psychopharmacology, 18,* 141-143.

Bouali, S., Evrard, A., Chastanet, M., Lesch, K. P., Hamon, M., & Adrien, J. (2003). Sex hormone-dependent desensitization of 5HT-1A autoreceptors in knockout mice deficient in the 5HT transporter. *European Journal of Neuroscience, 18,* 2203-2212.

Bourin, M. (1997). Animal models of anxiety: are they suitable for predicting drug action in humans? *Polish Journal of Pharmacology, 49,* 79-84.

Bourin, M. & Hascoet, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology, 463,* 55-65.

Bowles, E. J., Cheras, P., Stevens, J., & Myers, S. (2005). A survey of aromatherapy practices in aged care facilities in northern NSW, Australia. *International Journal of Aromatherapy, 15,* 42-50.

Bowles, E.J., Griffiths, D. M., Quirk, L., Brownrigg, A., & Croot, K. (2002). Effects of essential oils and touch on resistance to nursing care procedures and other dementia-related behaviours in a residential care facility. *International Journal of Aromatherapy,* 12, 22-29.

Bradley, B. F., Starkey, N. J., Brown, S. L., & Lea, R. W. (2007). Anxiolytic effects of Lavandula angustifolia odour on the Mongolian gerbil elevated plus-maze. *Journal of Ethnopharmacology, 111,* 517-525.

Bradley, B. F., Starkey, N. J., Brown, S. L., & Lea, R. W. (2007). The effects of prolonged rose odor inhalation in two animal models of anxiety. *Physiology and Behav*ior, 92 (5), 931-938.

Bradwejn, J. & Koszycki, D. (1996). CCK-B Agonists and Panic Induction in Humans. *European Neuropsychopharmacology, 6,* 213.

Brain, P. F. (1999). UFAW Handbook on the Care and Management of Laboratory Animals . In T.Poole (Ed.) (7th ed..

Bramness, J. G., Skurtveit, S., & Morland, J. (2002). Clinical impairment of BDZs--relation between BDZ concentrations and impairment in apprehended drivers. *Drug and Alcohol Dependence,68 (2),* 131-141.

Brandt, C., Glien, M., Potschka, H., Volk, H., & L÷scher, W. (2003). Epileptogenesis and neuropathology after different types of status epilepticus induced by prolonged electrical stimulation of the basolateral amygdala in rats. *Epilepsy Research, 55,* 83-103.

Bridges, N. J. & Starkey, N. J. (2004). Sex differences in Mongolian gerbils in four tests of anxiety. *Physiology and Behavior, 83,* 119-127.

Bristow, L. J., O'Connor, D., Watts, R., Duxon, M. S., & Hutson, P. H. (2000). Evidence for accelerated desensitisation of 5HT2C receptors following combined treatment with fluoxetine and the 5HT-1A receptor antagonist, WAY 100,635, in the rat. *Neuropharmacology, 39,* 1222-1236.

British Broadcasting Corporation , 2007. More funds for talking therapies. BBC News Channel. Retrieved July, 2008 from, http://news.bbc.co.uk/1/hi/health/7037400.stm

Brocco, M., Dekeyne, A., Mannoury la, C. C., Touzard, M., Girardon, S., Veiga, S. et al. (2008). Cellular and behavioural profile of the novel, selective neurokinin1 receptor antagonist, vestipitant: a comparison to other agents. *Eur.Neuropsychopharmacol., 18,* 729-750.

Brodkin, J., Busse, C., Sukoff, S. J., & Varney, M. A. (2002). Anxiolytic-like activity of the mGluR5 antagonist MPEP: A comparison with diazepam and buspirone. *Pharmacology Biochemistry and Behavior, 73,* 359-366.

Brosschot, J. F., Van Dijk, E., & Thayer, J. F. (2007). Daily worry is related to low heart-rate variability during waking and the subsequent nocturnal sleep period. *International Journal of Psychophysiology, 63,* 39-47.

Broughan, C. (2001). *The effects of the olfactory properties of essential oils on selected aspects of cognition.* Coventry University.

Brown, T. A. & Barlow, D. H. (2005). Dimensional Versus Categorical Classification of Mental Disorders in the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders and Beyond: Comment on the Special Section. *Journal of Abnormal Psychology, 114,* 551-556.

Brown, T. A., Chorpita, B. F., & Barlow, D. H. (1998). Structural relationships among dimensions of the DSM-IV anxiety and mood disorders and dimensions of negative affect, positive affect, and autonomic arousal. *J.Abnorm.Psychol., 107,* 179-192.

Brownfield, A. B. (1998). Aromatherapy in arthritis: a study. [Report]. *Nursing Standard, 13,* 34-35.

Bruce, S. E., Yonkers, K. A., Otto, M. W., Eisen, J. L., Weisberg, R. B., Pagano, M. et al. (2005). Influence of psychiatric comorbidity on recovery and recurrence in generalized anxiety disorder, social phobia, and panic disorder: a 12-year prospective study. *American Journal of Psychiatry, 162,* 1179-1187.

Brum, L. F., Elisabetsky, E., & Souza, D. (2001). Effects of linalool on [(3)H]MK801 and [(3)H] muscimol binding in mouse cortical membranes. *Phytotherapy .Research, 15,* 422-425.

Brunet, A., Orr, S. P., Tremblay, J., Robertson, K., Nader, K., & Pitman, R. K. (2007). Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *Journal of Psychiatric Research.*

Bruneton, J. (1999). Essential Oils Physical Properties. In J.Hatton (Ed.) *Pharmacognosy Phtyochemistry Medicinal Plants* (Second ed., pp. 488). Paris: Lavoisier.

Buchbauer, G., Jager, W., Jirovetz, L., Ilmberger, J., & Dietrich, H. (1993). Therapeutic Properties of Essential Oils and Fragrances. *Acs Symposium Series, 525,* 159-165.

Buchbauer, G. & Jirovetz, L. (1994). Aromatherapy - Use of fragrances and essential oils as medicaments. *Flavour and Fragrance Journal, 9,* 217-222.

Buchbauer, G., Jirovetz, L., Czejka, M., Nasel, C., & Dietrich, H. (1993). New Results in aromatherapy research. *International symposium on essential oils, 24,* 18.

Buchbauer, G., Jirovetz, L., Jager, W., Dietrich, H., & Plank, C. (1991). Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation. *Zeitschrift für Naturforschung. C, Journal of biosciences, 46,* 1067-1072.

Buchbauer, G., Jirovetz, L., Jager, W., Plank, C., & Dietrich, H. (1993). Fragrance compounds and essential oils with sedative effects upon inhalation. *Journal of Pharmaceutical Sciences, 82,* 660-664.

Buckle, J. (2001). The role of aromatherapy in nursing care. *Nursing Clinics of North America, 36,* 57-+.

Bunney, W. E. & McKinney, W. T. (1969). Animal Models of Depression. *Archives of General Psychiatry, 21,* 240-248.

Burnett, K. M., Solterbeck, L. A., & Strapp, C. M. (2004). Scent and mood state following an anxiety-provoking task. *Psychological Reports, 95,* 707-722.

Buyukokuroglu, M. E., Gepdiremen, A., Hacimuftuoglu, A., & Oktay, M. (2003). The effects of aqueous extract of Lavandula angustifolia flowers in glutamate-induced neurotoxicity of cerebellar granular cell culture of rat pups. *Journal of Ethnopharmacology, 84,* 91-94.

Cacioppo, J. & Tassinary, L. (1999). *Principles of Psychophysiology. Physical, Social and Inferential Elements*. Cambridge University Press.

Carey, G. J., Costall, B., Domeney, A. M., Jones, D. N. C., & Naylor, R. J. (1992). Behavioural effects of anxiogenic agents in the common marmoset. *Pharmacology Biochemistry and Behavior, 42,* 143-153.

Carobrez, A. P. & Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neuroscience and Biobehavioral Reviews, 29,* 1193-1205.

Carr, A. (2001). *Abnormal Psychology Psychology Focus*. Hove [England], Philadelphia Psychology Press.

Carrasco, G. A. & Van de Kar, L. D. (2003). Neuroendocrine pharmacology of stress. *European Journal of Pharmacology, 463,* 235-272.

Castle, J. & Lis-Balchin, M. (2002). History of usage of Lavandula species. In M.Lis-Balchin (Ed.) *Lavender The genus* ( Taylor and Francis).

Cavanagh, H. M. A. & Wilkinson, J. N. (2002). Biological activities of lavender essential oil. *Phytotherapy Research, 16,* 301-308.

Ceballos, R. H., Matthews, K., Catledge, C., & Geisler, M. W. (2000). Quantifying states of relaxation with EEG and ECG: A comparison of biofeedback and aromatherapy. *Psychophysiology, 37,* S32.

Chadda, R. & Devaud, L. L. (2004). Sex differences in effects of mild chronic stress on seizure risk and GABAA receptors in rats. *Pharmacology Biochemistry and Behavior, 78,* 495-504.

Chambers, J. A., Power, K. G., & Durham, R. C. (2004). The relationship between trait vulnerability and anxiety and depressive diagnoses at long-term follow-up of Generalized Anxiety Disorder. *Journal of Anxiety Disorders, 18,* 587-607.

Chaouloff, F. (1994). Failure to find behavioural differences between lean and obese Zucker rats exposed to novel environments. *International Journal of Obesity, 18,* 780-782.

Chaouloff, F., Castanon, N., & Mormede, P. (1994). Paradoxical differences in animal models of anxiety among the Roman rat lines. *Neuroscience Letters, 182,* 217-221.

Chaouloff, F., Durand, M., & Mormede, P. (1997). Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. *Behavioural Brain Research, 85,* 27-35.

Chaouloff, F., Kulikov, A., Sarrieau, A., Castanon, N., & Mormede, P. (1995). Male Fischer 344 and Lewis rats display differences in locomotor reactivity, but not in anxiety-related behaviours: relationship with the hippocampal serotonergic system. *Brain Research, 693,* 169-178.

Charney, D. S., Heninger, G. R., & Redmond, J. (1983). Yohimbine induced anxiety and increased noradrenergic function in humans: Effects of diazepam and clonidine. *Life Sciences, 33,* 19-29.

Cheal, M. & Domesick, V. B. (1979). Mating in male Mongolian gerbils after olfactory bulbectomy. .*Physiology and Behavior, 22,* 199-202.

Cheeta, S., Kenny, P. J., & File, S. E. (2000a). Hippocampal and septal injections of nicotine and 8-OH-DPAT distinguish among different animal tests of anxiety. *Progress in Neuropsychopharmacology, Biological Psychiatry, 24,* 1053-1067.

Cheeta, S., Tucci, S., Sandhu, J., Williams, A. R., Rupniak, N. M. J., & File, S. E. (2001). Anxiolytic actions of the SP (NK1) receptor antagonist L-760735 and the 5HT-1A agonist 8-OH-DPAT in the social interaction test in gerbils. *Brain Research, 915,* 170-175.

Chorpita, B. F. & Barlow, D. H. (1998). The Development of Anxiety: The Role of Control in the Early Environment. *Psychological Bulletin, 124*, 3-21.

Clark, L. A. & Watson, D. (1991). Tripartite Model of Anxiety and Depression: Psychometric Evidence and Taxonomic Implications. *Journal of Abnormal Psychology, 100*, 316-336.

Clark, L. A., Watson, D., & Mineka, S. 1994, "Temperament, Personality, and the Mood and Anxiety Disorders,", *Journal of Abnormal Psychology*, vol. 103, no. 1, pp. 103-116.

Cline, M., Taylor, J. E., Flores, J., Bracken, S., McCall, S., & Ceremuga, T. E. (2008). Investigation of the anxiolytic effects of linalool, a lavender extract, in the male Sprague-Dawley rat. Journal of the Association of American Anaesthetists, *76*, 47-52.

CNS Forum, 2008. The areas of the brain affected by gernal anxiety disorder. http://www.cnsforum.com/content/pictures/imagebank/hirespng/Neuro_biol_GAD.png

Cole, J. C. & Rodgers, R. J. (1994). Ethological evaluation of the effects of acute and chronic buspirone treatment in the murine elevated plus-maze test: comparison with haloperidol. *Psychopharmacologia, 114*, 288-296.

Cole, J. C. & Rodgers, R. J. (1995). Ethological comparison of the effects of diazepam and acute/chronic imipramine on the behaviour of mice in the elevated plus-maze. *Pharmacology Biochemistry and Behavior, 52*, 473-478.

Cooke, B. & Ernst, E. (2000). Aromatherapy: a systematic review. *The British Journal Of General Practice: The Journal Of The Royal College Of General Practitioners, 50*, 493-496.

Cools, R., Robinson, O. J., & Sahakian, B. (2007). Acute Tryptophan Depletion in Healthy Volunteers Enhances Punishment Prediction but Does not Affect Reward Prediction. *Neuropsychopharmacology*.

Costall, B., Jones, B. J., Kelly, M. E., Naylor, R. J., & Tomkins, D. M. (1989). Exploration of mice in a black and white test box: Validation as a model of anxiety. *Pharmacology Biochemistry and Behavior, 32*, 777-785.

Costantino, H. R., Illum, L., Brandt, G., Johnson, P. H., & Quay, S. C. (2007). Intranasal delivery: Physicochemical and therapeutic aspects. *International Journal of Pharmaceutics, 337*, 1-24.

Crawley, J. & Goodwin, F. K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of BDZs. *Pharmacology Biochemistry and Behavior, 13*, 167-170.

Crozier, A., Clifford, M. N., & Ashihara, H. (2006). *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human*. Blackwell, Publishing

Cruz, A. P. M., Frei, F., & Graeff, F. G. (1994b). Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacology Biochemistry and Behavior, 49,* 171-176.

Cruz-Morales, S. E., Santos, N. R., & Brandao, M. L. (2002). One-trial tolerance to midazolam is due to enhancement of fear and reduction of anxiolytic-sensitive behaviors in the elevated plus-maze retest in the rat. *Pharmacology Biochemistry and Behavior, 72,* 973-978.

Cryan, J. F. & Dev, K. K. (2008). Chapter 4.4 The glutamatergic system as a potential therapeutic target for the treatment of anxiety disorders. In J.B.Robert (Ed.) *Handbook of Behavioral Neuroscience Handbook of Anxiety and Fear* (Volume 17 ed., pp. 269-301). Elsevier.

Cutler, M. G. & Piper, D. C. (1990). Chronic administration of the 5HT3 receptor antagonist BRL 43694; effects on reflex epilepsy and social behaviour of the Mongolian gerbil. *Psychopharmacologia, 101,* 244-249.

Dalton, P. (2000). Psychophysical and behavioral characteristics of olfactory adaptation. *Chemical Senses, 25,* 487-492.

Davidson, R. J. (2002). Anxiety and affective style: role of prefrontal cortex and amygdala. *Biological Psychiatry, 51,* 68-80.

Davis, M. (1992). The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *Trends in Pharmacological Sciences, 13,* 35-41.

Davis, M. (1998). Are different parts of the extended amygdala involved in fear versus anxiety? *Biological Psychiatry, 44,* 1239-1247.

Davis, J. L. & Eichenbaum, H. (1999). *Olfaction a Model System for Computational Neuroscience.* Cambridge: M. I. T. Press.

Davis, M., Myers, K. M., Chhatwal, J., & Ressler, K. J. (2006). Pharmacological treatments that facilitate extinction of fear: relevance to psychotherapy. *The Journal of the American Society for Experimental Therapeutics, 3,* 82-96.

Davis, M., Ressler, K., Rothbaum, B. O., & Richardson, R. (2006). Effects of D-cycloserine on extinction: translation from preclinical to clinical work. *Biological Psychiatry, 60,* 369-375.

de Almeida, R. N., Motta, S. C., Brito Faturi, C., Catallani, B., & Leite, J. R. (2004). Anxiolytic-like effects of rose oil inhalation on the elevated plus-maze test in rats. *Pharmacology Biochemistry and Behavior, 77,* 361-364.

De Araújo, J. E., Huston, J. P., & Brandão, M. L. (2001). Opposite effects of SP fragments C (anxiogenic) and N (anxiolytic) injected into dorsal periaqueductal gray. *European Journal of Pharmacology, 432,* 43-51.

de Beurs, E., den Hollander-Gijsman, M. E., Helmich, S., & Zitman, F. G. (2007). The tripartite model for assessing symptoms of anxiety and depression: Psychometrics of the Dutch version of the mood and anxiety symptoms questionnaire. *Behaviour Research and Therapy, 45,* 1609-1617.

Degroot, A. & Treit, D. (2003). Septal gabaergic and hippocampal cholinergic systems interact in the modulation of anxiety. *Neuroscience, 117,* 493-501.

Degroot, A. & Treit, D. (2002). Dorsal and ventral hippocampal cholinergic systems modulate anxiety in the plus-maze and shock-probe tests. *Brain Research, 949,* 60-70.

del Turina, A. V. & Perillo, M. A. (2003). Monoterpenes affect chlorodiazepoxide-micelle interaction through micellar dipole potential modifications. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1616,* 112-120.

Delaveau, P., Guillemain, J., Narcisse, G., & Rousseau, A. (1989). Sur les propriétés neuro-dépressives de l'huile essentiale de Lavande. *Neurophysiologie, 183,* 342-348.

Diego, M. A., Jones, N. A., Field, T., Hernandez-Reif, M., Schanberg, S., Kuhn, C. et al. (1998). Aromatherapy positively affects mood, EEG patterns of alertness and math computations. *International .Journal of Neuroscience, 96,* 217-224.

Dijksterhuis, G. B., Moller, P., Bredie, W. L. P., Rasmussen, G., & Martens, M. (2002). Gender and handedness effects on hedonicity of laterally presented odours. *Brain and Cognition, 50,* 272-281.

Dixon, A. K. & Fisch, H. U. (1998). Animal Models and Ethological Strategies for Early Drug-Testing in Humans. *Neuroscience and Biobehavioral Reviews, 23,* 345-358.

Dixon, A. K., Huber, C., & Kaesermann, F. (1984). Urinary odours as a source of indirect drug effects on the behaviour of male mice. *Progress in Clinical Biology Research, 167,* 81-91.

Do-Rego, J. C., Viana, A. F., Le Maitre, E., Deniel, A., Rates, S. M. K., Leroux-Nicollet, I. et al. (2006). Comparisons between anxiety tests for selection of anxious and non anxious mice. *Behavioural Brain Research, 169,* 282-288.

Dobson, H. E. M., Danielson, E. M., & Wesep, I. D. V. (1999). Pollen odor chemicals as modulators of bumble bee foraging on Rosa rugosa Thunb. (Rosaceae). *Plant Species Biology, 14,* 153-166(14).

Dodd, G. & Squirrell, D. J. (1980). The structure and function of the mammalian olfactory system. In M.Stoddart (Ed.) *Olfaction in Mammals. Proceedings of the Zoological society of London. Number 45.* ( London & New York: Academic press).

Dominguez, R., Cruz-Morales, S. E., Carvalho, M. C., Xavier, M., & Brandao, M. L. (2003). Sex differences in serotonergic activity in dorsal and median raphé nucleus. *Physiology and Behavior, 80,* 203-210.

Doty, R. L. (2001). Olfaction. *Annual Review of Psychology, 52,* 423-452.

Douglas Bremner, J., Randall, P., Scott, T. M., Capelli, S., Delaney, R., McCarthy, G. et al. (1995). Deficits in short-term memory in adult survivors of childhood abuse. *Psychiatry Research, 59,* 97-107.

Dragich, A. M. & Halpern, B. P. (2008). An oral-cavity component in retronasal smelling of natural extracts. *Physiology and Behavior, 93,* 521-528.

Duan, X., Tashiro, M., Wu, D., Yambe, T., Wang, Q., Sasaki, T. et al. (2007). Autonomic nervous function and localization of cerebral activity during lavender aromatic immersion. *Technology and Health Care, 15,* 69-78.

Dubuc, 2002, The brain from top to bottom, Canadian Institute of Neurosciences, Mental Health and Addiction. Retrieved July, 2008 from, http://thebrain.mcgill.ca/flash/i/i_04/i_04_m/i_04_m_peu/i_04_m_peu_2a.jpg

Duffy, R. A., Varty, G. B., Morgan, C. A., & Lachowicz, J. E. (2002). Correlation of neurokinin (NK) 1 receptor occupancy in gerbil striatum with behavioral effects of NK1 antagonists. *The Journal of Pharmacology and Experimental Therapeutics, 301,* 536-542.

Dunn, C., Sleep, J., & Collett, D. (1995). Sensing an improvement: an experimental study to evaluate the use of aromatherapy, massage and periods of rest in an intensive care unit. *Journal of Advanced Nursing, 21,* 34-40.

Dye, J. (1992). *Aromatherapy for Women and Children*. The CW Daniel Company Ltd.

Ebner, K., Rupniak, N. M., Saria, A., & Singewald, N. (2004). Substance P in the medial amygdala: Emotional stress-sensitive release and modulation of anxiety-related behavior in rats. *Proceedings of the National Academy of Sciences of the United States of America, 101,* 4280-4285.

Ebner, K. & Singewald, N. (2006). The role of substance P in stress and anxiety responses. *Amino Acids, 31,* 251-272.

Edelmann, R. J. (1992). *Anxiety Theory, Research and Intervention in Clinical and Health Psychology.* Chichester: John Wiley & Sons.

Ehrlichmann, H. & Bastone, L. (1992). The Use of Odour in the Study of Emotion. In S.Van Toller & G. H. Dodd (Eds.) *Fragrance: The Psychology and Biology of Perfume.* (pp. 143-159). New York: Elsevier Applied Science.

Einat, H., Yuan, P., & Manji, H. K. (2005). Increased anxiety-like behaviors and mitochondrial dysfunction in mice with targeted mutation of the Bcl-2 gene: further support for the involvement of mitochondrial function in anxiety disorders. *Behavioral Brain Research, 165,* 172-180.

El Yacoubi, M., Ledent, C., Parmentier, M., Costentin, J., & Vaugeois, J. M. (2000). The anxiogenic-like effect of caffeine in two experimental procedures measuring anxiety in the mouse is not shared by selective A(2A) adenosine receptor antagonists. *Psychopharmacologia, 148,* 153-163.

Elisabetsky, E., Marschner, J., & Souza, D. O. (1995). Effects of Linalool on glutamatergic system in the rat cerebral cortex. *Neurochemical Research, 20,* 461-465.

Emslie, M. J., Campbell, M. K., & Walker, K. A. (2002). Changes in public awareness of, attitudes to, and use of complementary therapy in North East

Scotland: surveys in 1993 and 1999. *Complementary Therapies in Medicine, 10,* 148-153.

Endler, N. S. & Kocovski, N. L. (2001). State and trait anxiety revisited. *Journal of Anxiety Disorders, 15,* 231-245.

Engberg, S., Ahlstedt, I., Leffler, A., Lindstr÷m, E., Kristensson, E., Svensson, A. et al. (2007). Molecular cloning, mutations and effects of NK1 receptor antagonists reveal the human-like pharmacology of gerbil NK1 receptors. *Biochemical Pharmacology, 73,* 259-269.

Engen, T. (1982). *The perception of odors*. New York: Academic Press.

Engin, E. & Treit, D. (2008). The effects of intra-cerebral drug infusions on animals' unconditioned fear reactions: A systematic review. *Progress in Neuropsychopharmacology Biological Psychiatry*.

Ernst, E. (2003). Herbal medicines put into context: Their use entails risks, but probably fewer than with synthetic drugs. *British medical Journal, 327,* 881-882.

Ernst, E. & White, A. (2000). The BBC survey of complementary medicine use in the UK. *Complementary Therapies in Medicine, 8,* 32-36.

Ettinger, A. B. & Argoff, C. E. (2007). Use of Antiepileptic Drugs for Nonepileptic Conditions: Psychiatric Disorders and Chronic Pain. *Neurotherapeutics, 4,* 75-83.

Eysenck, H. J. (1957). *The dynamics of anxiety and hysteria*. London: Routledge.

Fenske, M. (1991). Protein binding of cortisol by means of competitive adsorption: application to cortisol binding by serum of sixteen eutherian mammals. *Comparative Biochemistry and .Physiology A, 98,* 61-66.

Fernandes, C., Gonzalez, M. I., Wilson, C. A., & File, S. E. (1999). Factor Analysis Shows That Female Rat Behaviour Is Characterized Primarily by Activity, Male Rats Are Driven by Sex and Anxiety. *Pharmacology Biochemistry and Behavior, 64,* 731-736.

Fernandes, C. & File, S. E. (1996). The influence of open arm ledges and maze experience in the elevated plus-maze. *Pharmacology Biochemistry and Behavior, 54,* 31-40.

Fernandez, M., Hernandez-Reif, M., Field, T., Diego, M., Sanders, C., & Roca, A. (2004). EEG during lavender and rosemary exposure in infants of depressed and non-depressed mothers. *Infant Behavior and Development, 27,* 91-100.

Field, T., Diego, M., Hernandez-Reif, M., Cisneros, W., Feijo, L., Vera, Y. et al. (2005). Lavender fragrance cleansing gel effects on relaxation. *International Journal of Neuroscience, 115,* 207-222.

File, S. E. (2001). Factors controlling measures of anxiety and responses to novelty in the mouse. *Behavioural Brain Research, 125,* 151-157.

File, S. E. (1997). Anxiolytic action of a neurokinin1 receptor antagonist in the social interaction test. *Pharmacology Biochemistry and Behavior, 58,* 747-752

File, S. E., Cheeta, S., & Akanezi, C. (2001). Diazepam and nicotine increase social interaction in gerbils: a test for anxiolytic action. *Brain Research, 888,* 311-313.

File, S. E., Kenny, P. J., & Cheeta, S. (2000). The Role of the Dorsal Hippocampal Serotonergic and Cholinergic Systems in the Modulation of Anxiety. *Pharmacology Biochemistry and Behavior, 66,* 65-72.

Firestein, S. (1996). Olfaction: Scents and sensibility. *Current Biology, 6,* 666-667.

Fitzgerald, M., Culbert, T., Finkelstein, M., Green, M., Johnson, A., & Chen, S. (2007). The Effect of Gender and Ethnicity on Children's Attitudes and Preferences for Essential Oils: A Pilot Study. *EXPLORE: The Journal of Science and Healing, 3,* 378-385.

Fowler, N. A. (2006). Aromatherapy, Used as an Integrative Tool for Crisis Management by Adolescents in a Residential Treatment Center. *Journal of Child and Adolescent Psychiatric Nursing, 19,* 69-76.

Franzini, L. & Spears, W. (2003). Contributions of social context to inequalities in years of life lost to heart disease in Texas, USA. *Social Science and Medicine, 57,* 1847-1861.

Frey, H.-H., Löscher, R., Reiche, R., & Schulz, D. (1983). Anticonvulsant potency of common antiepilptic drugs in the gerbil. *Pharmacology, 27,* 330-335.

Friedman, B. H. & Thayer, J. F. (1998). Autonomic balance revisited: panic anxiety and heart-rate variability. *Journal of Psychosomatic Research, 44,* 133-151.

Frokjaer, V. G., Mortensen, E. L., Nielsen, F. +., Haugbol, S., Pinborg, L. H., Adams, K. H. et al. (2008). Frontolimbic Serotonin 2A Receptor Binding in Healthy Subjects Is Associated with Personality Risk Factors for Affective Disorder. *Biological Psychiatry, 63,* 569-576.

Frussa-Filho, R. & Ribeiro, R. d. (2002). One-trial tolerance to the effects of chlordiazepoxide in the elevated plus-maze is not due to acquisition of a phobic avoidance of open arms during initial exposure. *Life Sciences, 71,* 519-525.

Furmark, T., Appel, L., Michelgσrd, +., Wahlstedt, K., +hs, F., Zancan, S. et al. (2005). Cerebral Blood Flow Changes After Treatment of Social Phobia with the Neurokinin-1 Antagonist GR205171, Citalopram, or Placebo. *Biological Psychiatry, 58,* 132-142.

Gale, C. & Davidson, O. (2007). Generalised anxiety disorder. *British Medical Journal, 334,* 579-581.

Garcia-Leal, C., Parente, A. C. B. V., Del Ben, C. M., Guimaraes, F. S., Moreira, A. C., Elias, L. L. K. et al. (2005). Anxiety and salivary cortisol in symptomatic and nonsymptomatic panic patients and healthy volunteers performing simulated public speaking. *Psychiatry Research, 133,* 239-252.

Gattefosse, R. M. (1937). *Aromatherapy. (p58)*. Saffron Walden the C. W. Daniel Company Ltd.

Geddes, S. M., Gray, W. M., Millar, K., & Asbury, A. J. (1993). Skin conductance responses to auditory stimuli and anticipatory responses before venepuncture in patients premedicated with diazepam or morphine. *British .Journal of Anaesthesia, 71,* 512-516.

Gedney, J. J., Glover, T. L., & Fillingim, R. B. (2004). Sensory and affective pain discrimination after inhalation of essential oils. *Psychosomatic Medicine, 66,* 599-606.

Ghelardini, C., Galeotti, N., Salvatore, G., & Mazzanti, G. (1999). Local anaesthetic activity of the essential oil of Lavandula angustifolia. *Planta Medica, 65,* 700-703.

Gilman, A. G. (1987). G Proteins: Transducers of Receptor-Generated Signals. *Annual Review Of Biochemistry, 56,* 615-649.

Gobbi, G. & Blier, P. (2005). Effect of neurokinin-1 receptor antagonists on serotoninergic, noradrenergic and hippocampal neurons: Comparison with antidepressant drugs. *Peptides, 26,* 1383-1393.

Goel, N. & Grasso, D. J. (2004). Olfactory discrimination and transient mood change in young men and women: variation by season, mood state, and time of day. *Chronobioogy .International, 21,* 691-719.

Gomez, C., Saldivar-Gonzalez, A., Delgado, G., & Rodriguez, R. (2002). Rapid anxiolytic activity of progesterone and pregnanolone in male rats. *Pharmacology Biochemistry and Behavior, 72,* 543-550.

Gorman, J. M. & Sloan, R. P. (2000). Heart-rate variability in depressive and anxiety disorders. *American Heart Journal, 140,* 77-83.

Graeff, F. G. (1994). Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. *Brazilian Journal of Medical and Biological Research, 27,* 811-829.

Graeff, F. G., Parente, A., Del Ben, C. M., & Guimaraes, F. S. (2003). Pharmacology of human experimental anxiety. *Brazilian Journal of Medical and Biological Research, 36,* 421-432.

Graeff, F. G., Ferreira Netto, C., & Zangrossi, J. (1998). The elevated T-maze as an experimental model of anxiety. *Neuroscience & Biobehavioral Reviews, 23,* 237-246.

Graeff, F. G., Guimaraes, F. S., De Andrade, T. G. C. S., & Deakin, J. F. W. (1996). Role of 5HT in stress, anxiety, and depression. *Pharmacology Biochemistry and Behavior, 54,* 129-141.

Graham, C. S., Graham, B. G., Bartlett, J. A., Heald, A. E., & Schiffman, S. S. (1995). Taste and smell losses in HIV infected patients. *Physiology and Behavior, 58,* 287-293.

Graham, L., Wells, D. L., & Hepper, P. G. (2005). The influence of olfactory stimulation on the behaviour of dogs housed in a rescue shelter. *Applied Animal Behaviour Science, 91,* 143-153.

Graham, P. H., Browne, L., Cox, H., & Graham, J. (2003). Inhalation aromatherapy during radiotherapy: results of a placebo-controlled double-blind randomized trial. *Journal of Clinical Oncology, 21,* 2372-2376.

Grassmann, J., Hippeli, S., & Elstner, E. F. (2002). Plant's defence and its beneseizures for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant Physiology and Biochemistry, 40,* 471-478.

Gray, E. K. & Watson, D. (2007). Assessing positive and negative affect via self-report. In J.A.Coan & J. J. B. Allen (Eds.) *The handbook of emotion elicitation and assessment* (pp. 171-183). New York: Oxford University Press.

Gray, J. A. & McNaughton, N. (2003). *The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-Hippocampal System*. (2 edition (August 7, 2003) ed.) Oxford University Press, USA.

Gray, J. A. (1970). The psychophysiological basis of introversion-extraversion. *Behaviour Research and Therapy, 8,* 249-266.

Graziadei, P. P., Levine, R. R., & Monti Graziadei, G. A. (1978). Regeneration of Olfactory Axons and Synapse Formation in the Forebrain after Bulbectomy in Neonatal Mice. *Proceedings of the National Academy of Sciences of the United States of America, 75,* 5230-5234.

Green, S. & Hodges, H. (1991). *Behavioural Models in Psychopharmacology.* Cambridge: Cambridge University Press.

Greenfield, S. M., Innes, M. A., Allan, T. F., & Wearn, A. M. (2002). First year medical students' perceptions and use of complementary and alternative medicine. *Complement Ther.Med, 10,* 27-32.

Griebel, G., Moindrot, N., Aliaga, C., Simiand, J., & Soubrie, P. (2001). Characterization of the profile of neurokinin-2 and neurotensin receptor antagonists in the mouse defense test battery. *Neuroscience & Biobehavioral Reviews, 25,* 619-626.

Griebel, G., Perrault, G., & Sanger, D. J. (1998). Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. *Psychopharmacology (Berl) 138,* 55-66.

Griebel, G., Blanchard, D. C., & Blanchard, R. J. (1997). Evidence that the Behaviors in the Mouse Defence Test Battery Relate to Different Emotional States: A Factor Analytic Study. *Physiology and Behavior, 60,* 1255-1260.

Griebel, G. (1999). Is There a Future for Neuropeptide Receptor Ligands in the Treatment of Anxiety Disorders? *Pharmacology and Therapeutics, 82,* 1-61.

Griebel, G., Rodgers, R. J., Perrault, G., & Sanger, D. J. (1997). Risk-assessment Behaviour: Evaluation of Utility in the Study of 5HT-Related Drugs in the Rat

Elevated Plus-Maze Test. *Pharmacology Biochemistry and Behavior, 57,* 817-827.

Grös, D. F., Antony, M. M., Simms, L. J., & McCabe, R. E. (2007). Psychometric properties of the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA): Comparison to the State-Trait Anxiety Inventory (STAI). *Psychological Assessment, 19,* 369-381.

Gross, J. J. & Levenson, R. W. (1995). Emotion elicitation using films. *International Journal of Neuroscience, 9,* 87-108.

Guillemain, J., Rousseau, A., & Delaveau, P. (1989). [Neurodepressive effects of the essential oil of Lavandula angustifolia Mill]. *Annales Pharmaceutique Francaise, 47,* 337-343.

Guimaraes, F. S., Zuardi, A. W., & Graeff, F. G. (1987). Effect of chlorimipramine and maprotiline on experimental anxiety in humans. *Journal of Psychopharmacology, 1,* 184-192.

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine, 27,* 1-93.

Gutknecht, L., Jacob, C., Strobel, A., Kriegebaum, C., Muller, J., Zeng, Y. et al. (2007). Tryptophan hydroxylase-2 gene variation influences personality traits and disorders related to emotional dysregulation. *International Journal of Neuropsychopharmacology, 10,* 309-320.

Haddjeri, N. & Blier, P. (2001). Sustained blockade of neurokinin-1 receptors enhances serotonin neurotransmission. *Biological Psychiatry, 50,* 191-199.

Hagenbuch, N., Feldon, J., & Yee, B. K. (2006). Use of the elevated plus-maze test with opaque or transparent walls in the detection of mouse strain differences and the anxiolytic effects of diazepam. *Behavioral Pharmacology, 17,* 31-41.

Haglund, M. E. M., Nestadt, P. S., Cooper, N. S., Southwick, S. M., & Charney, D. S. (2007). Psychobiological mechanisms of resilience: Relevance to prevention and treatment of stress-related psychopathology. *Development and Psychopathology, 19,* 889-920.

Halpern, M., Daniels, Y., & Zuri, I. (2005). The role of the vomeronasal system in food preferences of the gray short-tailed opossum, Monodelphis domestica. *Nutrition and Metabolism, 2,* 6.

Halpin, Z. T. (1976). The role of individual recognition by odors in the social interactions of the Mongolian gerbil (Meriones unguiculatus). *Behaviour, 58,* 117-130.

Handley, S. L. & Mithani, S. (1984). Effects of alpha adrenoceptor agonists and antagonists in a maze exploration model of 'fear' motivated behaviour. *Nauyn-Schmiedeberg's Archives of Pharmacology, **327**,* 1-5.

Hanson, L. R. & Frey, W. H. (2007). Strategies for intranasal delivery of therapeutics for the prevention and treatment of neuroAIDS. *Journal of Neuroimmune Pharmacology, 2,* 81-86.

Hardelin, J. P. (2001). Kallmann syndrome: towards molecular pathogenesis. *Molecular and Cellular Endocrino, 179,* 75-81.

Hardy, M., Kirk-Smith, M., & Stretch, D. (1995). Replacement of drug treatment for insomnia by ambient odour. *The Lancet, 346,* 701.

Harris, B. (2002). Research: Reports. *International Journal of Aromatherapy, 11,* 167-170.

Hascoet, M. & Bourin, M. (1998). A new approach to the light/dark test procedure in mice. *Pharmacology, Biochemistry and Behavior, 60,* 645-653.

Hascoet, M., Colombel, M. C., & Bourin, M. (1999). Influence of age on behavioural response in the light/dark paradigm. *Physiology and Behavior, 66,* 567-570.

Hawkes, C. H., Shephard, B. C., & Daniel, S. E. (1997). Olfactory dysfunction in Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 62,* 436-446.

Hayley, S., Borowski, T., Merali, Z., & Anisman, H. (2001). Central monoamine activity in genetically distinct strains of mice following a psychogenic stressor: effects of predator exposure. *Brain Research, 892,* 293-300.

Haze, S., Sakai, K., & Gozu, Y. (2002). Effects of fragrance inhalation on sympathetic activity in normal adults. *Japanese Journal of Pharmacology, 90,* 247-253.

Hendrie, C. A., Weiss, S. M., & Eilam, D. (1996). Exploration and predation models of anxiety: Evidence from laboratory and wild species. *Pharmacology Biochemistry and Behavior, 54,* 13-20.

Henley, D. V., Lipson, N., Korach, K. S., & Bloch, C. A. (2007). Prepubertal gynecomastia linked to lavender and tea tree oils. *New England Journal of Medicine, 356,* 479-485.

Herd, M. B., Belelli, D., & Lambert, J. J. (2007). Neurosteroid modulation of synaptic and extrasynaptic GABAA receptors. *Pharmacology and Therapeutics, 116,* 20-34.

Hertzberg, M. A., Butterfield, M. I., Feldman, M. E., Beckham, J. C., Sutherland, S. M., Connor, K. M. et al. (1999). A preliminary study of lamotrigine for the treatment of posttraumatic stress disorder. *Biological Psychiatry, 45,* 1226-1229.

Heuberger, E., Redhammer, S., & Buchbauer, G. (2004a). Transdermal absorption of (-)-linalool induces autonomic deactivation but has no impact on ratings of wellbeing in humans. *Neuropsychopharmacology, 29,* 1925-1932.

Hill, M. N., Karacabeyli, E. S., & Gorzalka, B. B. (2007). Estrogen recruits the endocannabinoid system to modulate emotionality. *Psychoneuroendocrinology, 32,* 350-357.

Hodges, H. & Joseph, M. H. (1992). Anxiety, depression and mania: Animal models of psychiatric disorders : Vol. 3. Edited by P. Soubrie. Basel: Karger, 1991.

Price [UK pound]64.40. 126 pp. *Journal of Psychosomatic Research, 36,* 697-698.

Hoebel, B. G., Avena, N. M., & Rada, P. (2007). Accumbens dopamine-acetylcholine balance in approach and avoidance. *Current Opinions in .Pharmacology, 7,* 617-627.

Hoferl, M., Krist, S., & Buchbauer, G. (2006). Chirality influences the effects of linalool on physiological parameters of stress. *Planta Medica.72(13):1188-92.*

Holland, R. W., Hendriks, M., & Aarts, H. (2005). Smells Like Clean Spirit. Nonconscious Effects of Scent on Cognition and Behavior. *Psychological Science, 16,* 689-693.

Hollander, E., DeCaria, C., Nitescu, A., Cooper, T., Stover, B., Gully, R. et al. (1991). Noradrenergic function in obsessive-compulsive disorder: Behavioral and neuroendocrine responses to clonidine and comparison to healthy controls. *Psychiatry Research, 37,* 161-177.

Holmes, A., Heilig, M., Rupniak, N. M. J., Steckler, T., & Griebel, G. (2003). Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends in Pharmacological Sciences, 24,* 580-588.

Holmes, A., Iles, J. P., Mayell, S. J., & Rodgers, R. J. (2001). Prior test experience compromises the anxiolytic efficacy of chlordiazepoxide in the mouse light/dark exploration test. *Behavioural Brain Research, 122,* 159-167.

Hongratanaworakit, T., Heuberger, E., & Buchbauer, G. (2004). Evaluation of the effects of East Indian sandalwood oil and alpha-santalol on humans after transdermal absorption. *Planta Medica, 70,* 3-7.

Hornung, J. P. (2003). The human raphé nuclei and the serotonergic system. *Journal of Chemical Neuroanatomy, 26,* 331-343.

Horst, W. D. & Preskorn, S. H. (1998). Mechanisms of action and clinical characteristics of three atypical antidepressants: venlafaxine, nefazodone, bupropion. *Journal of Affective Disorders, 51,* 237-254.

Howell, D. C. (2002). Statistical Methods for Psychology. (5th ed.) 2002. In (5 ed., Duxbury, Thomson Learning.

Howes, M.-J. R., Houghton, P. J., Barlow, D. J., Pocock, V. J., & Milligan, S. R. (2002). Assessment of estrogenic activity in some common essential oil constituents. *Journal of Pharmacy and Pharmacology, 54,* 1521-1528.

Hoyer, D., Hannon, J. P., & Martin, G. R. (2002). Molecular, pharmacological and functional diversity of 5HT receptors. *Pharmacology Biochemistry and Behavior, 71,* 533-554.

Hubert, W. & Jong-Meyer, R. (1989). Emotional stress and saliva cortisol response. *Journal of Clinical Chemistry and Clinical Biochemistry, 27,* 235-237.

Hubert, W. & Jong-Meyer, R. (1990). Psychophysiological response patterns to positive and negative film stimuli. *Biological Psychology, 31,* 73-93.

Hubert, W. & Jong-Meyer, R. (1991). Psychophysiological response patterns to positive and negative film stimuli. *Biological Psychology 31,* 73-93.

Hubert, W. & Jong-Meyer, R. (1992). Saliva cortisol responses to unpleasant film stimuli differ between high and low trait anxious subjects. *Neuropsychobiology, 25,* 115-120.

Hulshoff Pol, H. E., Hijman, R., Tulleken, C. A. F., Heeren, T. J., Schneider, N., & van Ree, J. M. (2002). Odor discrimination in patients with frontal lobe damage and Korsakoff's syndrome. *Neuropsychologia, 40,* 888-891.

Hummel, T., Futschik, T., Frasnelli, J., & Huttenbrink, K. B. (2003). Effects of olfactory function, age, and gender on trigeminally mediated sensations: a study based on the lateralization of chemosensory stimuli. *Toxicology Letters, 140-141,* 273-280.

Hwang, I. K., Park, S. K., An, S. J., Yoo, K. Y., Kim, D. S., Jung, J. Y. et al. (2004). GABAA, not GABAB, receptor shows subunit- and spatial-specific alterations in the hippocampus of seizure prone gerbils. *Brain Research, 1003,* 98-107.

Iannilli, E., Gerber, J., Frasnelli, J., & Hummel, T. (2007). Intranasal trigeminal function in subjects with and without an intact sense of smell. *Brain Research, 1139,* 235-244.

Illum, L. (2003). Nasal drug delivery--possibilities, problems and solutions. *Journal of Controlled Release, 87,* 187-198.

International organisation for Standardisation (1997). Aromatic Natural Raw Materials- Vocabulary. ISO 9235:1997 (E/F). Geneva, Switzerland: Technical Committee ISO/TC54

Itai, T., Amayasu, H., Kuribayashi, M., Kawamura, N., Okada, M., Momose, A. et al. (2000). Psychological effects of aromatherapy on chronic hemodialysis patients. *Psychiatry and Clinical Neurosciences, 54,* 393-397.

Izidio, G. S., Lopes, D. M., Spricigo, L., Jr., & Ramos, A. (2005). Common variations in the pretest environment influence genotypic comparisons in models of anxiety. *Genes Brain and Behavavior, 4,* 412-419.

Jacob, S., Garcia, S., Hayreh, D., & McClintock, M. K. (2002). Psychological effects of musky compounds: comparison of androstadienone with androstenol and muscone. *Hormones and Behavior, 42,* 274-283.

Jacob, S. & McClintock, M. K. (2000). Psychological state and mood effects of steroidal chemosignals in women and men. *Hormones and Behavior, 37,* 57-78.

Jager, W., Buchbauer, G., Jirovetz, L., & Fritzer, M. (1992). Percutaneous absorption of lavender oil from a massage oil. *Journal of the Society of Cosmetic Chemists, **43**,* 49-54.

Jellinek, J. S. (1998). Odours and mental states. *International Journal of Aromatherapy, 9,* 115-120.

Jenck, F., Moreau, J. L., & Martin, J. R. (1995). Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: Elements of face and predictive validity. *Psychiatry Research, 57,* 181-191.

Jirovetz, L., Buchbauer, G., Jager, W., Raverdino, V., & Nikiforov, A. (1990). Determination of lavender oil fragrance compounds in blood samples. *Fresenius Journal of Analytical Chemistry 338,* 922-923.

Jirovetz, L., Buchbauer, G., Ngassoum, M. B., Essia-Ngang, J. J., Tatsadjieu, L. N., & Adjoudji, O. (2002). Chemical composition and antibacterial activities of the essential oils of Plectranthus glandulosus and Cinnamomum zeylanicum from Cameroon. *Scientia Pharmaceutica, 70,* 93-99.

Jirovetz, L., Buchbauer, G., Stoyanova, A., Balinova, A., Zhang, G. J., & Ma, X. H. (2005). Solid phase microextraction/gas chromatographic and olfactory analysis of the scent and fixative properties of the essential oil of Rosa damascena L. from China. *Flavour and Fragrance Journal, 20,* 7-12.

Johnsen, B. H., Thayer, J. F., Laberg, J. C., Wormnes, B., Raadal, M., Skaret, E. et al. (2003). Attentional and physiological characteristics of patients with dental anxiety. *Journal of Anxiety Disorders, 17,* 75-87.

Johnston, G. A. (1996). GABAc receptors: relatively simple transmitter -gated ion-channels? *Trends Pharmacol.Sci., 17,* 319-323.

Jorm, A. F., Christensen, H., Henderson, A. S., Jacomb, P. A., Korten, A. E., & Rodgers, B. 1998, "Using the BIS/BAS scales to measure behavioural inhibition and behavioural activation: Factor structure, validity and norms in a large community sample", Personality and Individual Differences, vol. 26, no. 1, pp. 49-58.

Kalin, N. H., Shelton, S. E., & Davidson, R. J. (2007). Role of the Primate Orbitofrontal Cortex in Mediating Anxious Temperament. *Biological Psychiatry, 62,* 1134-1139.

Kalueff, A. V. & Nutt, D. J. (2007). Role of GABA in anxiety and depression. *Depression and .Anxiety, 24,* 495-517.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (2000). *The priciples of neural science* .

Kanno, T., Asada, N., Yanase, H., Iwanaga, T., Ozaki, T., Nishikawa, Y. et al. (1999). Salivary secretion of highly concentrated chromogranin a in response to noradrenalinee and acetylcholine in isolated and perfused rat submandibular glands. *Experimental Physiology, 84,* 1073-1083.

Kaplan, H. & Miezejeski, C. (1972). Development of seizures in the Mongolian gerbil (Meriones unguiculatus). *Journal of Comparative and Physiological Psychology, 81,* 267-273.

Kawakami, K., Takai-Kawakami, K., Okazaki, Y., Kurihara, H., Shimizu, Y., & Yanaihara, T. (1997). The effect of odors on human newborn infants under stress. *Infant Behavior and Development, 20,* 531-535.

Keegan, L. (2003). Therapies to reduce stress and anxiety. *Critical Care Nursing Clinics of North America, 15,* 321-327.

Kelley, S. P., Bratt, A. M., & Hodge, C. W. (2003). Targeted gene deletion of the 5HT3A receptor subunit produces an anxiolytic phenotype in mice. *European Journal of Pharmacology, 461,* 19-25.

Kennedy, D. O., Scholey, A. B., Tildesley, N. T. J., Perry, E. K., & Wesnes, K. A. (2002). Modulation of mood and cognitive performance following acute administration of Melissa officinalis (lemon balm). *Pharmacology Biochemistry and Behavior, 72,* 953-964.

Kent, J. M., Mathew, S. J., & Gorman, J. M. (2002). Molecular targets in the treatment of anxiety. *Biological Psychiatry, 52,* 1008-1030.

Kerl, S. (1997). Can learning processes be influenced by olfacactory means. *Dragoco Report., 2,* 45-49.

Kessler, R. C., Davis, R. B., Foster, D. F., Van Rompay, M. I., Walters, E. E., Wilkey, S. A. et al. (2001). Long-term trends in the use of complementary and alternative medical therapies in the United States. *Annals of Internal Medicine, 135,* 262-268.

Keverne, B. (1980). Olfaction in the behaviour of non human primates. *Symposia of the zooological society London,, 45,* 313-327.

Keverne, E. B. (2002). Mammalian pheromones: from genes to behaviour. *Current Biology: CB, 12,* R807-R809.

Khalaj, L. & Farzin, D. (2006). Evaluation of antidepressant activities of rose oil and geranium oil in the forced swim test in mouse. *European Neuropsychopharmacology, 16,* S334-S335.

Khoshbouei, H., Cecchi, M., Dove, S., Javors, M., & Morilak, D. A. (2002). Behavioral reactivity to stress: Amplification of stress-induced noradrenergic activation elicits a galanin-mediated anxiolytic effect in central amygdala. *Pharmacology Biochemistry and Behavior, 71,* 407-417.

Kilfoil, T., Michel, A., Montgomery, D., & Whiting, R. L. (1989). Effects of anxiolytic and anxiogenic drugs on exploratory activity in a simple model of anxiety in mice. *Neuropharmacology, 28,* 901-905.

Kim, J. & Gorman, J. (2005). The psychobiology of anxiety. *Clinical Neuroscience Research, 4,* 335-347.

Kim, J. T., Wajda, M., Cuff, G., Serota, D., Schlame, M., Axelrod, D. M. et al. (2006). Evaluation of Aromatherapy in Treating Postoperative Pain: Pilot Study. *Pain Practice, 6,* 273-277.

Kim, N. S. & Lee, D. S. (2002). Comparison of different extraction methods for the analysis of fragrances from Lavandula species by gas chromatography-mass spectrometry. *Journal of Chromatography A, 982,* 31-47.

Kim, S. I., Roh, J. Y., Kim, D. H., Lee, H. S., & Ahn, Y. J. (2003). Insecticidal activities of aromatic plant extracts and essential oils against Sitophilus oryzae and Callosobruchus chinensis. *Journal of Stored Products Research, 39,* 293-303.

King, K. B. (1988). Anxiety reduction using fragrances. In S.Van Toller & G. Dodd (Eds.) *Perfumery: The Psychology and Biology of Fragrance* (pp. 147-165). London: Chapman.

Kinrys, G. & Wygant, L. E. (2005). Anxiety disorders in women: does gender matter to treatment?. *Revista Brasileira de Psiquiatria, 27 Suppl 2,* S43-S50.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28,* 76-81.

Knasko, S. C. (1992). Ambient odor's effect on creativity, mood, and perceived health. *Chemical Senses, 17,* 27-35.

Kohl, J. V., Atzmueller, M., Fink, B., & Grammer, K. (2001). Human pheromones: integrating neuroendocrinology and ethology. *Neuroendocrinology Letters, 22,* 309-321.

Kohlert, C., van Rensen, I., März, R., Schindler, G., Graefe, E. U., & Veit, M. (2000). Bioavailability and Pharmacokinetics of Natural Volatile Terpenes in Animals and Humans. *Planta Medica, 06,* 495-505.

Kornstein, S. G., Schatzberg, A. F., Thase, M. E., Yonkers, K. A., McCullough, J. P., Keitner, G. I. et al. (2000). Gender differences in treatment response to sertraline versus imipramine in chronic depression. *American Journal of Psychiatry, 157,* 1445-1452.

Korpi, E. R., Grunder, G., & Luddens, H. (2002). Drug interactions at GABAA receptors. *Progress in Neurobiology, 67,* 113-159.

Korte, S. M. (2001). Corticosteroids in relation to fear, anxiety and psychopathology. *Neuroscience & Biobehavioral Reviews, 25,* 117-142.

Kosel, M., Rudolph, U., Wielepp, P., Luginbuhl, M., Schmitt, W., Fisch, H. U. et al. (2004). Diminished GABA(A) receptor-binding capacity and a DNA base substitution in a patient with treatment-resistant depression and anxiety. *Neuropsychopharmacology, 29,* 347-350.

Kozlovskii, V. L. & Prakh'e IV (1995). [The effect of calcium channel blockers on animal behavior in tests to study their anxiolytic activity]. *Eksp.Klin.Farmakol., 58,* 18-20.

Koss, W. A., Gehlert, D. R., & Shekhar, A. (2004). Different effects of subchronic doses of 17-beta estradiol in two ethologically based models of anxiety utilizing female rats. *Hormones and Behavior, 46,* 158-164.

Koto, R., Imamura, M., Watanabe, C., Obayashi, S., Shiraishi, M., Sasaki, Y. et al. (2006). Linalyl-acetate as a major ingredient of lavender essential oil relaxes the rabbit vascular smooth muscle through dephosphorylation of myosin light chain. *Journal of Cardiovascular.Pharmacology, 48,* 850-856.

Kovacs, T., Cairns, N. J., & Lantos, P. L. (2001). Olfactory centres in Alzheimer's disease: olfactory bulb is involved in early Braak's stages. *NeuroReport, 12,* 285-288.

Kovar, K. A., Gropper, B., Friess, D., & Ammon, H. P. T. (1987). Blood levels of 1,8- cineole and locomotor activity of mice after inhalation and oral administration of rosemary oil. *Planta Medica, 53,* 315-318.

Kreibig, S. D., Wilhelm, F. H., Roth, W. T., & Gross, J. J. (2007). Cardiovascular, electrodermal, and respiratory response patterns to fear- and sadness-inducing films. *Psychophysiology, 44,* 787-806.

Kroeze, W. K. & Roth, B. L. (1998). The molecular biology of serotonin receptors: therapeutic implications for the interface of mood and psychosis. *Biological Psychiatry, 44,* 1128-1142.

Kulikov, A., Castanon, N., Mormede, P., & Chaouloff, F. (1995). Cerebral tryptophan hydroxylase activity, and 5HT(1A) receptor, 5HT(2A) receptor, and 5HT transporter binding in grouped and isolated Roman RHA and RLA rats: Relationships with behaviours in two models of anxiety. *Psychopharmacology, 121,* 385-395.

Kulkarni, S. K., Singh, K., & Bishnoi, M. (2007). Involvement of adenosinergic receptors in anxiety related behaviours. *Indian Journal of Experimental Biology, 45,* 439-443.

Lader, M. H. (1995). Clinical Pharmacology of Anxiolytic Drugs: Past, Present, and Future. In G.Biggio, E. Sanna, & E. Costa (Eds.) *$GABA_A$ Receptors and Anxiety: From neurobiology to Treatment* (pp. 135-149). New York: Raven Press.

Lamparsky, D. & Müller, P. M. (1994). *Perfumes, Art, Science and Technology*. Kluwer Academic Publishers.

Lang, P. J. (1968). Fear Reduction and fear behaviour: Problems in treating a construct. In J.M.Schlien (Ed.) *Research in Psychotherapy.* ( Washington D.C.: American Psychological Association.

Lang, P. J., Davis, M., & Ohman, A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *Journal of Affective Disorders, 61,* 137-159.

Lapiz, M. D. S. & Hogg, S. (2001). Behavioural response of gerbils to anxiolytics and antidepressants in the light and dark test. *European Neuropsychopharmacology, 11,* S189-S190.

Lawford, B. R., Young, D., Noble, E. P., Kann, B., Arnold, L., Rowell, J. et al. (2003). D2 dopamine receptor gene polymorphism: paroxetine and social functioning in posttraumatic stress disorder. *European Neuropsychopharmacology, 13,* 313-320.

Lawless, J. (1994). *Aromatherapy and the Mind.* London: Thorsons.

Lee, C. O. (2003). Clinical Aromatherapy Part II:Safe Guidelines for Integration Into Clinical Practice. *Clinical Journal of Oncology Nursing* 7 (5), 597-598.

Lehrner, J., Marwinski, G., Lehr, S., Johren, P., & Deecke, L. (2005). Ambient odors of orange and lavender reduce anxiety and improve mood in a dental office. *Physiology and Behavior, 86,* 92-95.

Lemon, K. (2004). An assessment of treating depression and anxiety with aromatherapy. *International Journal of Aromatherapy, 14,* 63-69.

Leonard, B. E. (1994). Anxiolytics and the Treatment of Anxiety Disorders. In *Fundamentals of Psychopharmacology* (pp. 99-112). New York: John Wiley & Sons.

Lerner, J.S., Dahl, R.E., Hariri, A.R. Taylor, S.E. 2007 Facial Expressions of Emotion Reveal Neuroendocrine and Cardiovascular Stress Responses. *Biological Psychiatry,* 61, 253-260.

Lesch, K. P., Zeng, Y., Reif, A., & Gutknecht, L. (2003). Anxiety-related traits in mice with modified genes of the serotonergic pathway. *European Journal of Pharmacology, 480,* 185-204.

Letizia, C. S., Cocchiara, J., Lalko, J., & Api, A. M. (2003). Fragrance material review on linalool. *Food and Chemical Toxicology, 41,* 943-964.

L'Hommedieu, G. & Hull, E. M. (1978). Role of vomeronasal system in gerbil male sexual behavior. *Eastern Conference on Reproductive Behavior*.

Lim, L. W., Temel, Y., Sesia, T., Vlamings, R., Visser-Vandewalle, V., Steinbusch, H. W. M. et al. (2008). Buspirone induced acute and chronic changes of neural activation in the periaqueductal gray of rats. *Neuroscience, 155,* 164-173.

Lin, D. & Parsons, L. H. (2002). Anxiogenic-like effect of serotonin1B receptor stimulation in the rat elevated plus-maze. *Pharmacology Biochemistry and Behavior, 71,* 581-587.

Liotti, M., Mayberg, H. S., Brannan, S. K., McGinnis, S., Jerabek, P., & Fox, P. T. (2000). Differential limbic-cortical correlates of sadness and anxiety in healthy subjects: implications for affective disorders. *Biological Psychiatry, 48,* 30-42.

Lis-Balchin, M. (1995). *The chemistry and bioactivity of essential oils*. (reprint ed.) Amberwood Publishing Limited.

Lis-Balchin, M. (2004). *Lavender: The genus*. (2nd ed.) Taylor & Francis Group.

Lis-Balchin, M. & Hart, S. (1999). Studies on the mode of action of the essential oil of lavender (Lavandula angustifolia P. Miller). *Phytotherapy Research, 13,* 540-542.

Lis-Balchin, M. & Hart, S. (1997). A preliminary study of the effect of essential oils on skeletal and smooth muscle in vitro. *Journal of Ethnopharmacology, 58,* 183-187.

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacology and Therapeutics, 46,* 321-340.

Long, L., Huntley, A., & Ernst, E. (2001). Which complementary and alternative therapies benefit which conditions? A survey of the opinions of 223 professional organizations. *Complementary Therapies in Medicine, 9,* 178-185.

Longo, L. P. & Johnson, B. (2000). Addiction: Part I. BDZs side-effects, abuse risk and alternatives. *American Family Physician, 61*, 2121-2128.

Loskota, W. J., Lomax, P., & Rich, S. T. (1974). The gerbil as a model for the study of the epilepsies. Seizure patterns and ontogenesis. *Epilepsia, 15*, 109-119.

Lucki, I. (1996). Serotonin receptor specificity in anxiety disorders. *Journal of Clinical Psychiatry, 57,* 5-10.

Ludvigson, H. W. & Rottman, T. R. (1989). Effects of ambient odors of lavender and cloves on cognition, memory, affect and mood. *Chemical Senses, 14*, 525-536.

Lukkes, J. L., Forster, G. L., Renner, K. J., & Summers, C. H. (2008). Corticotropin-releasing factor 1 and 2 receptors in the dorsal raphθ differentially affect serotonin release in the nucleus accumbens. *European Journal of Pharmacology, 578,* 185-193.

Luo, L. & Wiesenfeld-Hallin, Z. (1993). Low-dose intrathecal clonidine release tachykinins in rat spinal cord. *European Journal of Pharmacology, 235,* 157-159.

Lydiard, R. B. (2000). An overview of generalized anxiety disorder: disease state--appropriate therapy. *Clinical Therapeutics, 22,* A3-A24.

Maddocks-Jennings, W. & Wilkinson, J. M. (2004). Aromatherapy practice in nursing: literature review. Journal of Advanced Nursing 48[1], 93-103. Blackwell Publishing Limited.

Makatsori, A., Duncko, R., Schwendt, M., Moncek, F., Johansson, B. B., & Jezova, D. (2003). Voluntary wheel running modulates glutamate receptor subunit gene expression and stress hormone release in Lewis rats. *Psychoneuroendocrinology, 28,* 702-714.

Maldonado, E. & Navarro, J. F. (2000). Effects of 3,4-methylenedioxy-methamphetamine (MDMA) on anxiety in mice tested in the light-dark box. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 24,* 463-472.

Malik, M., Bigger, J., Camm, J., Kleiger, R., Malliani, A., Moss, A. et al. (1996). Heart-rate variability: Standards of measurement, physiological interpretation, and clinical use. *European Heart Journal, 17,* 354-381.

Mansour, A. A., Babstock, D. M., Penney, J. H., Martin, G. M., McLean, J. H., & Harley, C. W. (2003). Novel objects in a holeboard probe the role of the locus coeruleus in curiosity: support for two modes of attention in the rat. *Behavioral Neuroscience, 117,* 621-631.

Marsland, A. L., Salmon, P., Terry, P., & Stanford, S. C. (1990). Effects of propranolol on, and noradrenergic correlates of, the response to nonreward. *Pharmacology Biochemistry and Behavior, 35,* 41-46.

Marston, J. H. & Chang, M. C. (1965). The Breeding, Management and Reproductive Physiology of the Mongolian gerbil (*Meriones unguiculatus*). *Laboratory Animal Care, 15,* 34-48.

Martenyi, F., Dossenbach, M., Mraz, K., & Metcalfe, S. (2001). Gender differences in the efficacy of fluoxetine and maprotiline in depressed patients: a double-blind trial of antidepressants with serotonergic or norepinephrinergic reuptake inhibition profile. *European Neuropsychopharmacology, 11,* 227-232.

Matthews, G. & Gilliland, K. (1999). The personality theories of H. J. Eysenck and J. A. Gray: a comparative review. *Personality and Individual Differences, 26,* 583-626.

Mathews, A., Mackintosh, B., & Fulcher, E. P. (1997). Cognitive biases in anxiety and attention to threat. *Trends in Cognitive Sciences, 1,* 340-345.

Matuszewich, L., Karney, J. J., Carter, S. R., Janasik, S. P., O'Brien, J. L., & Friedman, R. D. (2007). The delayed effects of chronic unpredictable stress on anxiety measures. *Physiology and Behavior, 90,* 674-681.

Mauri, M. C., Laini, V., Cerveri, G., Scalvini, M. E., Volonteri, L. S., Regispani, F. et al. (2002). Clinical outcome and tolerability of sertraline in major depression: A study with plasma levels. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 26,* 597-601.

McCraty, R., Atkinson, M., Tiller, W. A., Rein, G., & Watkins, A. D. (1995). The effects of emotions on short-term power spectrum analysis of heart-rate variability. *American Journal of Cardiology, 76,* 1089-1093.

McEwen, B. S. & Alves, S. E. (1999). Estrogen Actions in the Central Nervous System. *Endocrine Reviews, 20,* 279-307.

McGrath, C. & Norman, T. R. (1998). The effect of venlafaxine treatment on the behavioural and neurochemical changes in the olfactory bulbectomised rat. *Psychopharmacology (Berl) 136,* 394-401.

McHugh, S. B., Deacon, R. M. J., Rawlins, J. N. P., & Bannerman, D. M. (2004). Amygdala and Ventral Hippocampus Contribute Differentially to Mechanisms of Fear and Anxiety. *Behavioral Neuroscience, 118,* 63-78.

McHugh, S. B., Niewoehner, B., Rawlins, J. N. P., & Bannerman, D. M. (2008). Dorsal hippocampal N-methyl-d-aspartate receptors underlie spatial working memory performance during non-matching to place testing on the T-maze. *Behavioural Brain Research, 186,* 41-47.

McKinney, J., Gardner, J., Barlow, G. W., & McGuire, M. T. (1994). Conceptual basis of animal models in psychiatry: A conference summary. *Ethology and Sociobiology, 15,* 369-382.

McLean, R. G. (2001). *Anxiety Disorders in Adults : An Evidence-Based Approach to Psychological Treatment* . Oxford University Press, Incorporated.

McNaughton, N. & Corr, P. J. (2004). A two-dimensional neuropsychology of defence: fear/anxiety and defensive distance. *Neuroscience and Biobehavioral Reviews, 28,* 285-305.

Mercuro, G., Podda, A., Pitzalis, L., Zoncu, S., Mascia, M., Melis, G. B. et al. (2000). Evidence of a role of endogenous estrogen in the modulation of autonomic nervous system. *The American Journal of Cardiology, 85,* 787-789.

Milad, M. R., Quirk, G. J., Pitman, R. K., Orr, S. P., Fischl, B., & Rauch, S. L. (2007). A Role for the Human Dorsal Anterior Cingulate Cortex in Fear Expression. *Biological Psychiatry, 62,* 1191-1194.

Millan, M. J. (2003). The neurobiology and control of anxious states. *Progress in Neurobiology, 70,* 83-244.

Mitchell, E. A., Herd, M. B., Gunn, B. G., Lambert, J. J., & Belelli, D. (2008). Neurosteroid modulation of GABAA receptors: Molecular determinants and significance in health and disease. *Neurochemistry International, 52,* 588-595.

Moghaddam, B. (2002). Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. *Biological Psychiatry, 51,* 775-787.

Mombaerts, P. (1999). Odorant receptor genes in humans. *Current Opinion in Genetics & Development, 9,* 315-320.

Montgomery, K. C. (1955). The relationship between fear induced by novel stimulation and exploratory behaviour. *Journal of Comparative and Physiological Psychology, 48,* 254-260.

Morgan, P. F. & Durcan, M. J. (1990). Caffeine-induced seizures: Apparent proconvulsant action of n-ethyl carboxamidoadenosine (NECA). *Life Sciences, 47,* 1-8.

MorganIII, C. A., Wang, S., Mason, J., Southwick, S. M., Fox, P., Hazlett, G. et al. (2000). Hormone profiles in humans experiencing military survival training. *Biological Psychiatry, 47,* 891-901.

Morilak, D. A., Cecchi, M., & Khoshbouei, H. (2003). Interactions of norepinephrine and galanin in the central amygdala and lateral bed nucleus of the stria terminalis modulate the behavioral response to acute stress. *Life Sciences, 73,* 715-726.

Morris, N. (2002). The effects of lavender (Lavendula angustifolium) baths on psychological well-being: two exploratory randomised control trials. *Complementary Therapies in Medicine, 10,* 223-228.

Morse, J. K. & Davis, J. N. (1990). Regulation of ischemic hippocampal damage in the gerbil: adrenalectomy alters the rate of CA1 cell disappearance. *Experimental Neurology, 110,* 86-92.

Moss, M., Cook, J., Wesnes, K., & Duckett, P. (2003). Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults. *International Journal of Neuroscience, 113,* 15-38.

Motomura, N., Sakurai, A., & Yotsuya, Y. (2001). Reduction of mental stress with lavender odorant. *Perceptual and Motor Skills, 93,* 713-718.

Mravec, B. (2005). A new focus on interoceptive properties of adrenal medulla. *Autonomic Neuroscience, 120,* 10-17.

Mukai, H., Takata, N., Ishii, H., Tanabe, N., Hojo, Y., Furukawa, A. et al. (2006). Hippocampal synthesis of estrogens and androgens which are paracrine modulators of synaptic plasticity: Synaptocrinology. *Neuroscience, 138,* 757-764.

Murphy, D. L., Mueller, E. A., Hill, J. L., Tolliver, T. J., & Jacobsen, F. M. (1989). Comparative anxiogenic, neuroendocrine, and other physiologic effects of m-chlorophenylpiperazine given intravenously or orally to healthy volunteers. *Psychopharmacology, 98,* 275-282.

Muzzarelli, L., Force, M., & Sebold, M. (2006). Aromatherapy and reducing preprocedural anxiety: A controlled prospective study. *Gastroenterology Nursing Journal, 29,* 466-471.

Nagai, Y., Critchley, H. D., Featherstone, E., Fenwick, P. B. C., Trimble, M. R., & Dolan, R. J. (2004). Brain activity relating to the contingent negative variation: an fMRI investigation. *NeuroImage, 21,* 1232-1241.

Narishetty, S. T. K. & Panchagnula, R. (2005). Effect of L-menthol and 1,8-cineole on phase behavior and molecular organization of SC lipids and skin permeation of zidovudine. *Journal of Controlled Release, 102,* 59-70.

Nasel, C., Nasel, B., Samec, P., Schindler, E., & Buchbauer, G. (1994). Functional imaging of effects of fragrances on the human brain after prolonged inhalation. *Chemical Senses, 19,* 359-364.

Nash, J. & Nutt, D. (2007). Antidepressants. *Psychiatry, 6,* 289-294.

The National Institute for Clinical Health and Excellence, 2007. Anxiety (amended) Management of anxiety disorder (panic disorder with or without agrophobia, and generalized anxiety disorder) in adults in primary, secondary and community care. Retrieved July, 2008 from http://www.nice.org.uk/nicemedia/pdf/CG022NICEguidelineamended.pdf

Nemeroff, C. B. (2003). The role of GABA in the pathophysiology and treatment of anxiety disorders. *Psychopharmacology Bulletin, 37,* 133-146.

Nishino, N. & Totsukawa, K. (1996). Study on the oestrous cycle in the Mongolian gerbil (Meriones unguiculatus). *Experimental Animals, 45,* 283-288.

Niskanen J-P, Tarvainen MP, Ranta-aho PO, & Karjalainen PA (2004). Software for Advanced HRV Analysis. *Computer Methods and Programs in Biomedicine, 76,* 73-81.

Nutt, D. (1990). The Pharmacology of Human Anxiety. *Pharmacology and Therapeutics, 47,* 233-266.

Nutt, D. (1995). The role of SSRIs in anxiety disorders. *European Neuropsychopharmacology, 5,* 260-261.

Nutt, D. (2005). Overview of Diagnosis and Drug Treatments of Anxiety Disorders. *CNS.Spectrums*. 10[1], 49-56. Elsevier.

Nutt, D. *(*2006). GABAA receptors: subtypes, regional distribution, and function. *Journal of Clinical Sleep Medicine,* 2, S7-11.

Ohl, F., Toschi, N., Wigger, A., Henniger, M. S. H., & Landgraf, R. (2001). Dimensions of Emotionality in a Rat Model of Innate Anxiety. *Behavioral Neuroscience, 115,* 429-436.

Ohloff, G. (1986). Chemistry of odor stimuli. *Experientia, 42,* 271-279.

Ohman, A. & Mineka, S. (2001). Fears, Phobias, and Preparedness: Toward an Evolved Module of Fear and Fear Learning. *Psychological Review, 108,* 483-522.

Oliver, J. T. & Peron, F. G. (1964). 19-hydroxy-11-deoxycortisol, a major steroid secreted by the adrenal gland of the Mongolian gerbil. *Steroids, 4,* 351-362.

Olsen, R. W., Wamsley, J. K., Lee, R. J., & Lomax, P. (1986). BDZ/barbiturate/GABA receptor-chloride ionophore complex in a genetic model for generalized epilepsy. *Advances in neurology, 44,* 365-378.

Olsson, M. J., Lundstrom, J. N., Diamantopoulou, S., & Esteves, F. (2006). A putative female pheromone affects mood in men differently depending on social context. *Revue Europeenne de Psychologie Appliquee/European Review of Applied Psychology, 56,* 279-284.

Opalchenova, G. & Obreshkova, D. (2003). Comparative studies on the activity of basil--an essential oil from Ocimum basilicum L.--against multidrug resistant clinical isolates of the genera Staphylococcus, Enterococcus and Pseudomonas by using different test methods. *Journal of Microbiological Methods, 54,* 105-110.

Overall, K. L. (2000). Natural Animal Models of Human Psychiatric Conditions: Assessment of Mechanism and Validity. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 24,* 727-776.

Ozel, M. Z., Gogus, F., & Lewis, A. C. (2006). Comparison of direct thermal desorption with water distillation and superheated water extraction for the analysis of volatile components of Rosa damascena Mill. using GCxGC-TOF/MS. *Analytica Chimica Acta, 566,* 172-177.

Pandaranandaka, J., Poonyachoti, S., & Kalandakanond-Thongsong, S. Differential effects of exogenous, endogenous estrogen on anxiety as measured by elevated T-maze in relation to the serotonergic system. *Behavioural Brain Research, In Press, Accepted Manuscript*.

Papadopoulos, V., Lecanu, L., Brown, R. C., Han, Z., & Yao, Z. X. (2006). Peripheral-type BDZ receptor in neurosteroid biosynthesis, neuropathology and neurological disorders. *Neuroscience, 138,* 749-756.

Pardon, M. C., Ma, S., & Morilak, D. A. (2003). Chronic cold stress sensitizes brain noradrenergic reactivity and noradrenergic facilitation of the HPA stress response in Wistar Kyoto rats. *Brain Research, 971,* 55-65.

Parke, D. V., Rahman, KH. M., & Walker, R. (1974). The absorption, distribution and excretion of linalool in the rat. *Biochemical Society Transactions, 2,* 612-615.

Peana, A. T., Marzocco, S., Popolo, A., & Pinto, A. (2006). (-)-Linalool inhibits in vitro NO formation: Probable involvement in the antinociceptive activity of this monoterpene compound. *Life Sciences, 78,* 719-723.

Peana, A. T., D'Aquila, P. S., Chessa, M. L., Moretti, M. D. L., Serra, G., & Pippia, P. (2003). (-)-Linalool produces antinociception in two experimental models of pain. *European Journal of Pharmacology, 460,* 37-41.

Peana, A. T., De Montis, M. G., Nieddu, E., Spano, M. T., D'Aquila, P. S., & Pippia, P. (2004). Profile of spinal and supra-spinal antinociception of (-)-linalool. *European Journal of Pharmacology, 485,* 165-174.

Pan, Y., Khalili, P., Ripps, H., & Qian, H. (2005). Pharmacology of GABAC receptors: responses to agonists and antagonists distinguish A- and B-subtypes of homomeric [rho] receptors expressed in Xenopus oocytes. *Neuroscience Letters, 376,* 60-65.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods, 14,* 149-167.

Penttila, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P. et al. (2001). ime domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clinical Physiology, 21*, 365-376.

Percival, A. (1995). The Use of Essential Oils. In *Aromatherapy A Nurses Guide* (pp. 8-11). Amberwood.

Perez, C. (2003). Clinical Aromatherapy Part I: An Introduction Into Nursing Practice. Clinical Journal of Oncology Nursing 7[5], 595-596. Oncology Nursing Society.

Peroutka, S. J. (1985). Selective interaction of novel anxiolytics with 5-hydroxytryptamine1A receptors. *Biological Psychiatry, 20,* 971-979.

Perry, N. & Perry, E. (2006). Aromatherapy in the management of psychiatric disorders - Clinical and neuropharmacological perspectives. *Cns Drugs, 20,* 257-280.

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional Neuroanatomy of Emotion: A Meta-Analysis of Emotion Activation Studies in PET and fMRI. *NeuroImage, 16,* 331-348.

Pieper, S., Brosschot, J. F., van der, L. R., & Thayer, J. F. (2007). Cardiac effects of momentary assessed worry episodes and stressful events. *Psychosomatic Medicine, 69,* 901-909.

Pitman, R. K., Sanders, K. M., Zusman, R. M., Healy, A. R., Cheema, F., Lasko, N. B. et al. (2002). Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biological Psychiatry, 51,* 189-192.

Pollack, M. H., van Amsterdam, H., Snyder, M., Brown, C., Ondrasik, J., Roy-Byrne, R. et al. (2005). P.4.025 Tiagabine in generalized anxiety disorder: a randomized, double-blind, placebo-controlled study. *European Neuropsychopharmacology, 15,* S538.

Pralong, E., Magistretti, P., & Stoop, R. (2002). Cellular perspectives on the glutamate-monoamine interactions in limbic lobe structures and their relevance for some psychiatric disorders. *Progress in Neurobiology, 67,* 173-202.

Prashar, A., Locke, I. C., & Evans, C. S. (2004). Cytotoxicity of lavender oil and its major components to human skin cells. *Cell Proliferation, 37,* 221-229.

Prediger, R. D., Batista, L. C., & Takahashi, R. N. (2004). Adenosine A1 receptors modulate the anxiolytic-like effect of ethanol in the elevated plus-maze in mice. *European Journal of Pharmacology, 499,* 147-154.

Price, S. (1998). *Aromatherapy Workbook*. London: Thorsons.

Price, S. & Price, L. (1999). *Aromatherapy for Health Professionals.* (Second ed.) London: Churchill Livingstone.

Prinssen, E. P. M., Ballard, T. M., Kolb, Y., & Nicolas, L. B. (2006). The effects of serotonin reuptake inhibitors on locomotor activity in gerbils. *Pharmacology Biochemistry and Behavior, 85,* 44-49.

Purdon, S. E. & FlorHenry, P. (2000). Asymmetrical olfactory acuity and neuroleptic treatment in schizophrenia [Full text delivery]. *Schizophrenia Research, 44,* 221-232.

Quiroga, E. N., Sampietro, A. R., & Vattuone, M. A. (2001). Screening antifungal activities of selected medicinal plants. *Journal of Ethnopharmacology, 74,* 89-96.

Rachmann, S. (2002). *Anxiety. Clinical psychology a modular Course.* Psychology Press. Taylor and Francis Group.

Raguso, R. A. (2004). Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Current Opinion in Plant Biology, 7,* 434-440.

Rakhshandah, H. & Hosseini, M. (2006). Potentiation of pentobarbital hypnosis by Rosa damascena in mice. *Indian Journal of Experimental Biology, 44,* 910-912.

Ramos, A., Berton, O., Mormede, P., & Chaouloff, F. (1997). A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behavioural Brain Research, 85,* 57-69.

Rawlings, F. & Meerabeau, L. (2003). Implementing aromatherapy in nursing and midwifery practice. *Journal of Clinical Nursing, 12,* 405-411.

Raymond, J. R., Mukhin, Y. V., Gelasco, A., Turner, J., Collinsworth, G., Gettys, T. W. et al. (2001). Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacology and Therapeutics, 92,* 179-212.

Re, L., Barocci, S., Sonnino, S., Mencarelli, A., Vivani, C., Paolucci, G. et al. (2000). Linalool modifies the nicotinic receptor-ion channel kinetics at the mouse neuromuscular junction. *Pharmacological Research, 42,* 177-182.

Reddy, G. V. P. & Guerrero, A. (2004). Interactions of insect pheromones and plant semiochemicals. *Trends in Plant Science, 9,* 253-261.

Refinetti, R. (2006). Variability of diurnality in laboratory rodents. *Journal of Comparative Physiology A,. Neuroethology, Sensory Neural and Behavioral Physiol*ogy, 1-14.

Ressler, K. J. & Mayberg, H. S. (2007). Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nature Neuroscience, 10,* 1116-1124.

Risberg, T., Kolstad, A., Bremnes, Y., Holte, H., Wist, E. A., Mella, O. et al. (2004). Knowledge of and attitudes toward complementary and alternative therapies; a national multicentre study of oncology professionals in Norway. *Eur J Cancer, 40,* 529-535.

Rodgers, R. J. (1997). Animal models of 'anxiety': where next? [Review]. *Behavioural Pharmacology, 8,* 477-496.

Rodgers, R. J. (2006). Animal models of human behaviour: can they help in understanding pathology and in drug development? *European Neuropsychopharmacology, 16,* S37-S39.

Rodgers, R. J. & Cole, J. C. (1993). Influence of social isolation, gender, strain, and prior novelty on plus-maze behaviour in mice. *Physiology and Behavior, 54,* 729-736.

Rodgers, R. J. & Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neuroscience and Biobehavioral Reviews, 21,* 801-810.

Rodgers, R. J. & Johnson, N. J. (1995). Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacology, Biochemistry and Behavior, 52,* 297-303.

Rodriguez, I., Greer, C. A., Mok, Y. M., & Mombaerts, P. (2000). A Putative Pheromone Receptor Gene Expressed in Human Olfactory Mucosa. *Nature Genetics, 26,* 18-19.

Rokers, B., Mercado, I. I. I., Allen, M. T., Myers, C. E., & Gluck, M. A. (2002). A Connectionist Model of Septohippocampal Dynamics During Conditioning: Closing the Loop. *Behavioral Neuroscience, 116,* 48-62.

Rosa Brito-Zurita, O., Posadas-Romero, C., Hermosillo, A. G., Zamora-Gonzalez, J., Hernandez-Ono, A., Cardoso-Saldana, G. et al. (2003). Estrogen effect on heart-rate variability in hypertensive postmenopausal women. *Maturitas, 44,* 39-48.

Roth, B. L., Willins, D. L., Kristiansen, K., & Kroeze, W. K. (1998). 5-Hydroxytryptamine2-Family Receptors (5-Hydroxytryptamine2A, 5-Hydroxytryptamine2B, 5-Hydroxytryptamine2C): Where Structure Meets Function. *Pharmacology andTherapeutics, 79,* 231-257.

Rottenberg, J., Ray, R. R., & Gross, J. J. (2007). Emotion elicitation using films. In J.A.Coan & J. J. B. Allen (Eds.) *The handbook of emotion elicitation and assessment* (pp. 9-28). New York: Oxford University Press.

Royal College of Psychiatrists, 1997. http://www.rcpsych.ac.uk/files/pdfversion/cr59.pdf

Royet, J. P., Hudry, J., Zald, D. H., Godinot, D., Gregoire, M. C., Lavenne, F. et al. (2001). Functional neuroanatomy of different olfactory judgments. *NeuroImage, 13,* 506-519.

Rupprecht, R. (2003). Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. *Psychoneuroendocrinology, 28,* 139-168.

Saeki, Y. (2000). The effect of foot-bath with or without the essential oil of lavender on the autonomic nervous system: a randomized trial. *Complementary Therapies in Medicine, 8,* 2-7.

Saeki, Y. & Shiohara, M. (2001). Physiological effects of inhaling fragnances. *International Journal of Aromatherapy, 11,* 118-125.

Saias, T. & Gallarda, T. Reactions d'agressivite sous BDZs : une revue de la litterature. *L'Encephale,* 34 (4), 330-336.

Salinas, E. O. & Hackett, D. (2001). Venlafaxine in treatment of GAD. *European Neuropsychopharmacology, 11,* S157-S158.

Salkovskis, P. M., Clark, D. M., & Gelder, M. G. (1996). Cognition-behaviour links in the persistence of panic. *Behaviour Research and Therapy, 34,* 453-458.

Salome, N., Viltart, O., Darnaudery, M., Salchner, P., Singewald, N., Landgraf, R. et al. (2002). Reliability of high and low anxiety-related behaviour:: influence of laboratory environment and multifactorial analysis. *Behavioural Brain Research, 136,* 227-237.

Salzman, C. (1993). BDZ treatment of panic and agoraphobic symptoms: Use, dependence, toxicity, abuse. *Journal of Psychiatric Research, 27,* 97-110.

Samuelsson, G. (1992). *Drugs of natural Origin*. (Third ed.) Stockholm: Swedish Pharmaceutical Press.

Sanchez, C. (1996). 5HT(1A) receptors play an important role in modulation of behavior of rats in a two-compartment black and white box. *Behavioural Pharmacology, 7,* 788-797.

Sanchez, C. (1997). Acute stress enhances anxiolytic-like drug responses of mice tested in a black and white test box. *European Neuropsychopharmacology, 7,* 283-288.

Sanchez, C. & Meier, E. (1997). Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? *Psychopharmacology, 129,* 197-205.

Sanchez, C. (2003). Stress-induced vocalisation in adult animals. A valid model of anxiety? *European Journal of Pharmacology, 463,* 133-143.

Sanders, C., Diego, M., Fernandez, M., Field, T., Hernandez-Reif, M., & Roca, A. (2002). EEG asymmetry responses to lavender and rosemary aromas in adults and infants. *International Journal of Neuroscience, 112,* 1305-1320.

Sandford, J. J., Argyropoulos, S. V., & Nutt, D. J. (2000). The psychobiology of anxiolytic drugs: Part 1: Basic neurobiology. *Pharmacology and Therapeutics, 88,* 197-212.

Santagostino, G., Amoretti, G., Frattini, P., Zerbi, F., Cucchi, M. L., Preda, S., & Corona, G. L. 1996, "Catecholaminergic, neuroendocrine and anxiety responses to acute psychological stress in healthy subjects: influence of alprazolam administration", *Neuropsychobiology*, vol. 34, no. 1, pp. 36-43.

Savic, I. (2001). Processing of Odorous Signals in Humans. *Brain Research Bulletin., 54,* 307-312.

Savic, I. & Gulyas, B. (2000). PET shows that odors are processed both ipsilaterally and contralaterally to the stimulated nostril. *NeuroReport, 11,* 2861-2866.

Savic, I. (2002). Imaging of brain activation by odorants in humans. *Current Opinion in Neurobiology, 12,* 455-461.

Sawynok, J. & Reid, A. (1986). Role of ascending and descending noradrenergic pathways in the antinociceptive effect of baclofen and clonidine. *Brain Research, 386,* 341-350.

Schelling, G., Roozendaal, B., Krauseneck, T., Schmoelz, M., DE Quervain, D., & Briegel, J. (2006). Efficacy of hydrocortisone in preventing posttraumatic stress disorder following critical illness and major surgery. *Annals of the New York Academy of Sciences, 1071,* 46-53.

Schelling, G. (2007). Post-traumatic stress disorder in somatic disease: lessons from critically ill patients. In E.R.de Kloet (Ed.) *Progress in Brain Research Stress Hormones and Post Traumatic Stress Disorder Basic Studies and Clinical Perspectives* (Volume 167 ed., pp. 229-237). Elsevier.

Schiffman, S. S., Sattely-Miller, E. A., Suggs, M. S., & Graham, B. G. (1995). The effect of pleasant odors and hormone status on mood of women at midlife. *Brain Research Bulletin, 36,* 19-29.

Schiffman, S. S., Suggs, M. S., & Sattely-Miller, E. A. (1995). Effect of pleasant odors on mood of males at midlife: Comparison of African-American and European-American men. *Brain Research Bulletin, 36,* 31-37.

Schmidt, M. V. & Muller, M. B. (2006). Animal models of anxiety. *Drug Discovery Today: Disease Models, 3,* 369-374.

Schnaubelt, K. (1998). *Advanced aromatherapy, the science of essential oil therapy.* (First ed.) Healing Arts Press.

Schnaubelt, K. (2005). Essential oil therapy according to traditional Chinese medical concepts. *International Journal of Aromatherapy, 15,* 98-105.

Setem, J., Pinheiro, A. P., Motta, V. A., Morato, S., & Cruz, A. P. M. (1999). Ethopharmacological Analysis of 5HT Ligands on the Rat Elevated Plus-Maze. *Pharmacology Biochemistry and Behavior, 62,* 515-521.

Shansky, R. M., Glavis-Bloom, C., Lerman, D., McRae, P., Benson, C., Miller, K. et al. (2004). Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. *Molecular Psychiatry, 9,* 531-538.

Shaw, D., Annett, J. M., Doherty, B., & Leslie, J. C. (2007). Anxiolytic effects of lavender oil inhalation on open-field behaviour in rats. Phytomedicine.14(9), 613-620..

Shellie, R., Mondello, L., Marriott, P., & Dugo, G. (2002). Characterisation of lavender essential oils by using gas chromatography-mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography. *Journal of Chromatography A, 970,* 225-234.

Shen, J., Niijima, A., Tanida, M., Horii, Y., Maeda, K., & Nagai, K. (2005). Olfactory stimulation with scent of lavender oil affects autonomic nerves, lipolysis and appetite in rats. *Neuroscience Letters, 383,* 188-193.

Shen, J., Niijima, A., Tanida, M., Horii, Y., Nakamura, T., & Nagai, K. (2007). Mechanism of changes induced in plasma glycerol by scent stimulation with grapefruit and lavender essential oils. *Neuroscience Letters, 416,* 241-246.

Shepherd, J. K., Flores, T., Rodgers, R. J., Blanchard, R. J., & Caroline Blanchard, D. (1992). The anxiety/defence test battery: Influence of gender and ritanserin treatment on antipredator defensive behavior. *Physiology and Behavior, 51,* 277-285.

Shiina, Y., Funabashi, N., Lee, K., Toyoda, T., Sekine, T., Honjo, S. et al. Relaxation effects of lavender aromatherapy improve coronary flow velocity reserve in healthy men evaluated by transthoracic Doppler echocardiography. *International Journal of Cardiology,129 (2),* 193-7.

Shimada, T., Matsumoto, K., Osanai, M., Matsuda, H., Terasawa, K., & Watanabe, H. (1995). The Modified Light/Dark Transition Test in Mice: Evaluation of Classic and Putative Anxiolytic and Anxiogenic Drugs. *General Pharmacology, 26,* 205-210.

Siegel, S. & Castellan, N. J. (1998). The case of K independent samples. In *Nonparametric Statistics for the Behavioural Sciences,* (2nd ed., pp. 190-223). McGraw Hill.

Silva Brum, L. F., Emanuelli, T., Souza, D. O., & Elisabetsky, E. (2001). Effects of linalool on glutamate release and uptake in mouse cortical synaptosomes. *Neurochemical Research, 26,* 191-194.

Silva, R. C. & Brandao, M. L. (2000). Acute and chronic effects of gepirone and fluoxetine in rats tested in the elevated plus-maze: an ethological analysis. *Pharmacology, Biochemistry, and Behavior, 65,* 209-216.

Sinclair, L. & Nutt, D. (2007). Anxiolytics. *Psychiatry, 6,* 284-288.

Singewald, N., Salchner, P., & Sharp, T. (2003). Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. *Biological Psychiatry, 53,* 275-283.

Slotnick, B., Glover, P., & Bodyak, N. (2000). Does intranasal application of zinc sulfate produce anosmia in the rat? *Behavioral Neuroscience, 114,* 814-829.

Smith, A. & Nutt, D. (1996). Noradrenalinee and attention lapses. *Nature, 380,* 291.

Smith, B. A. & Block, M. L. (1991). Male saliva cues and female social choice in Mongolian gerbils. *Physiology and Behavior, 50,* 379-384.

Smith, J. C., Bradley, M. M., & Lang, P. J. (2005). State anxiety and affective physiology: effects of sustained exposure to affective pictures. *Biological Psychology, 69,* 247-260.

Smythe, J. W., Bhatnagar, S., Murphy, D., Timothy, C., & Costall, B. (1998). The effects of intrahippocampal scopolamine infusions on anxiety in rats as measured by the black-white box test. *Brain Research Bulletin, 45,* 89-93.

Smythe, J. W., Murphy, D., Timothy, C., & Costall, B. (1997). Hippocampal mineralocorticoid, but not glucocorticoid, receptors modulate anxiety-like behavior in rats. *Pharmacology Biochemistry and Behavior, 56,* 507-513.

Smythe, J. W., Murphy, D., Bhatnagar, S., Timothy, C., & Costall, B. (1996). Muscarinic antagonists are anxiogenic in rats tested in the black-white box. *Pharmacology Biochemistry and Behavior, 54,* 57-63.

Snow, A. L., Hovanec, L., & Brandt, J. (2004). A controlled trial of aromatherapy for agitation in nursing home patients with dementia. *Journal of Alternative and Complementary Medicine, 10,* 431-437.

Sobel, N., Prabhakaran, V., Desmond, J. E., Glover, G. H., Goode, R. L., Sullivan, E. V. et al. (1998). Sniffing and smelling: separate subsystems in the human olfactory cortex. *Nature, 392,* 282-286.

Sobel, N., Prabhakaran, V., Hartley, C. A., Desmond, J. E., Glover, G. H., Sullivan, E. V. et al. (1999). Blind smell: brain activation induced by an undetected air-borne chemical. *Brain; a Journal of Neurology, 122 Part 2,* 209-217.

Soden, K., Vincent, K., Craske, S., Lucas, C., & Ashley, S. (2004). A randomized controlled trial of aromatherapy massage in a hospice setting. *Palliative Medicine, 18,* 87-92.

Song, C. & Leonard, B. E. (2005). The olfactory bulbectomised rat as a model of depression. *Neuroscience and Biobehavioral Reviews, 29,* 627-647.

Souetre, E., Lozet, H., Cimarosti, I., Martin, P., Chignon, J. M., Ades, J. et al. (1994). Cost of anxiety disorders: Impact of comorbidity. *Journal of Psychosomatic Research, 38,* 151-160.

Spiacci, J., Kanamaru, F., Guimarπes, F. S., & Oliveira`, R. M. W. (2008). Nitric oxide-mediated anxiolytic-like and antidepressant-like effects in animal models of anxiety and depression. *Pharmacology Biochemistry and Behavior, 88,* 247-255.

Spielberger, C., Gorusch, R., Lushene, R., Vagg, P., & Jacobs, G. (1983). *State-trait anxiety inventory for adults*. Mind Garden Inc.

Spinella, M. (2002). A relationship between smell identification and empathy. *International Journal of Neuroscience, 112,* 605-612.

Stanford, S. C., Baldwin, H. A., & File, S. E. (1989). Effects of a single or repeated administration of the BDZ inverse agonist FG7142 on behaviour and cortical adrenoceptor binding in the rat. *Psychopharmacologia, 98,* 417-424.

Starkey, N. J. (2000). *Ethological and Pharmacological Examination of Social Behaviour in Gerbils (Meriones unguicalatus).* PhD Thesis, Leeds University.

Starkey, N. J., Normington, G., & Bridges, N. J. (2007). The effects of individual housing on `anxious' behaviour in male and female gerbils. *Physiology and Behavior, 90,* 545-552.

Stathis, M., Scheffel, U., Lever, S. Z., Boja, J. W., Carroll, F. I., & Kuhar, M. J. (1995). Rate of binding of various inhibitors at the dopamine transporter in vivo. *Psychopharmacology (Berl) 119,* 376-384.

Stern, R., RAY, W., & Quigley, K. (2001). *Psychophysiological Recording*. (Second ed.) New York: Oxford University Press.

Stockhorst, U. & Pietrowsky, R. (2004). Olfactory perception, communication, and the nose-to-brain pathway. *Physiology and Behavior, 83,* 3-11.

Stoddart, M. (1976). *Mammalian Odours and Pheromones.* Camelot Press Limited.

Strous, R. D., Maayan, R., & Weizman, A. (2006). The relevance of neurosteroids to clinical psychiatry: From the laboratory to the bedside. *European Neuropsychopharmacology, 16,* 155-169.

Struzik, L., Vermani, M., Coonerty-Femiano, A., & Katzman, M. A. (2004). Treatments for generalized anxiety disorder. *Expert Review of Neurotherapeutics, 4,* 285-294.

Sullivan, G. M., Coplan, J. D., Kent, J. M., & Gorman, J. M. (1999). The noradrenergic system in pathological anxiety: a focus on panic with relevance to generalized anxiety and phobias. *Biological Psychiatry, 46,* 1205-1218.

Swaab, D. F. (2004). Neuropeptides in Hypothalamic Neuronal Disorders. In W.J.Kwang (Ed.) *International Review of Cytology* (Volume 240 ed., pp. 305-375). Academic Press.

Tabachnick, B. & Fidell, S. L. (2001). *Using Multivariate Statistics*. (4th International ed.).

Takahashi, L. K. (2001). Role of CRF1 and CRF2 receptors in fear and anxiety. *Neuroscience and Biobehavioral Reviews, 25,* 627-636.

Tanida, M., Niijima, A., Shen, J., Nakamura, T., & Nagai, K. (2006). Olfactory stimulation with scent of lavender oil affects autonomic neurotransmission and blood pressure in rats. *Neuroscience Letters*.

Tannenbaum, B. & Anisman, H. (2003). Impact of chronic intermittent challenges in stressor-susceptible and resilient strains of mice. *Biological Psychiatry, 53,* 292-303.

Tapiero, H., Mathe, G., Couvreur, P., & Tew, K. D. (2002). II. Glutamine and glutamate. *Biomedecine and Pharmacotherapy, 56,* 446-457.

Tassone, D. M., Boyce, E., Guyer, J., & Nuzum, D. (2007). Pregabalin: a novel gamma-aminobutyric acid analogue in the treatment of neuropathic pain, partial-onset seizures, and anxiety disorders. *Clinical Therapeutics, 29,* 26-48.

Tatsadjieu, L. N., Essia Ngang, J. J., Ngassoum, M. B., & Etoa, F.-X. (2003). Antibacterial and antifungal activity of Xylopia aethiopica, Monodora myristica, Zanthoxylum xanthoxyloides and Zanthoxylum leprieurii from Cameroon. *Fitoterapia, 74,* 469-472.

Teixeira-Silva, F., Prado, G. B., Ribeiro, L. C. G., & Leite, J. R. (2004). The anxiogenic video-recorded Stroop Color-Word Test: psychological and physiological alterations and effects of diazepam. *Physiology and Behavior, 82,* 215-230.

Teuscher, E., Melzig, M., & Villmann, E. (1989). Components of essential oils as membrane -active compounds. *Planta Medica, 55,* 660.

Teuscher, E., Melzig, M., Villmann, E., & Moritz, K. U. (1990). Experiments concerning the mechanisms of action of essential oils. *Zeitschrift fur Phytotherapie, 11,* 87-92.

Thayer, J. F. & Friedman, B. H. (2002). Stop that! Inhibition, sensitization, and their neurovisceral concomitants. *Scandinavian Journal of Psychology, 43,* 123-130.

Thayer, J. F. & Lane, R. D. (2007). The role of vagal function in the risk for cardiovascular disease and mortality. *Biological Psychology, 74,* 224-242.

Thiessen, D. D. & Yahr, P. (1977). *The gerbil in behavioural investigations: Mechanisms of territoriality and olfactory communication.* Austin, Texas, USA.: University of Texas press, Austin.

Tildesley, N. T., Kennedy, D. O., Perry, E. K., Ballard, C. G., Wesnes, K. A., & Scholey, A. B. (2005). Positive modulation of mood and cognitive performance following administration of acute doses of Salvia lavandulaefolia essential oil to healthy young volunteers. *Physiology and Behavior, 83,* 699-709.

Tildesley, N. T. J., Kennedy, D. O., Perry, E. K., Ballard, C. G., Savelev, S., Wesnes, K. A. et al. (2003). Salvia lavandulaefolia (Spanish Sage) enhances memory in healthy young volunteers. *Pharmacology Biochemistry and Behavior, 75,* 669-674.

Timothy, C., Costall, B., & Smythe, J. W. (1999). Effects of SCH23390 and Raclopride on Anxiety-Like Behavior in Rats Tested in the Black-White Box. *Pharmacology Biochemistry and Behavior, 62,* 323-327.

Tisserand, R. (1977). *The Art of Aromatherapy.* Saffron-walden. The C.W. Daniel Company Ltd.

Tisserand, R. (1988). *Aromatherapy for everyone.* Penguin Health.

Tisserand, R. & Balacs, T. (1995). *Essential oil safety*. Elsevier.

Toda, M. & Morimoto, K. (2008). Effect of lavender aroma on salivary endocrinological stress markers. *Archives of Oral Biology*.

Torii, S., Fukuda, H., Kanemoto, H., Miyanchi, R., Hamauzu, Y., & Kawasaki, M. (1988). Contingent negative variation (CNV) and the psychological effects of odour. In S.Van Toller & G. Dodd (Eds.) *Perfumery. The psychology and biology of fragrance* (pp. 107-120). Chapman Hall.

Toth, M., Sibille, E., Gyulai, Z., & Gal, J. (1996). Genetic approaches to elucidate serotonin2A (5HT-2A) receptor function. *Biological Psychiatry, 39,* 532.

Toth, M. (2003). 5HT-1A receptor knockout mouse as a genetic model of anxiety. *European Journal of Pharmacology, 463,* 177-184.

Totten, G. L. & France, C. R. (1995). Physiological and subjective anxiety responses to caffeine and stress in nonclinical panic. *Journal of Anxiety Disorders, 9,* 473-488.

Toufexis, D. J., Myers, K. M., & Davis, M. (2006). The effect of gonadal hormones and gender on anxiety and emotional learning. *Hormones and Behavior, 50,* 539-549.

Tronson, D. (2001). The odour, the animal and the plant. *Molecules, 6,* 104-116.

Tull, M. T. & Roemer, L. 2007, "Emotion regulation difficulties associated with the experience of uncued panic attacks: Evidence of experiential avoidance, emotional nonacceptance, and decreased emotional clarity", *Behavior Therapy*, vol. 38, no. 4, pp. 378-391.

Turina, A. D., Nolan, M. V., Zygadlo, J. A., & Perillo, M. A. (2006). Natural terpenes: Self-assembly and membrane partitioning. *Biophysical Chemistry, 122,* 101-113.

Tye, N. C., Iversen, S. D., & Green, A. R. (1979). The effects of BDZs and serotonergic manipulations on punished responding. *Neuropharmacology, 18,* 689-695.

Tyrer, P. J. & Lader, M. H. (1974). Response to propranolol and diazepam in somatic and psychic anxiety. *British Medical Journal, 2,* 14-16.

Tyrer, P. (1989). Choice of treatment in anxiety. In P.Tyrer (Ed.) *Psychopharmacology of Anxiety* (pp. 255-282). Oxford University Press.

Uchiyama, H., Toda, A., Hiranita, T., Watanabe, S., & Eyanagi, R. (2008). Role of amygdaloid nuclei in the anxiolytic-like effect of nociceptin/orphanin FQ in rats. *Neuroscience Letters, 431,* 66-70.

Umezu, T. (1999). Anticonflict effects of plant-derived essential oils. *Pharmacology, Biochemistry, and Behavior, 64,* 35-40.

Umezu, T. (2000). Behavioural effects of plant- derived essential oils in the Geller type conflict test in mice. *Japanese Journal of Pharmacology, 83,* 150-153.

Umezu, T., Ito, H., Nagano, K., Yamakoshi, M., Oouchi, H., Sakaniwa, M. et al. (2002). Anticonflict effects of rose oil and identification of its active constituents. *Life Sciences, 72,* 91-102.

Umezu, T., Nagano, K., Ito, H., Kosakai, K., Sakaniwa, M., & Morita, M. (2006). Anticonflict effects of lavender oil and identification of its active constituents. *Pharmacology, Biochemistry, and Behavior, 85,* 713-721.

Upson, T. M., Andrews, T., Harris, B., King, C., & Langhorne, J. (2004). *The Genus Lavender*. Timber press.

Valls-Sole, J. (2005). Neurophysiological assessment of trigeminal nerve reflexes in disorders of central and peripheral nervous system. *Clinical Neurophysiology, 116,* 2255-2265.

Valnet, J. (1980). . *The Practice of Aromatherapy.*

van der Watt, G., Laugharne, J., & Janca, A. (2008). Complementary and alternative medicine in the treatment of anxiety and depression. *Current Opinions in Psychiatry, 21,* 37-42.

Van Toller, S. (1988). Emotion and the brain. In S.Van Toller & G. H. Dodd (Eds.) *Perfumery* (First ed., pp. 121-140). London: Chapman & Hall.

Varty, G., Morgan, C., Cohen-Williams, M., Coffin, V., & Carey, G. (2002). The gerbil elevated plus-maze I. Behavioral characterization and pharmacological validation. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 27,* 357.

Varty, G., Cohen-Williams, M., Morgan, C., Pylak, U., Duffy, R., Lachowicz, J. et al. (2002). The Gerbil Elevated Plus-maze II. Anxiolytic-like Effects of Selective Neurokinin NK1 Receptor Antagonists. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 27,* 371.

Vaswani, M., Linda, F. K., & Ramesh, S. (2003). Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 27,* 85-102.

Vernet-Maury, E., Alaoui-Ismaili, O., Dittmar, A., Delhomme, G., & Chanel, J. (1999). Basic emotions induced by odorants: a new approach based on autonomic pattern results. *Journal of the .Autonomic Nervous System, 75,* 176-183.

Verpoorte, R. & Memelink, J. (2002). Engineering secondary metabolite production in plants. *Current Opinion in Biotechnology, 13,* 181-187.

Vgontzas, A. N., Kales, A., & Bixler, E. O. (1995). BDZ side-effects: role of pharmacokinetics and pharmacodynamics. *Pharmacology, 51,* 205-223.

von- Bingen, H. (1998). *Physica*. Editor Throop, Rochester, Vermont: Healing Arts Press.

Wall, P. M. & Messier, C. (2001). Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. *Neuroscience and Biobehavioral Reviews, 25,* 275-286.

Wall, P. M. & Messier, C. (2000). Ethological confirmatory factor analysis of anxiety-like behaviour in the murine elevated plus-maze. *Behavioural Brain Research, 114,* 199-212.

Walsh, E. R. M. M. & Wilson, C. B. M. C. (1999). Complementary therapies in long-stay neurology in-patient settings. [Miscellaneous]. *Nursing Standard April 28-May 4, 13*, 32-35.

Watkins, L. L., Grossman, P., Krishnan, R., & Sherwood, A. (1998). Anxiety and vagal control of heart-rate. *Psychosomatic Medicine.60(4),* 498-502.

Watson, D. & Clark, L. A. 1992, On traits and temperament: general and specific factors of emotional experience and their relation to the five-factor model, *J.Pers.*, vol. 60, no. 2, pp. 441-476.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology, 54,* 1063-1070.

Watson, D. & Tellegen, A. 1985, "Toward a consensual structure of mood", *Psychol.Bull.*, vol. 98, no. 2, pp. 219-235.

Watson, D., Weber, K., Assenheimer, J. S., Clark, L. A., Strauss, M. E., & McCormick, R. A. 1995, "Testing a tripartite model: I. Evaluating the convergent and discriminant validity of anxiety and depression symptom scales", *J.Abnorm.Psychol.*, vol. 104, no. 1, pp. 3-14.

Watson, L. (1999). *Jacobson's Organ and the Remarkable Nature of Smell.* Allen Lane: The Penguin Press.

Weber, S. T. & Heuberger, E. (2008). The Impact of Natural Odors on Affective States in Humans. *Chemical Senses*.

Webster, M. (2003). Miriam Webster Dictionary. www.uclan.ac.uk [On-line]. Available: http://www.m-w.com/cgi-bin/dictionary

Wegener, G., Volke, V., Harvey, B. H., & Rosenberg, R. (2003). Local, but not systemic, administration of serotonergic antidepressants decreases hippocampal nitric oxide synthase activity. *Brain Research, 959,* 128-134.

Weier, K. M. & Beal, M. W. (2004). Complementary therapies as adjuncts in the treatment of postpartum depression. *Journal of Midwifery and Women's Health, 49,* 96-104.

Weintraub, M. I. (1973). Hysteria. *American Family Physician, 8,* 91-95.

Weiser, M. J., Foradori, C. D., & Handa, R. J. (2008). Estrogen receptor beta in the brain: From form to function. *Brain Research Reviews, 57,* 309-320.

Weiss, S. M., Lightowler, S., Stanhope, K. J., Kennett, G. A., & Dourish, C. T. (2000). Measurement of Anxiety in transgenic Mice. *Reviews in the Neurosciences, 11,* 59-74.

Wells, R. (2005). Lovely is the rose. *Soap, Perfumery and Cosmetics, 78,* 32-34.

Westen, D. & Morrison, K. (2001). A multidimensional meta-analysis of treatments for depression, panic, and generalized anxiety disorder: An empirical

examination of the status of empirically supported therapies. *Journal of Consulting and Clinical Psychology, 69,* 875-899.

Whiting, P. J. (2003). GABAA receptor subtypes in the brain: a paradigm for CNS drug discovery? *Drug Discovery Today, 8,* 445-450.

Wildwood, C. (2003). *Mood Enhancing Plants*. The CWDaniel Company Ltd.

Wilkinson, S. M., Love, S. B., Westcombe, A. M., Gambles, M. A., Burgess, C. C., Cargill, A. et al. (2007). Effectiveness of Aromatherapy Massage in the Management of Anxiety and Depression in Patients With Cancer: A Multicenter Randomized Controlled Trial. *Journal of Clinical Oncology, 25,* 532-539.

Wilner, P. (1991). Behavioural Models in Psychopharmacology. In *Behavioural Models in Psychopharmacology: Theoretical Industrial and Clinical Perspectives.* (pp. 91-125). Cambridge University Press.

Wilson, D. A. (2008). Olfaction as a model system for the neurobiology of mammalian short-term habituation. *Neurobiol.Learn.Mem.*.

Wilson, M. A., Burghardt, P. R., Ford, K. A., Wilkinson, M. B., & Primeaux, S. D. (2004). Anxiolytic effects of diazepam and ethanol in two behavioral models: comparison of males and females. *Pharmacology, Biochemistry, and Behavior, 78,* 445-458.

Wootton, J. C. & Sparber, A. (2003). Surveys of complementary and alternative medicine usage: a review of general population trends and specific patient populations. *Seminars in Integrative Medicine, 1,* 10-24.

Worwood, V. W. (1997). *The Fragrant Mind*. (Second ed.) London: Bantam Books.

Wu, J. C., Buchsbaum, M. S., Hershey, T. G., Hazlett, E., Sicotte, N., & Chad Johnson, J. (1991). PET in generalized anxiety disorder. *Biological Psychiatry, 29,* 1181-1199.

Wyatt, T. D. (2003). *Pheromones and animal behaviour.* Cambridge University Press.

Xue, C. C., Zhang, A. L., Lin, V., Da Costa, C., & Story, D. F. (2007). Complementary and alternative medicine use in Australia: a national population-based survey. *J Alternative and Complementary Medicine, 13,* 643-650.

Yagyu, T. (1994). Neurophysiological findings on the effects of fragrance: Lavender and jasmine. *Integrative Psychiatry.10(2)()(pp 62-67) 1994.Date of Publication: 1994.,* 62-67.

Yamada, K., Mimaki, Y., & Sashida, Y. (2005). Effects of inhaling the vapor of Lavandula burnatii super-derived essential oil and linalool on plasma adrenocorticotropic hormone (ACTH) catecholamine and gonadotropin levels in experimental menopausal female rats. *Biological and Pharmaceutical .Bulletin, 28,* 378-379.

Yasumatsu, K., Uchida, S., Sugano, H., & Suzuki, T. (1994). The effect of the odour of mother's milk and orange on the spectral power of EEG in infants. *Journal of UOEH, 16,* 71-83.

Yocca, F. & Altar, C. A. (2006). Partial agonism of dopamine, serotonin and opiate receptors for psychiatry. *Drug Discovery Today: Therapeutic Strategies, 3,* 429-435.

Yoshitake, T., Yoshitake, S., Yamaguchi, M., Ogren, S. O., & Kehr, J. (2003). Activation of 5HT-1A autoreceptors enhances the inhibitory effect of galanin on hippocampal 5HT release in vivo. *Neuropharmacology, 44,* 206-213.

Yousem, D. M., Williams, S. C., Howard, R. O., Andrew, C., Simmons, A., Allin, M. et al. (1997). Functional MR imaging during odor stimulation: preliminary data. *Radiology, 204,* 833-838.

Zald, D. H. & Kim, S. W. (1996). Anatomy and function of the orbital frontal cortex, I: anatomy, neurocircuitry; and obsessive-compulsive disorder. *The Journal of Neuropsychiatry and Clinical Neurosciences, 8,* 125-138.

Zald, D. H. & Pardo, J. V. (1997). Emotion, olfaction, and the human amygdala: amygdala activation during aversive olfactory stimulation. *Proceedings of the National Academy of Sciences of the United States of America, 94,* 4119-4124.

Zernig, G., Troger, J., & Saria, A. (1993). Different behavioral profiles of the non-peptide SP (NK1) antagonists CP-96,345 and RP 67580 in Swiss albino mice in the black-and-white box. *Neuroscience Letters, 151,* 64-66.

Zhuang, X., Gross, C., Santarelli, L., Compan, V., Trillat, A. C., & Hen, R. (1999). Altered Emotional States in Knockout Mice Lacking 5HT-1A or 5HT1B Receptors. *Neuropsychopharmacology, 21,* 52S-60S.

Zuardi, A. W. (1990). 5HT-related drugs and human experimental anxiety. *Neuroscience and Biobehavioural Reviews, 14,* 507-510.

# APPENDICES

# APPENDIX 1

## TABLE 1.1 An example of the suggested ubiquitous healing properties of essential oils: Reputed Properties of essential oils from 4 internet web sites in 2007

| Essential Oils | **www.aromatherapygoddess.com** | **www.thesoulelement.com** | **www.mnwelldir.org** | **http://www.itsmynature.net/html/HerbalProperties.html** |
|---|---|---|---|---|
| Bergamot | Encouraging, Anti-Depressant, Balances | Aggression, anxiety, balancing, calming, cheering, concentration, confidence, creativity, encouraging, frustration, grief, memory loss, nervous tension, normalizing, stimulating, stress, uplifting | Skin conditions associated with an oily complexion; soothes insect bites, insect repellent. Refreshing, mood-lifting; beneseizures nervous and digestive system. | Sedating to nervous system, but reviving to the spirit. |
| Cedarwood | Calming, Comforting, Strengthening | Not mentioned | Calming, purifying benefit the skin and tissues near the surface of the skin; calms nervous tension; beneseizures the digestive system; supplies oxygen to tissues and erases DNA damage; cancer. | Good for easing colds, flu and bronchitis; arthritis and rheumatism; a natural anti-depressant and a sedative, good for nervous tension, stress and anxiety. |
| Chamomile, Roman | Not mentioned | Calming, creativity, rejuvenating, relaxing. acne, allergies, analgesic, antibacterial, antidepressant, anti-infectious, anti-inflammatory, antiseptic, antispasmodic, black eyes, blisters, boils, bruises, burns, bursitis, chapped lips, chapped skin, chilblains, cold sores, colic, conjunctivitis, coughs, diarrhea, disinfectant, diuretic, dry skin, earaches, eczema, fainting, febrifuge, fibrosis, gingivitis, hair loss, headaches, heatstroke, hiccups, influenza, inflamed joints, insect bites, insomnia, laryngitis, nausea, neuralgia, psoriasis, rashes, rheumatism, rheumatoid arthritis, shock, sprains, sties, stress, | Calming relieving restlessness and tension; cosmetically for the skin. | Nerve sedative, helps menopausal problems. Eases depression, stress related complaints, insomnia and migraines. Facilitates meditation. Relaxing, and soothing, |

| | | | | |
|---|---|---|---|---|
| | | sunburns, toothaches, upper abdominal pain, whitlows, wounds | | |
| Cinnamon | Stimulates creativity, Anti-depressant | Aphrodisiac, energizing, invigorating, refreshing, stimulating, vitalizing, warming. antibacterial, antibiotic, anti-fungal, antiseptic, antispasmodic, antiviral, bronchitis, carbuncles, colds, cystitis, diarrhea, digestive, hair loss, influenza, insect repellent, muscular aches, rheumatism, vaginal infections, warts, whooping cough | Antiseptic essential oil with a high antioxidant rating. Invigorates and rejuvenates mind and body. . | Not mentioned |
| Eucalyptus | Stimulant, mental focus | Balancing, cooling, invigorating, stimulating, acne, analgesic, animal bites, antibacterial, antibiotic, anti-fungal, anti-inflammatory, antiseptic, antispasmodic, antiviral, asthma, athlete's feet, bedbugs, bleeding gums, blisters, bronchitis, burns, candida, chapped lips, colds, coughs, cuts, cystitis, dandruff, decongestant, deodorant, diabetes, diarrhea, disinfectant, diuretic, drug withdrawal, ear infections, earaches, | (Radiata) suitable for topical use, diffusing, and even direct inhalation. Relatively gentle and nonirritating. | Promotes health, purification and healing. Is a stimulant and aids memory. Also rids negative psychic energy. Balancing. |
| Lavender | Calming, Balancing, Strengthening, Stimulating, Healing, PMS | Aggression, anxiety, balancing, calming, concentration, cooling, exhaustion, fatigue, hysteria, nervous tension, relaxing, soothing, stress; abrasions, abscesses, acne, animal bites, antibacterial, antibiotic, antidepressant, anti-fungal, anti-inflammatory, antiseptic, antispasmodic, antiviral, athlete's foot, bleeding, blisters, boils, bruises, burns, catarrh, chapped skin, chilblains, colds, convalescence, coughs, cuts, dandruff, dermatitis, detoxifying, diaper rash, diarrhoea, disinfectant, ear infections, fainting, febrifuge, frostbite, gingivitis, hay fever, headaches, heartburn, hiccups, immunity | The most versatile of all essential oils. Highly regarded for the skin; clinically evaluated for its relaxing effects. It might be used to cleanse cuts, bruises, and skin irritations. The fragrance is calming, relaxing, and balancing -- physically and emotionally. Makes an excellent rub for sprains, strains, and sore muscles (used in carrier oil) and goes well mixed with Tea Tree Oil (Melaleuca). Can be taken internally (1 to 3 drops in a cup of water) for headaches and even migraines. A few drops for a gargle works really peachy. | Relieves headaches, migraines, insomnia, PMS and shock. Promotes peace, love and health. Dispels depression, brings feelings under conscious control. Lavender is soothing, relaxing and balancing. |

| | | | | |
|---|---|---|---|---|
| | | stimulant, influenza, insect bites, insect repellent, insomnia, itching, laryngitis, lumbago, muscular aches, neuralgia, normalizes skin, palpitations, rashes, scalds, scars, scrapes, sedative, shock, sinusitis, sties, sunburns, swelling, tendonitis, tonic, toothaches, ulcers, vomiting, whitlows, whooping cough, windburns, wounds. | | |
| Lemon | Clarity, Calming, Mental Powers | Anxiety, balancing, calming, cheering, cooling, exhaustion, memory loss, refreshing, relaxing, stress, uplifting; abscesses, acne, antibacterial, anti-fungal, anti-infectious, antiseptic, antispasmodic, antiviral, arthritis, asthma, astringent, athlete's foot, bleeding, blisters, boils, cellulite, cerebral palsy, chapped skin, chilblains, circulatory stimulant, cold sores, constipation, coughs, dandruff, detoxifying, diarrhea, digestive, disinfectant, diuretic, fainting, febrifuge, fever, gallstones, gout, hair loss, hangovers, hay fever, headaches, hiccups, hot flashes, insect bites, insomnia, jet lag, kidney stones, laryngitis, lice, mumps, muscular dystrophy, normal hair, normal skin, normalizes blood pressure, oily hair, oily skin, osteo-arthritis, Parkinson's disease, rheumatoid arthritis, sedative, shock, sore throat, tendonitis, throat infections, thrush, toning, tonsillitis, varicose veins, warts, water purifier, water retention, whitlows, wrinkles | Antiseptic-like properties and contains compounds that have been studied for their effects on immune function, lymphatic, circulatory, and digestive systems. Is antibacterial and might serve as an insect repellent as well as being beneficial for the skin. Diffuse or add a few drops to a spray bottle to deodorize and sterilize the air. Add two drops to soy or rice milk for purification or combine with peppermint (Mentha piperita) to provide a refreshing lift. Use for removing gum, oil, or grease spots. Add to food or soy or rice milk as a dietary supplement or flavoring. | Not mentioned |
| Orange, Sweet | Relaxing, Balancing, Stimulating, Sensual | Anxiety, calming, cheering, inspiring, invigorating, refreshing, relaxing, stress, uplifting; acne, antidepressant, antiseptic, antispasmodic, astringent, cellulite, | Brings peace and happiness to the mind and body. It has been recognized to help a dull, oily complexion. Diffuse or apply topically on location, or add to food | Not mentioned |

| | | | | |
|---|---|---|---|---|
| | | constipation, diarrhea, drug withdrawal, muscular aches, muscular dystrophy, normal skin, oily skin, palpitations, Parkinson's disease, poor circulation, scars, sedative, spasm, stretch marks, toning, wrinkles | or soy or rice milk as a dietary supplement or flavoring. | |
| Patchouli | Aphrodisiac | Aphrodisiac, anxiety, calming, clearing, concentration, exhaustion, relaxing, self-hypnosis, soothing, stress; acne, antibiotic, antidepressant, anti-fungal, anti-infectious, anti-inflammatory, antiseptic, astringent, athlete's foot, carminative, cellulite, chapped skin, constipation, dandruff, deodorant, dermatitis, diuretic, dry skin, eczema, fixative, ganglion, jock itch, lower abdominal pain, normal skin, oily skin, seborrhea, sores, thrush, wrinkles . | Contains 71% sesquiterpenes, is very beneficial for the skin and might help prevent wrinkled or chapped skin. It is a general tonic and stimulant, helps the digestive system, fighting candida (yeast) infections, and beneseizures the nervous and glandular systems. It has antiseptic properties and helps relieve itching. | Helps frigidity, nervous exhaustion and stress related complaints. Is appeasing, calming and uplifting. |
| Peppermint | Mental Clarity | Concentration, cooling, exhaustion, invigorating, memory loss, refreshing, relaxing, revitalizing, stimulating; ant repellent, antibiotic, anti-inflammatory, antiseptic, antispasmodic, bronchitis, carminative, catarrh, cellulite, circulatory stimulant, colds, constipation, deodorant, dermatitis, digestive, disinfectant, emollient, fainting, febrifuge, fibrosis, flatulence, gingivitis, hay fever, headaches, heartburn, heatstroke, hemorrhoids, indigestion, insect repellent, itching, jet lag, lumbago, mosquito repellent, muscular aches, nausea, neuralgia, oily skin, osteoporosis, palpitations, raises blood pressure, rheumatoid arthritis, ringworm, scabies, sinusitis, sunburns, tendonitis, ton ing, toothaches, upper abdominal pain, varicose veins, vomiting, water retention | Contains 45% monoterpenes, 25% phenylpropanoids. It is one of the oldest and most highly regarded herbs for soothing digestion. Jean Valnet, M.D., studied peppermint's effect on the liver and respiratory systems. Other scientists have also researched peppermint's role in affecting impaired taste and smell when inhaled. Dr. William N. Dember of the University of Cincinnati studied peppermint's ability to improve concentration and mental accuracy. Alan Hirsch, M.D., studied peppermint's ability to directly affect the brain's satiety center, which triggers a sense of fullness after meals. It is beneficial to the sinuses and muscular system and especially useful for women during monthly cycles or menopause. Diffuse. Massage on the stomach or add to water or tea for supporting normal digestion. Apply | Stimulant that helps headaches, migraines, mental fatigue, nervous stress and fainting. Affects conscious mind and halts negative thoughts. |

| | | | | |
|---|---|---|---|---|
| | | | to bottom of feet to cool off on a hot day. Rub on temples for a calming effect, or place several drops on the tongue as an invigorating pick-me-up. A wonderful flavoring and preservative. Avoid contact with eyes, mucus membranes, or sensitive skin areas. | |
| Rose | Strengthens heart chakra, and spirit. | Not Mentioned | Has a beautiful fragrance that is intoxicating and aphrodisiac-like. Rose helps bring balance and harmony. It is stimulating and elevating to the mind, creating a sense of wellbeing. It has been called the Queen of oils for women's concerns, establishing harmony throughout the body no matter what life brings. It is also great for circulation and skin care. . | Depression, impotence, insomnia, frigidity, headaches, nervous tension. Promotes a feeling of wellbeing. Influences love, peace and happiness. An aphrodisiac acting directly upon brain and sexual centers of the body. Help alleviate sexual problems of women, helps psychological impotence in men. Increases sperm count. |
| Ylang-Ylang | Balancing, Strengthens Spirit, Aphrodisiac, Magnetic | Aggression, aphrodisiac, anxiety, balancing, calming, cooling, euphoric, relaxing; acne, antidepressant, antiseptic, disinfectant, hair growth, high blood pressure, insect bites, lowers blood pressure, normal skin, oily skin, sedative, shock | Might be extremely effective in calming, balancing (the nervous system) and bringing about a sense of relaxation. Ylang-Ylang affects the glandular system, great for hair and skin, stimulates adrenal glands, but at the same time can be used for insomnia and pain. Has been known to have good results for impotence and frigidity. Taken internally, it has been said to lower blood pressure, alleviate problems with PMS, and ease intestinal infections. For depression, rub a drop or two beTween your palms and inhale the warm aroma. | Eases nervous tension, soothes and inhibits anger born of frustration. Promotes peace, sex, and love. Calms anger and negative emotional states. Inclines to rest, comfort and sleep. |

# APPENDIX 2

## GERBIL ODOUR PILOT STUDY

### The effects of acute exposure to *L. angustifolia* and *R. damascena* on gerbil behaviour in the EPM and BWB.

This study was conducted as a feasibility study to determine whether or not to proceed with chronic odour exposure studies.

**Alterations to the validation methods**

(For materials and methods see chapters 4 and 5).

**Animals**

Gerbils, 30 male and 30 female obtained from a breeding stock at UCLAN (mean weight males 83.55g (+/- 8.80); females 71.90g (+/- 5.7)) were individually housed for one week prior to testing on the EPM and BWB.

**Odours**

Health Aid EOs of rose and lavender were bought from a local distributor.

Watch glasses containing the neat odours of the oils were placed in the air conditioning inlets in the holding, weighing rooms. While Ambipure plug-ins (kindly supplied by Mr Eippe at Ambipure) were plugged into two sockets in each of the weighing and holding rooms. The plug-ins contained a 10% solution of EO in distilled water. This was to maximize any potential odour effects.

**Procedure**

On the day of testing the gerbils were taken from the housing room, to the holding room and left to acclimatise for one hour. Following this the gerbils were exposed to lavender odour, rose odour or no odour for one hour, weighed and placed on the EPM for 5 minutes followed by the BWB for 5 minutes. Each condition was tested at least two weeks apart in order to allow any residual odour in the rooms to clear. For this pilot

study gerbils were tested in random order for males and females. The controls were tested first, this was carried out by a student (Mr. G. Normington) using this condition as part of his final year project, (video scoring the data from this group was carried out by myself). Two weeks following this lavender odour was tested using a separate set of gerbils, followed by the rose odour study two weeks later. The rest of the procedure follows that of the validation procedure.

**Data screening**

a.) EPM

No of gerbils which had seizures in the elevated plus-maze: controls, male 4, female 3; lavender, male 3, females 1; rose oil males 2, females 0.

b.) BWB

In the black white box controls, male 2, females 0; lavender males 0, females 1; rose oil, males 2, females 0.

## Results

### Lavender oil odour

Lavender odour EPM

Following exclusion of gerbils which had seizures or were off the EPM for longer than 200 seconds, there was a total of 15 gerbils in the no-odour control group and 21 gerbils in the lavender odour group (males no odour n = 6, lavender n = 11. Females: no odour n = 9, lavender n = 10). Again due to the small sample sizes in each cell, analysis was via non parametric means. (For median and quartile ranges see Table 2.1 below). The Mann Whitney U test between males and females for each variable in each odour group revealed that percent open duration was higher in females than males ($U = 2$, $p<0.01$) and percent protected head-dips were lower in females than males ($U = 2$, $p<0.01$) in the no-odour control group. These variables were analysed separately for each sex.

For the remaining variables which did not differ between the sexes there were significant decreases in total entries ($U = 75$, $p < 0.01$) percentage duration closed ($U =$

102, $p < 0.05$) rear frequency ($U = 81$, $p < 0.05$), immobile duration ($U = 103$, $p < 0.05$) locomotor duration ($U = 59$, $p < 0.01$) when gerbils were exposed to acute lavender odour.

In males acute lavender caused a significant decrease in percentage protected head-dip ($U = 15$, $p < 0.05$) when compared with male no-odour controls. However in females lavender caused an increase in percent protected head-dips ($U = 16$, $p < 0.05$).

**Appendix Table 2.1 The effects of one hour's exposure to lavender EO odour on gerbil behaviour in theEPM. (Table of median inter-quartile ranges).**

| Dependent variable | Odour group | Median and inter-quartile ranges | | |
|---|---|---|---|---|
| | | **All gerbils** | **Males** | **Females** |
| **Total entries** | **no odour** | 34.32, **43.66,** 45.69 | 34.64, **44.46,** 46.47 | 34.0 0, **40.39**, 43.89 |
| | **lavender** | 25.00, **29.88**,** 36.00 | 14.10, **27.00,** 32.94 | 26.00, **33.82**, 44.00 |
| **% open entries** | **no odour** | 40.63, **45.71,** 55.05 | 34.09, **40.63,** 45.24 | 45.71, **51.85**, 56.25 |
| | **lavender** | 40.00, **50.00,** 56.41 | 36.09, **44.74,** 59.78 | 43.48, **51.39**, 56.41 |
| **% closed entries** | **no odour** | 44.95, **54.29,** 59.37 | 54.76, **59.37,** 65.91 | 43.75,**48.15**, 54.29 |
| | **lavender** | 43.18, **47.22,** 60.00 | 34.67, **44.44,** 61.48 | 43.59,**48.61**, 56.52 |
| **% open duration** | **no odour** | 20.10, **27.24,** 35.74 | 12.94, **15.57,** 24.19 | 27.72, **35.54**, 42.65 |
| | **lavender** | 22.11, **38.61,** 47.35 | 17.48, **34.47,** 47.18 | 35.21, **40.43**, 47.35 |
| **% closed duration** | **no odour** | 35.18, **44.82,** 56.93 | 49.49, **56.93,** 59.13 | 30.37, **42.63**, 44.82 |
| | **lavender** | 28.80, **36.93*,** 43.62 | 15.48, **36.12,** 41.93 | 28.80**, 38.30**, 43.62 |
| **Rear frequency** | **no odour** | 22.09, **26.11,** 36.81 | 23.00, **31.87,** 43.03 | 21.32**, 26.00**, 35.00 |
| | **lavender** | 12.17, **19.00*,** 24.00 | 7.19, **23.00,** 29.00 | 13.00, **18.50**, 20.51 |
| **Immobile duration** | **no odour** | 0.00, **0.00,** 0.97 | 0.00, **0.77,** 5.66 | 0.00, **0.00**, 0.00 |
| | **lavender** | 0.00, **3.24*,** 15.70 | 0.00, **8.77,** 32.05 | 0.00**, 1.52**, 14.55 |
| **Locomotor duration** | **no odour** | 157.48, **167.06,** 173.19 | 133.08, **159.99,** 174.10 | 161.03, **168.84**, 172.29 |
| | **lavender** | 138.12, **147.84*,** 154.87 | 72.79, **146.02,** 155.40 | 141.85, **150.20**, 154.87 |
| **Head-dip frequency** | **no odour** | 17.00, **25.00,** 30.60 | 11.55, **16.50,** 29.87 | 24.69, **25.00**, 36.38 |
| | **lavender** | 21.90, **34.00,** 41.00 | 13.27, **25.00,** 34.16 | 26.00, **39.00**, 43.00 |
| **Stetch-attend frequency** | **no odour** | 9.40, **13.00,** 17.38 | 9.45, **11.98,** 17.76 | 9.35, **13.00**, 17.00 |
| | **lavender** | 8.85, **14.00,** 20.51 | 5.71, **9.00,** 17.00 | 13.37, **18.08**, 21.00 |
| **% protected head-dip frequency** | **no odour** | 9.81, **22.22,** 38.42 | 36.84, **47.27,** 57.14 | 6.67, **10.53,** 21.43 |
| | **lavender** | 18.42, **22.58,** 29.27 | 13.07, 22.50, 35.74 | 20.00, **25.71**, 29.27 |

Gerbils in each group: all gerbils no odour n = 15, lavender odour n = 21. Male gerbils no odour n= 6, lavender odour n= 11. Female gerbils no odour n = 9, lavender odour n = 10. Levels of significance results of Mann Whitney U tests * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Lavender Odour BWB

The number of gerbils included in this study after removal of those which had seizures from the data set were, no-odour control n = 19, lavender odour n = 21 (males control n = 9, lavender n = 11; females control n = 10, lavender n = 10).

An examination of each odour group for differences revealed no differences between males and females for any variable. Data from males and females were combined and analysed together. Lavender caused mobile duration white to decrease significantly ($U = 109$, $p<0.05$); while latency black increased almost reaching significance ($U = 141$, $p < 0.055$).

**Appendix Table 2.2 The effects of acute (one hour's exposure) to lavender EO odour on gerbil behaviour in the black white box. (Table of median and inter-quartile ranges).**

| Dependent variable | Odour group | Median and inter-quartile ranges. | | |
|---|---|---|---|---|
| | | **All gerbils** | **Males** | **Females** |
| **Latency black** | **control** | 1.43. **2.52,** 7.86 | 1.48, **5.11,** 7.42 | 1.32, **2.08,** 8.29 |
| | **lavender** | 1.92, **7.97,** 9.56 | 2.47, **8.02,** 9.59 | 1.92, **7.11,** 9.56 |
| **Crossing frequency** | **control** | 42.00, **47.00,** 55.00 | 40.00, **45.00,** 60.00 | 44.00, **49.00,** 55.00 |
| | **lavender** | 38.00, **47.00,** 56.00 | 35.00, **45.00,** 51.00 | 44.00, **53.50,** 59.00 |
| **% White duration** | **control** | 46.91, **51.85,** 57.77 | 47.17, **53.14,** 58.87 | 46.64, **49.80,** 52.14 |
| | **lavender** | 45.22, **52.01,** 60.49 | 43.44, **52.01,** 65.41 | 46.35, **51.60,** 60.49 |
| **% White duration** | **control** | 24.18, **30.18,** 35.89 | 22.14, **24.18,** 32.37 | 25.73, **30.48,** 37.11 |
| | **lavender** | 24.66, **30.61,** 36.90 | 18.57, **32.43,** 37.02 | 25.54, **29.83,** 34.83 |
| **Mobile duration White** | **control** | 80.78, **89.02,** 97.64 | 86.72, **96.33,** 106.00 | 77.97, **84.29,** 93.40 |
| | **lavender** | 67.87, **77.60,** 85.96 | 67.04, **78.98,** 84.43 | 67.87, **70.66,** 88.36 |
| **Exploration Frequency white** | **control** | 52.50, **65.00,** 84.50 | 64.00, **82.00,** 89.00 | 51.00, **59.50,** 65.00 |
| | **lavender** | 49.00, **71.00,** 87.00 | 46.00, **70.00,** 79.00 | 69.00, **76.00,** 97.00 |
| **Exploration Duration white** | **control** | 55.00, **63.18,** 69.12 | 56.00, **63.18,** 80.86 | 54.04, **62.16,** 66.77 |
| | **lavender** | 50.12, **59.65,** 74.49 | 39.04, **52.32,** 70.21 | 57.36, **63.79,** 75.77 |
| **Immobile duration white** | **Control** | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 |
| | **lavender** | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 |

Gerbils in each group: all gerbils no odour n = 19, lavender odour n = 21. Male gerbils no odour n= 9, lavender odour n= 11. Female gerbils no odour n = 10, lavender odour n = 10. Results of Mann Whitney U tests, levels of significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Rose oil odour**

Rose odour pilot EPM study

The number of gerbils included in this study was controls n = 15, rose odour n = 20 (males: controls n = 6, rose odour n =6; females: controls n = 9, rose odour n = 11). In the rose odour group females entered the open arms more than males ($U = 15$, $p < 0.01$) and the closed arms less than males ($U = 15$, $p < 0.01$). These variables were analysed separately for sex each sex. Data from all other variables were combined prior to analysis. Overall rose oil odour caused a decrease in total entries ($U = 51.5$, $p < 0.001$) and locomotor duration ($U = 79$, $p < 0.01$) and an increase in immobile duration ($U = 79$, $p < 0.01$). In both males and females there was an increase in percent protected head-dips ($U = 12$, $p < 0.05$ and $U = 9$, $p < 0.001$ respectively).

**Appendix Table 2.3 The effects of one hour's exposure to rose EO odour on gerbil behaviour in theEPM. (Table of median and inter-quartile ranges).**

| Dependent variable | Odour group | **Median** and inter-quartile ranges | | |
|---|---|---|---|---|
| | | **All gerbils** | **Males** | **Females** |
| **Total entries** | **control** | 34.32, **43.66,** 45.69 | 34.64, **44.46,** 46.47 | 34.00, **40.39,** 43.89 |
| | **rose** | 17.00, **22.00,** 25.00 | 16.00, **17.00,** 24.00 | 21.00, **23.00,** 25.00 |
| **% open entries** | **control** | 40.63, **45.71,** 55.05 | 34.09, **40.63,** 45.24 | 45.71, **51.85,** 56.25 |
| | **rose** | 30.00, **43.70,** 53.36 | 23.81, **25.00,** 37.50 | 44.50, **46.43,** 55.27 |
| **% closed entries** | **control** | 44.95, **54.29,** 59.37 | 54.76, **59.37,** 65.91 | 43.75, **48.15,** 54.29 |
| | **rose** | 46.64, **56.30,** 70.00 | 62.50, **75.00,** 76.19 | 44.73, **53.57,** 55.50 |
| **% duration open** | **control** | 20.10, **27.24,** 35.74 | 12.94, **15.57,** 24.19 | 27.72, **35.54,** 42.65 |
| | **rose** | 14.43, **26.89,** 39.85 | 9.27, **17.67,** 25.64 | 26.43, **30.91,** 40.45 |
| **% duration closed** | **control** | 35.18, **44.82,** 56.93 | 49.49, **56.93,** 59.13 | 30.37, **42.63,** 44.82 |
| | **rose** | 37.23, **44.60,** 52.48 | 39.01, **46.75,** 63.33 | 32.98, **39.40,** 48.85 |
| **Rear frequency** | **control** | 22.09, **26.11,** 36.81 | 23.00, **31.87,** 43.03 | 21.32, **26.00,** 35.00 |
| | **rose** | 21.95, **28.28,** 34.50 | 23.00, **28.00,** 33.00 | 22.45, **28.56,** 34.50 |
| **Immobile duration** | **control** | 0.00, **0.00,** 0.97 | 0.00, **0.77,** 5.66 | 0.00, **0.00,** 0.00 |
| | **rose** | 0.00, **8.21,** 26.97 | 0.00, **11.47,** 30.17 | 0.52, **5.08,** 17.68 |
| **Locomotor duration** | **control** | 157.48, **167.06,** 173.19 | 133.08, **159.99,** 174.10 | 161.03, **168.84,** 172.29 |
| | **rose** | 113.96, **133.69,** 155.34 | 113.54, **115.97,** 149.19 | 127.69, **135.84,** 155.34 |
| **Head-dip frequency** | **control** | 17.00, **25.00,** 30.60 | 11.55, **16.50,** 29.87 | 24.69, **25.00,** 36.38 |
| | **rose** | 8.00, **22.00,** 28.25 | 6.44, **10.00,** 19.00 | 22.00, **23.00,** 28.56 |
| **Stretch- attend frequency** | **control** | 9.40, **13.00,** 17.38 | 9.45, **11.98,** 17.76 | 9.35, **13.00,** 17.00 |
| | **rose** | 10.61, **15.93,** 25.00 | 14.00, **22.00,** 34.00 | 10.61, **15.87,** 18.00 |
| **% protected headdip** | **control** | 9.81, **22.22,** 38.42 | 36.84, **47.27,** 57.14 | 6.67, **10.53,** 21.43 |
| | **rose** | 100.00, **100.00,** 107.14 | 100.00, **100.00,** 100.00 | 100.00, **100.00,** 119.64 |

Gerbils in each group: all gerbils no odour n = 15, rose odour n = 20. Male gerbils no odour n= 6, lavender odour n= 6. Female gerbils no odour n = 9, lavender odour n = 11. Levels of significance results of Mann Whitney U tests * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Rose odour pilot BWB study

Included in the rose BWB pilot were controls n=19, rose odour n = 20 (males: control n = 9, rose n = 9; females: controls n= 10, rose odour n = 11). There were no differences between males and females therefore data from both males and females were combined. Overall rose oil caused a decrease in time spent in the white compartment ($U = 125$, $p < 0.05$) and a decrease in locomotor activity (mobile duration white $U = 86$, $p < 0.01$).

**Appendix Table 2.4 The effects of acute (one hour's exposure) to rose EO odour on gerbil behaviour in the black white box. (Table of median and inter-quartile ranges).**

| Dependent variable | Odour group | **Median** and inter-quartile ranges | | |
|---|---|---|---|---|
| | | **All gerbils** | **Males** | **Females** |
| **Latency black** | **control** | 1.43, **2.52,** 7.86 | 1.48, **5.11,** 7.42 | 1.32, **2.08,** 8.29 |
| | **rose** | 1.21, **2.03,** 4.37 | 1.43, **1.93,** 5.61 | 1.19, **2.14,** 2.96 |
| **Total entries** | **control** | 42.00, **47.00,** 55.00 | 40.00, **45.00,** 60.00 | 44.00, **49.00,** 55.00 |
| | **rose** | 31.00, **34.00,** 55.00 | 34.00, **51.00,** 59.00 | 28.00, **32.00,** 36.00 |
| **% White Duration** | **control** | 46.91, **51.85,** 57.77 | 47.17, **53.14,** 58.87 | 46.64, **49.80,** 52.14 |
| | **rose** | 36.90, **45.30,** 52.44 | 30.54, **42.40,** 50.81 | 41.63, **46.79,** 58.63 |
| **% Black Duration** | **control** | 24.18, **30.18,** 35.89 | 22.14, **24.18,** 32.37 | 25.73, **30.48,** 37.11 |
| | **rose** | 24.23, **34.39,** 41.08 | 28.22, **34.38,** 42.61 | 19.27, **34.39,** 38.19 |
| **Mobile duration white** | **control** | 80.78, **89.02,** 97.64 | 86.72, **96.33,** 106.00 | 77.97, **84.29,** 93.40 |
| | **rose** | 52.72, **70.96,** 81.29 | 43.10, **70.70,** 74.92 | 58.81, **71.97,** 88.87 |
| **Exploration Frequency white** | **control** | 52.50, **65.00,** 84.50 | 64.00, **82.00,** 89.00 | 51.00, **59.50,** 65.00 |
| | **rose** | 53.00, **64.00,** 76.00 | 58.00, **70.00,** 83.00 | 53.00, **62.00,** 68.50 |
| **Exploration Duration white** | **control** | 55.00, **63.18,** 69.12 | 56.00, **63.18,** 80.86 | 54.04, **62.16,** 66.77 |
| | **rose** | 48.53, **65.21,** 74.50 | 47.22, **55.45,** 69.60 | 54.01, **67.78,** 77.20 |
| **Immobile duration white** | **control** | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 0.00 |
| | **rose** | 0.00, **0.00,** 0.30 | 0.00, **0.00,** 0.00 | 0.00, **0.00,** 1.37 |

Gerbils in each group: all gerbils no odour n = 19, rose odour n = 20. Male gerbils no odour n= 9, lavender odour n= 9. Female gerbils no odour n = 10, lavender odour n = 11. Levels of significance results of Mann Whitney U tests * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Conclusions**

Lavender caused mild anxiolysis and sedation particularly in male gerbils. This was evidenced by decreases in the time spent in the close arm in all gerbils and decreased percentage protected head-dips in all male gerbils in the EPM. While there were decreases in any kind of movement in the BWB reflecting sedation and an almost significant increase in the time taken to escape to the black compartment indicative of decreased anxiety. Interestingly females appeared more anxious following acute lavender exposure, as the EPM risk-assessment behaviour, protected head-dips, increased in female gerbils. While these effects were anxioytic they were also very different to diazepam's and buspirone's effects in both models (see Gerbil validation chapter, chapter 4). Although possibly lavenders effects could be compared to diazepam which at the lowest concentration had an effect on exploration only (head-dips increased)

In contrast to lavender, rose oil appeared to increase anxiety, causing an increase in protected head-dips in both males and females in response to the EPM and a decrease in time spent in the white compartment in the BWB.

Since these were both pleasant odours with very different effects acutely it was decided to proceed with the chronic study to examine the effects of prolonged exposure to lavender to answer the question do lavender's anxiolytic effects fade as habituation to the odour takes place or do they increase? Since rose oils profile was so different following acute exposure it was decided that this would be a second odour to use, in case any long term effects were due to the pleasant odour.

# APPENDIX 3

# FILM PILOT STUDY

Aim to validate the use of two film-clips for use as anxiety/ fear mood elicitors for use in laboratory based studies of anxiety.

## Materials and methods

### Design

The target population was taken from the staff and student population at UCLAN. For practical reasons film-clips for the selection of clips to use in the study were viewed in groups rather than on an individual basis.

### Participants

17 male and 17 female healthy students within the age range 18-24 with a mean age of 20.7 +/- 0.19 SEM for males and 22.9 +/- 0.67 SEM for females. Ethical approval was obtained from the Department of Psychology ethics committee. Participants were fully briefed, gave written informed consent and were free to withdraw at anytime, the study was conducted in accordance with the declaration of Helsinki.

### Questionnaires

The questionnaire employed by Gross and Levenson for film selection was used here along with the PANAS (Tellegen and Watson) (for questionnaire used see appendix to this section; and for permissions to use the questionnaires).

### Physiological Measures

In addition some participants, those who volunteered also wore portable blood pressure monitors to assess blood pressure and heart-rate pre and post each film-clip viewing.

**Film-clips**

Film-clips were edited and created following recommendations in the cited papers.

Millers crossing (Kaviani et al., 1999)
Silence of the Lambs (Gross & Levenson, 1995)
The Hitcher (Stalk et al., 2005)
Shining (Gross and Levenson, 1995)
Open water, self selected. Scenes selected
Laurel and Hardy (Light hearted relief film)

**Millers crossing**

Clip length: 4' 30": edited 1:0:0 – 1:04:31 minutes (Kaviani, Gray, Checkley, Veena, & Wilson, 1999)

**Silence of the Lambs**

Clip length: 3'29"
The clip was edited following Gross and Levenson's instructions as follows:

Advance to the first frame of the film in which the words "A STRONG HEART DEMME PRODUCTION" appear.
Reset the timer to 00:00:00:00 (hours: minutes: seconds: frames). Begin the clip at 01:40:16:29. At this point, a dirt road and trees are in the forefront and a mint green trailer is in the background.

Stop recording at 01:43:44:23. At this point, the profile of a dark-haired woman is visible. There is a metal wire hanging from the ceiling that appears to almost (but not quite) touch her nose and chin.

Begin recording at 01:46:36:24. At this point, hands holding a gun are moving rapidly into the scene from the right of the screen. In the background, there is dirty yellow wallpaper.

End the clip at 01:46:38:19. At this point, the dark-haired woman has her back to the yellow wallpaper, and has pointed her gun between the upper-middle and the upper-right hand portions of the screen. Her right hand obscures most of the left half of her face and we hear her exclaim as the lights go out. (Gross and Levenson, 1995).

**Hitcher**

Clip length: 3' 30" minutes (Stark, Schienle, Sarlo, Palomba, Walter, & Vaitl 2005)

**The Shining**

Clip length: 1'22"

Edited following Gross and Levenson's instructions:

Advance to the first frame of the film, which shows a body of water surrounded by mountains. Reset the timer to 00:00:00:00 (hours: minutes: seconds: frames).
Begin the clip at 00:56:51:15. At this point, a boy's hands are visible (one flat on the floor and the other in a fist). There are toy trucks and cars on a red, brown, and orange carpet. End the clip at 00:58:12:18. At this point, an open door with a key in the lock is visible, and one full second has passed since the boy has said "Mom, are you in there?" (Gross and Levenson, 1995).

**Open Water**

Clip Length: 9': scenes were selected to create a mini film depicting a couple being left behind by the boat, stranded out at sea and drifting with the tide, and finally being attacked fatally by sharks. All blood and gore were edited out in order to prevent elicitation of emotions related to disgust.
Open Water was edited as follows:
33:3- 34:07 minute's boat leaving
34:43 – 35:26 stranded together in sea
52:34-54:30 fell asleep, separated alone in water

57:50 – 59:04 shark

01:09:25 – 1:10:15 shark bites Daniel

01:11:53 – 1:12:26 calming Daniel down

01:14:05 – 01:15:50 storm – not much visible apart from circling sharks when lightening illuminates water, sounds of thunder. In addition the sound track was also edited to make the mini film more coherent. (Editing for this clip was in house (Adam Palmer Psychology AV technician).

#### Laurel and Hardy

10 minutes light relief film taken from 'Towed in a hole' 1932 (see appendix)

#### Neutral film

Nature Watch: 30 minutes TV documentary program.

## Procedure

Participants from undergraduate classes were asked to take part in the film validation study and offered popcorn and Fanta, a soft fizzy drink, following viewing of the film-clips, as an incentive to take part. Clips were projected onto a projector screen via a lap top using MS PowerPoint. Following completion of the initial screening questionnaire, participants who volunteered to wear the blood pressure monitor put it on their non dominant arm and took a reading. After which participants were allowed to habituate to the environment while being instructed to fill in the questionnaire after each film-clip and asked to complete the questionnaire according to how they were feeling at that moment in time and to take a blood pressure reading as soon as they were instructed to, following viewing of the clip. Participants were also asked if they minded waiting until the final clip (Laurel and Hardy) to eat their popcorn and drink their Fanta, all participants complied with these instructions. After this all participants took a blood pressure reading which was recorded and then watched the first clip.

### Data analysis

Following the method developed by Gross and Levenson (1995) to select suitable film-clips to elicit their stated target emotions, questionnaire data were analysed for intensity of the target emotion, in this case anxiety and fear and discreteness, that is the purity of the target emotion elicited by the film-clip, did it create pure anxiety and fear or a mixture of anxiety and disgust.

Intensity was determined by looking at the mean scores, for the emotions of anxiety, fear and also of negative affect, elicited by each film. This data were taken from participants' questionnaire data from the five film-clips.

Additionally the film which elicited the most fear in each individual participant and the film which elicited the most anxiety in each participant was tallied to determine which films created the most intense anxiety and fear overall.

## Results

For mean and standard error results of intensity of anxiety, fear, negative and positive affect see Tables 3.1, 3.2 and 3.3.

**Appendix Table 3.1 Measurement of the intensity of anxiety and fear emotions by selected films using Gross and Levenson's screening questionnaire. Data are presented as means and standard errors of total sample.**

| Dependent variable | Millers crossing | Hitcher | Silence of the lambs | Shinning | Open water |
|---|---|---|---|---|---|
| **Anger** | 2.23 (+/-0.39) | 1.35 (+/0.33) | 0.53(+/-0.18) | 0.38 (+/0.22) | 0.56 (+/-0.18) |
| **Anxiety** | 3.00 (+/-0.36) | 2.56 (+/0.40) | 3.41(+/-0.41) | 2.62 (+/0.41) | 3.79 (+/-0.44) |
| **Confusion** | 1.67 (+/-0.44) | 1.5 (+/-0.39) | 1.21(+/-0.33) | 1.88 (+/0.40) | 1.79 (+/-0.36) |
| **Contempt** | 1.59 (+/-0.35) | 1.82 (+/0.40) | 0.82 (+/0.27) | 0.79 (+/0.29) | 0.68 (+/-0.24) |
| **Disgust** | 2.65 (+/-0.41) | 2.26 (+/0.44) | 2.00 (+/0.41) | 0.68 (+/0.28) | 0.71 (+/-0.26) |
| **Fear** | 1.62 (+/-.32) | 1.91 (+/0.35) | 2.65 (+/0.39) | 2.18 (+/0.37) | 3.47 (+/-0.47) |
| **Guilt** | 1.09 (+/-0.33) | 0.44 (+/0.22) | 0.47 (+/0.20) | 0.44 (+/0.24) | 0.74 (+/-0.29) |
| **Sadness** | 3.09 (+/-0.45) | 1.38(+/-0.35) | 0.85 (+/0.24) | 0.50 (+/0.24) | 1.76 (+/-0.45) |
| **Shame** | 1.53 (+/-0.42) | 0.88(+/-0.31) | 0.38 (+/0.16) | 0.35 (+/0.19) | 0.68 (+/-0.25) |
| **Negative affect** | 14.38 (+/- 3.00) | 13.47 (+/-2.55) | 15.03(+/-2.49) | 11.88(+/-2.10) | 19.03(+/- 2.88) |

Sample size n = 34

**Appendix Table 3.2 Measurement in male participants of elicitation of anxiety and fear emotions by selected films using Gross and Levenson's screening questionnaire.**

| Dependent variable | Millers crossing | Hitcher | Silence of the lambs | Shinning | Open water |
|---|---|---|---|---|---|
| **Anger** | 2.71(+/-0.57) | 2.00(+/-0.54) | 0.47(+/-0.24) | 0.18 (+/0.13) | 0.59(+/-0.29) |
| **Anxiety** | 2.88(+/-0.49) | 2.41(+/-0.63) | 3.41(+/-0.59) | 2.06 (+/-0.57) | 3.47(+/- 0.64) |
| **Confusion** | 1.76(+/-0.71) | 2.12(+/-0.67) | 1.47(+/-0.56) | 2.53 (+/- 0.71) | 2.12(+/0.57) |
| **Contempt** | 1.47(+/-0.52) | 1.88(+/-0.57) | 1.06(+/-0.44) | 0.76 (+/-0.43) | 1.12 (+/-0.41) |
| **Disgust** | 3.06(+/-0.65) | 2.29(+/-0.55) | 1.41(+/-0.42) | 0.76 (+/-0.44) | 0.82 (+/-0.41) |
| **Fear** | 1.35(+/-0.44) | 1.71(+/-0.49) | 2.41(+/-0.54) | 2.06 (+/-0.49) | 3.35 (+/-0.63) |
| **Guilt** | 1.06(+/-0.39) | 0.41(+/-0.26) | 0.65(+/-0.36) | 0.47 (+/-0.36) | 0.71 (+/-0.44) |
| **Sadness** | 3.18(+/-0.71) | 1.41(+/-0.54) | 0.71(+/- 0.35) | 0.59 (+/-0.44) | 1.88 (+/-0.69) |
| **Shame** | 1.76(+/-0.63) | 0.77(+/-0.44) | 0.35(+/- 0.26) | 0.41 (+/-0.35) | 0.65 (+/-0.39) |
| **Negative affect** | 16.06 (+/- 4.97) | 12.76 (+/-3.91) | 14.35 (+/-3.88) | 10.76(+/-3.43) | 16(+/-4.23) |

Sex = male n =17

**Appendix Table 3.3 Measurement in female participants of elicitation of anxiety and fear emotions by selected films using Gross and Levenson's screening questionnaire.**

| | Millers crossing | Hitcher | Silence of the lambs | Shinning | Open water |
|---|---|---|---|---|---|
| **Anger** | 1.76(+/-0.52) | 0.71(+/-0.32) | 0.59(+/0.26) | 0.59(+/-0.42) | 0.53(+/-0.23) |
| **Anxiety** | 3.12(+/-0.54) | 2.71(+/-0.51) | 3.41(+/0.59) | 3.18(+/-0.56) | 4.12(+/-0.62) |
| **Confusion** | 1.59(+/-0.54) | 0.88(+/-0.35) | 0.94(+/0.35) | 1.24(+/-0.34) | 1.47(+/-0.45) |
| **Contempt** | 1.71(+/-0.48) | 1.76(+/-0.58) | 0.59(+/-0.3) | 0.82(+/-0.4) | 0.24(+/-0.24) |
| **Disgust** | 2.24(+/-0.49) | 2.23(+/-0.72) | 2.59(+/-0.7) | 0.59(+/-0.34) | 0.59(+/-0.32) |
| **Fear** | 1.88(+/-0.46) | 2.12(+/-0.49) | 2.88(+/-0.6) | 2.29(+/-0.57) | 3.59(+/-0.71) |
| **Guilt** | 1.12(+/-0.55) | 0.47(+/-0.37) | 0.29(+/-0.19) | 0.41(+/-0.31) | 0.76(+/-0.39) |
| **Sadness** | 3.00(+/-0.57) | 1.35(+/-0.46) | 1.00(+/-0.34) | 0.41(+/-0.2) | 1.65(+/-0.59) |
| **Shame** | 1.29(+/-0.56) | 1.00(+/-0.45) | 0.41(+/-0.21) | 0.29(+/-0.17) | 0.71(+/-0.33) |
| **Negative affect** | 12.71 (+/-3.49) | 14.18(+/-3.41) | 15.72 (+/- 3.23) | 13(+/-2.49) | 22.06(+/3.09) |

Sex = female n = 17

The intensity of the fear and anxiety experienced was highest for Open Water followed by Silence of the Lambs and elicited scores for anxiety of (3.79 (+/-0.44) and 3.41(+/-0.41) respectively) and fear (3.47 (+/-0.47) and 2.65 (+/0.39) respectively) target while being relatively low on all other negative emotions such as sadness, disgust and confusion.

To determine the discreteness of the intensity to which subjects report feeling the target emotion more intensely than all other target emotions, data from the five films were compared by tally chart across subjects for the film which had elicited the target emotion more intensely than other non target emotions of both anxiety and fear. Open Water gave the highest values for fear with (17 participants reporting it as the most fearful film-clip of the five) followed by Silence of the Lambs (8 participants reported that it was the most fear eliciting film-clip). For anxiety Open water created the most anxiety (15 participants reported it as the most anxiety eliciting film-clip) again followed by Silence of the Lambs (13 reported it as the most anxiety eliciting film-clip). Thus Open water followed by Silence of the Lambs clearly elicited the target emotions more intensely than any of the other measured emotions.

On the pleasant – unpleasant scale Open water again gave the lowest mean score of 3.082 compared with the highest of 6.88 for Millers Crossing.

Furthermore, PANAS scores for negative and positive effect, when standardised as Z scores between each film for each participant gave the highest NA and ZNA scores for Open Water, although interestingly Open water followed by Silence of the Lambs also gave the highest PA scores, this is possibly because many participants scored high on the interest factor which forms part of the positive affect scale.

## Conclusion

Thus, from these results the two film-clips to be used in the main study will be Open water and Silence of the Lambs, permission for use of these film-clips was granted from the licensing organizations for each film (see appendix 4 section 3).

# APPENDIX 4

## Appendix Figure 1 RECRUITMENT POSTER


**Appendix Figure 1 Recruitment poster**

# 2 PARTICIPATION PACK AND RECRUITMENT ADVERTS

## Participant briefing information

The aim of the study is to look at the effects of an EO on mood, and involves taking capsules which contain either the EO or sunflower oil. Following this you will be asked to watch a series of film-clips while various physiological and psychological measures are taken. The study will take approximately one hour, but please allow longer in case it over runs (90 minutes).

It is very important that if you have any allergies to perfumes, soap, EOs, food additives or sunflower oil; or any health conditions such as epilepsy, heart problems or pregnancy; or are taking any prescribed or unprescribed medication, that you tell Belinda Hornby immediately. Likewise if you are a smoker then you might not be able to take part in this study, please tell Belinda immediately.

Since many food additives contain EOs, it is important for you to keep a record of what you eat for 24 hours prior to the study (on the enclosed diary) and also carry out as closely as possible the instruction included in this pack.

You will be asked to under take a patch test a few days prior to the study, to screen for any potential allergies to the capsules. A patch test involves rubbing the contents of a capsule on your lower inner forearm and rubbing it in and then observing any reactions for 20 minutes. If you are allergic to it then there might be some discomfort/ itching, or even hay fever type symptoms. If you are allergic to the contents of the capsule then please inform Belinda immediately.

During the study you will be asked to watch a selection of film-clips from commercially available films (permission has been granted by the licence holders of these films for use in this study) some of these are thriller/ horror type films. While watching these film-clips a number of physiological recordings will be taken, including ECG, GSR, EMG, blood pressure and a small camera will be placed in the laboratory to record your facial responses to the films. In addition before and after watching the clips you will be asked to complete various psychological instruments including questionnaires and saliva samples might also be taken for later cortisol analysis. All data collected will be

anonymised and will only be viewed by myself (Belinda Hornby) and possibly my supervisor (Dr Stephen Brown) and possibly other researchers with a legitimate interest in the study.

The study itself will take between one hour and ninety minutes, for taking the time to take part if you are a psychology student you will be rewarded with 8 Course credits.

If at any time during the study you no longer wish to participate then please tell Belinda immediately and once you have been disconnected from the physiological recording equipment you will be allowed to leave and your data will not be saved.
If you find the film-clips in any way disturbing and feel the need to discuss this with someone then please contact the student counsellors on Tel: 01772 892572 or email CRecep@uclan.ac.uk. Or alternatively contact your general practitioner. Likewise should any other problems arise as a result of your participation in this study, please contact either of the aforementioned or Belinda Hornby email: bfhornby@uclan.ac.uk or Tel: 01772 893737. Thank you for agreeing to take part in this study.

## Final Study participant diet instruction sheet

Thank you for volunteering to take part in this study, for psychology students please bring your participation points paper work to the study and then the points can be awarded for taking part.

The aim of this study is to examine the effects of a commonly used food additive and EO on various physiological and psychological parameters in a laboratory setting. On arrival at the laboratory, various physiological and psychological measures will be taken and you will be asked if you have managed to keep to the diet restrictions. Next you will be asked to swallow four capsules containing either one of two doses of the additive or a placebo.
During the study you will be asked to watch a series of film-clips while being connected to a series of physiological transducers designed to measure blood pressure, heart-rate (ECG) galavanomic skin response and eye blink rate. You will also be asked to complete two questionnaires at various intervals, saliva samples will be taken for later cortisol analysis and you will be asked to take part in a computer based task.

Since the study is about naturally derived food additives it is important that you follow strict dietary guide lines for the twenty four hours preceding the study. Please follow the following instructions very closely, thank you.

Please refrain from **alcohol, tea coffee**, **tobacco, fruit and vegetables** products for at least 24 hours prior to the study and if possible it would be preferable to avoid them for a couple of days before hand if you can, if not do not worry the last 24 hours are the most important. Please avoid any scented teas or fruit drinks too. Drink plenty of water.

Foods which you can eat are, for example: potatoes, such as chips, baked boiled or fried rice, preferably white; white bread, lentils, pulses and dried beans, roasted nuts, soya, plain crisps, digestive biscuits, fresh unprocessed meat, eggs, dairy or fish based foods. Meals such as fish and chips, *without* the mushy peas, porridge, egg on toast, non-coloured cheese on toast, cornflakes, chicken and chips, peanut butter, salted peanuts, sugar *but not honey*. A meal plan for the day before would be porridge or corn flakes with sugar on them and milk, egg or cheese on toast and a glass of milk or water, or almond milk, followed by a ham and or cheese sandwich at lunchtime with

mightonnaise if required (no pepper) and fish or steak or steak pie or butter pie and chips for tea, toast and milk for supper.

Please avoid any food containing additives and food colourings such as sweets, coloured cheeses, and fish with breadcrumbs on it, such as fish fingers, chewing gum.

The day before the study please refrain from juices, fruits, sweets, curries, anything with a strong flavour. Likewise, processed foods such as sausages, burgers pizzas, also those which contain flavourings or additives such as those found in EOs.

Also, for two days please refrain from the use of **perfumes, deodorants, shampoos, antiperspirants and cleaning products in contact with the skin** and **use only simple unscented soap** (I can provide this if you have difficulty finding it). If at all possible try to avoid the use of cosmetics.

It would be helpful if you could keep a food and perfume/ shampoo/ soap exposure, cleaning products in contact with the skin diary for the next two days (see attached sheet).

Do not worry if something on the list is unavoidable, just record it and let me know that you have been exposed to it.

The most important time to refrain from these things is for the last twelve hours prior to the study.

For the meal before the study please eat only a light meal containing no fruit and vegetables, for example white bread toast and a boiled egg, or a cheese sandwich without any salad, or fish and chips with no vinegar, or peas, gravy is ok.

## Food / Additive Diary

Please list any products, their commercial name and the manufacturers name which you have eaten or been exposed to in the last 24 hours.

| **Food** | **Cosmetics/ soaps/ perfumes/hair care products etc.** | **Household cleaning products in contact with the skin** |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

## Participation Consent form and participant details

Please complete as much of the information below as possible and bring this form to the experiment with you. Thankyou.

Name:
Age: Sex:

Please answer the following questions:

Have you read and adhered to the diet instruction sheet?

Have you made an appointment for a patch test at least three days prior to your study appointment time? (Contact Belinda: bfhornby@uclan.ac.uk Tel: 01772 893737)

Do you have any allergies: Yes/ No.

If so, what are you allergic to?

Is there any likelihood that you might be pregnant? Yes/No

For female participants: when was the date of you last period?

When is the expected date of your next period?

Are you taking any form of contraceptive drug, implants or the pill?

If so could you indicate which sort for example: progesterone only, high oestrogen?

Do you have any health conditions such as epilepsy or heart condition which could prevent you from taking part in this study or interfere with the physiological recording?

Are you left or right handed? L / R.

Have you read the diet sheet?

Is there any reason why you will find it difficult or will not be able to follow the instructions?

As part of the study I will want to take height, weight and body fat measurements, and saliva samples using a salivette (a cotton swab placed under the tongue and then stored in a sealed tube for later analysis). In addition I will also want to take various on-line recordings of ECG, GSR, eye blinks and blood pressure measurements, this data will be entirely confidential and will only be seen by me, the experimentor, and once collected will be totally anonymised.

Have you been patch tested? Yes/ No Date:
If you still consent to participate in this study please sign and date below.

Signature: Date:
Patch test results: Date:

To be completed during the study:

Date of study

Participant number:

Height (cm):

Weight (Kg):

Stage of ooestrous (if female):

% Body fat content:

% Body water content

% Lean mass

# 3 PERMISSION TO USE THE FILM-CLIPS AND QUESTIONNAIRES

## i). Permission to use the Positive and Negative Affect Scales (PANAS)

INVOICE NO. **N/A**
Federal Tax I.D. 53-0205890

AMERICAN PSYCHOLOGICAL ASSOCIATION

Date: November 17, 2005

**IF THE TERMS STATED BELOW ARE ACCEPTABLE, PLEASE SIGN AND RETURN ONE COPY TO APA. RETAIN ONE COPY FOR YOUR RECORDS. PLEASE NOTE THAT PERMISSION IS NOT OFFICIAL UNTIL APA RECEIVES THE COUNTERSIGNED FORM AND ANY APPLICABLE FEES.**

Belinda Fay Hornby, Senior Psychology Technician
Permissions Office
Department of Psychology
University of Central Lancashire
Preston
5541
UNITED KINGDOM

APA
750 First Street, NE
Washington, DC 20002-4242
202-336-

**IN MAKING PAYMENT REFER TO THE ABOVE INVOICE NUMBER**

Request is for the following APA-copyrighted material: Appendix, page 1070, from JOURNAL OF PERSONALITY AND SOCIAL PSYCHOLOGY, 1988, *54*, 1063-1070

For the following use: **(Print use only)** PhD Study

File: Hornby, Belinda Fay (author)

Permission is granted for the nonexclusive use of APA-copyrighted material specified on the attached request contingent upon fulfillment of the conditions indicated below:

X A fee of $ 0 shall be paid to APA on or before publication.

This fee is ~~based on~~ waived

___X___ The reproduced material must include a full bibliographic citation and the following notice:
Copyright © 1988 by the American Psychological Association. Reprinted with permission.

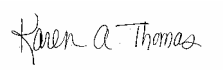
___X___ You must obtain the author's (or, in the case of multiple authorship, one author's) permission. APA's permission is subject to the condition that the author of the cited material does not object to your usage.

_____ A complimentary copy of the work shall be sent to the APA Permissions Office upon publication.

___X___ Other/Comments: **It is noted that author permission has been obtained.**

**This agreement constitutes permission to reprint only for the purposes specified on the attached request and does not apply to subsequent uses nor any form of electronic use. Permission applies solely to publication and distribution in the English language throughout the world, unless otherwise stated. No changes, additions, or deletions to the material other than any authorized in this correspondence shall be made without prior written consent by APA. This permission does not include permission to use any copyrighted matter obtained by APA or the author(s) from other sources that might be incorporated in the material. It is the responsibility of the applicant to obtain permission from such other sources.**

ACCEPTED AND AGREED TO BY: PERMISSION GRANTED ON ABOVE TERMS:



_____ _____

Applicant for the American Psychological Association

November 17, 2005 November 17, 2005

Date Date

_____ I wish to cancel my request for permission at this time.

**The Positive and Negative Affect Scales (PANAS)**

Watson, D., Clark, L. A., & Tellegen, A. (1988b). Development and validation of brief measures of
positive and negative affect: The PANAS Scales. Journal of Personality and Social Psychology, 47, 1063–1070.

Please tick the appropriate box as an indicator of how you feel just now:

| | **1. very slightly or not at all** | **2. a little** | **3. moderately** | **4. quite a bit** | **5. very much** |
|---|---|---|---|---|---|
| **alert** | | | | | |
| **excited** | | | | | |
| **determined** | | | | | |
| **proud** | | | | | |
| **attentive** | | | | | |
| **inspired** | | | | | |
| **enthusiastic** | | | | | |
| **interested** | | | | | |
| **strong** | | | | | |
| **afraid** | | | | | |
| **upset** | | | | | |
| **guilty** | | | | | |
| **jittery** | | | | | |
| **distressed** | | | | | |
| **nervous** | | | | | |
| **hostile** | | | | | |
| **ashamed** | | | | | |
| **irritable** | | | | | |
| **scared** | | | | | |

**Post-film questionnaire**

The following questions refer to how you *felt while watching the film.*

0 1 2 3 4 5 6 7 8
not at all / somewhat/ extremely/ a
none some great deal

Using the scale above, please indicate the greatest amount of EACH emotion you **experienced while watching the film.**

___ amusement
_____ anger
_____ anxiety
_____ confusion
_____ contempt
_____ disgust
____ embarrassment
_____ fear
_____ guilt
_____ happiness
_____ interest
_____ joy
_____ love
_____ pride
_____ sadness
_____ shame
_____ surprise
_____ unhappiness
_____ afraid
_____ upset
_____jittery
_____ distressed
_____ nervous
_____ hostile
_____ashamed
_____ irritable
_____ scared
_____ alert
_____ strong
_____ excited
_____ determined
_____ proud
_____ attentive
_____ inspired
_____enthousiastic

Did you feel any other emotion during the film? O No O Yes

If so, what was the emotion? _____

How much of this emotion did you feel? _____

Please use the following pleasantness scale to rate the feelings you had during the **film. Circle your answer:**

0 1 2 3 4 5 6 7 8

unpleasant pleasant

Had you seen this film before? O No O Yes
Did you close your eyes or look away during any scenes? O No O Yes

## ii). Permission to use the film-clips

**Open water**

Dear Belinda,

Permission is granted provided that the clip is going to be shown only as part of the PHD project in the UK and that the footage shall be as specified by you in previous emails.

Please call when you receive this message.

Kind regards

Guy Avshalom

**Silence of the Lambs**

Belinda -

As long as the Clip is only used in your experiment on the University Campus and the Clip is never copied to any other medium and never distributed in any way, MGM has no objection to your use of the Clip in the manner listed below.

Best,
Felicia Davis
Coordinator - Clip+Still Licensing
MGM Consumer Products
310.449.3572 PH
310.449.3277 FX

## 4. RECRUITMENT POSTER TO TAKE PART IN THE FILM SELECTION STUDY

Do you like watching films, can you spare 45 minutes to watch a few film-clips and answer a short questionnaire?

Are you aged between 18 and 25?

Please contact: Belinda Hornby Darwin room 338 ext 3737.

Alternatively meet at Darwin Room 035 on the ground floor any lunchtime, between 1pm and 2pm this week (November21-26$^{th}$) and please bring along all your friends.

# APPENDIX 5

# INVESTIGATION OF THE SYSTEMIC ABSORPTION RATE OF ORALLY DOSED LAVENDER CAPSULES

## Introduction

The aim was to obtain an estimate of when orally-administered lavender essential oil reached the bloodstream in an average participant. Linalool, one of the major components of lavender, was used as the marker for lavender. The verification of linalool in the blood was identified using gas chromatography and mass spectrometry (GC/MS). The peak concentration of linalool in the blood samples was estimated by the peak area of the linalool peaks to the area of a constant amount of internal standard added to each sample, as an estimate of the quantity of linalool reaching the bloodstream.

## Methods

### Participants

Three healthy female undergraduate students aged between 18 and 28 years, who had abstained from tea, coffee, fruit and vegetables, wearing cosmetics or perfumes for at least 24 hours prior to the study and fasted on the day of the study, following advice from Professor Gerhard Buchbauer, University of Vienna, Austria (personal communication *via* email). Diet diaries, height, weight and body fat content were all assessed on arrival at the laboratory. Ethical approval was obtained from the Universtiy of Central Lancashire, School of Psychology ethics committee. Participants were fully briefed, gave written informed consent and were free to withdraw at anytime, the study was conducted in accordance with the declaration of Helsinki and approval was also gained from the University insurers. Written informed consent was obtained from the participants prior to taking part in the study.

### Location

Faculty of health clinical skills lab, which provided hospital type beds for participants to recline on. To help them relax participants watched a film, provided by one of the participants, while they had blood taken[2].

### Capsules

Lavender capsules were produced by Power Health according to the author's directions: 50 µL lavender oil in 150 µL of sunflower oil encapsulated in a gelatin capsule. Capsules were expected to take approximately 30 minutes to digest (personal communication Vicki McIvor, MD Power Health).

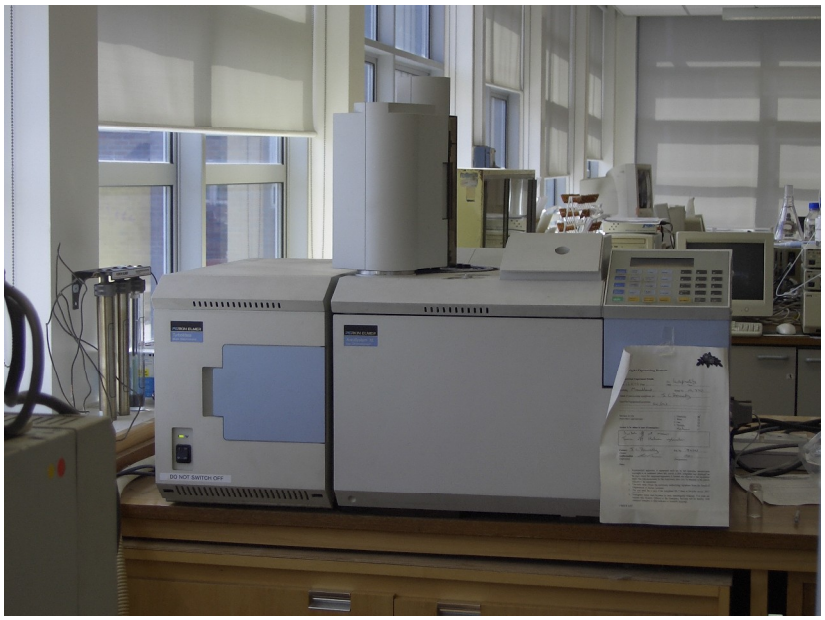
### Venipuncture equipment

Venflon cannulas (pink), 5 ml syringes, 17 gauge needles, tourniquet, were kindly supplied by the Faculty of Health, UCLAN. Heparin tubes: Bector and Dickinson. Saline syringes, dressings, sterets, adaptors for tubes: Southern syringe Ltd.

### GC/MS

Solid phase microextraction (SPME), 100µ coated with polydimethylsiloxane (PDMS), (Sigma Aldrich) 10ml SPME vials Sigma Aldrich, (pre-pierced with a hypodermic needle, in house). Internal standard: nondecane. GC/MS: Perkin Elmer Turbo mass, column: SGE, BPX5(non polar) bonded phase fused silica; 0.22mm I.D.; 25m length; 0.25µ film thickness; injector 50$^{o}$C, splitless. Carrier: Helium. Injector 250 $^{O}$C, Detector 250 $^{O}$C. Column 50 $^{O}$C, 2 minutes; 5 $^{O}$C / minute to 100 $^{O}$C; 20 $^{O}$C/ minute to 250 $^{O}$C for 0.5 minutes.

[2] Acknowledgement: I would like to thank Angela Edmonds, clinical skills tutor, Matt clinical skills technician, and Robin (surname) clinical skills manger for their help and support in this study for allowing me to use their laboratory. Also Jim Donelly and Richard McCabe for their help and support with the GC/MS work



**Appendix Figure 2 Perkin Elmer Turbomass GC/MS**

## Procedure

On arrival at the laboratory participants were directed to a waiting room until required for the study and asked to fill in the pro-forma to include details of the contents of food eaten in the last 12 hours and time eaten; any health problems and details of medication. Participants were also assured that all data would remain confidential and anonymous and that they could drop out of the study at any point. Measures of weight, height, and body fat content were recorded and then they were assigned to a bed. Cannulas were inserted in to the antecubital fossa and the time zero blood sample taken. After this participants were given either two (100µl), four (200µl ) or six (300µl) lavender capsules and a glass of water. Blood was taken 5, 10, 15, 30, 45, 60, 90 and 120 minutes following capsule administration. 5ml of saline was introduced into the cannula following the 30 minute and the 90 minute blood sample. This was to prevent clotting and allow enough time for the saline to disperse prior to the next sample being taken, so that the sample was not diluted. This procedure followed the advice given by the local haematology clinician, Dr Flaherty at the Royal Preston Hospital, Black Bull Lane, Preston. Blood samples were inverted 10-12 times immediately after taking each sample in order to prevent clotting, and then frozen ready for analysis.

**Analysis of blood**

Blood was analysed using SPME to extract the lavender components from the blood following the method of Boyle et al., (Boyle, McLean, Brandon, Pass, Davies, 2002): 50µL of blood and 1µL of an internal standard, nondecane (stock solution 2µL in 5mls of distilled water) was placed into the SPME vial (10ml) which had been pre-punctured with a hypodermic needle to facilitate insertion of the SPME into the vial to a depth of 1.5cm above the base.

The vial and SPME were placed into a carbon bath at 55 $^{O}$C, for exactly 10minutes, the SPME was supported with a clamp at the same height each time. Following this the SPME was inserted into the GC/MS injector and the adsorbed volatiles were analysed, following the method of Jäger, Buchbauer, Jirovetz, and Fritzer, (1992) which was adapted for optimum use in our laboratory. The method used was as follows: GC/MS: Perkin Elmer Turbo mass; column: SGE, BPX5(non polar) bonded phase fused silica; 0.22mm I.D.; 25m length; 0.25µ film thickness: injector 50$^{o}$C, splitless. Carrier: Helium; injector 250 $^{O}$C, detector 250 $^{O}$C. column 50 $^{O}$C, 2 minutes; 5 $^{O}$C / minute to 100 $^{O}$C; 20 $^{O}$C/ minute to 250 $^{O}$C for 0.5 minutes. The position of the peak expected for linalool following GC/MS had previously been determined using blood spiked with these two analytes.

**Appendix Figure 3. Carbon bath for warming essential oils to 55 $^{o}$C prior to and during solid phase microextraction**.

## Results

The values obtained with the participant who only took two capsules (100μl) were too small to detect.

Body fat and weight data from participants who took the 200 and 300μl doses :

200μl:

Height 1.55m

Weight 65.6Kg

Body fat 38.4%

Lean 40.4Kg

300μl

Height 1.65m

Weight 65.6kg

Body fat 29.7%

Lean 46.1%

**Appendix table 5.1 Linalool integrated peak area expressed as a percentage of the internal standard peak area.**

| **Time (minutes)** | **Lavender concentration** | |
|---|---|---|
| | **200μl** | **300μl** |
| **5** | 0.14 | 13.59 |
| **10** | 0.53 | 165.2 |
| **15** | 10.74 | Missing data |
| **30** | 197.76 | 103.17 |
| **45** | 0.15 | 145.85 |
| **60** | 1.88 | 0.14 |
| **90** | 0.71 | Missing data |

## Discussion

From the results the components of lavender appear to be at a maximum in the bloodstream at 30 minutes in the 200μl dose and between 10 and 45 minutes at the 300 μl dose. As this pilot study is just an estimate the time when the lavender will reach a peak in the bloodstream of the average participant, then the anxiety film-clip will be pitched after 30 minutes. Thirty minutes also co-incides with the manufacturer's advice on the digestibility of the capsules too. However, for the actual study lower doses of 100 and 200μL will be used and the effects on anxiety will be measured after 30 minutes. Thus allowing the lavender time to reach the bloodstream and any organs that it might exert its effects upon.

## Reference List

Boyle, R. R., McLean, S., Brandon, S., Pass, G. J., & Davies, N. W. (2002). Application of solid-phase microextraction to the quantitative analysis of 1,8-cineole in blood and expired air in a Eucalyptus herbivore, the brushtail possum (Trichosurus vulpecula). *J.Chromatogr.B Analyt.Technol.Biomed.Life Sci., 780*, 397-406.

Jager, W., Buchbauer, G., Jirovetz, L., & Fritzer, M. (1992). Percutaneous absorption of lavender oil from a massage oil. *Journal of the Society of Cosmetic Chemists, **43***, 49-54.

## Informed consent letter

*Pilot study: Assessment of the rate absorption into the bloodstream, of orally ingested lavender oil capsules.*

Dear Participant:

As outlined in the title, the aim of this pilot study is to assess the time taken for lavender oil to get into the bloodstream when ingested in capsular form. The lavender oil capsules are made from gelatin and contain lavender oil which is diluted in sun flower oil.

If you agree to take part in this study you will be asked to swallow not more than 10 lavender oil capsules and possibly have a cannula inserted into a vein in your non dominant lower arm. This procedure is commonly used in many different circumstances to take blood samples or insert drips into people's arms. I have undertaken special training to carry out this procedure it should cause minimal discomfort.

Seven small blood samples (5mL) will be taken over a one and a half hour time period via the cannula, not more than 100ml (0.1L) of blood will be taken. The study will last approximately 1.5hours, during which time you will have to remain in the laboratory while the cannula is still in place.

It is very important that you are in good health and have no allergies to soap, perfumes, flavours or fragrances for example lavender, linalool, or linalyl-acetate. It is also important that your non dominant hand has no bruising or damage to it. If you have had a mastectomy in the past or any condition or operation which might have affected your lymph glands in this arm it is not advisable to take blood from this arm, therefore please tell the experimenter about this immediately. **Similarly, please make the experimenter aware of any health conditions that you might be experiencing.**

**If at any point you wish to withdraw from this study please make the experimenter aware of this then the cannula can be removed. There is no obligation to take part in or complete the study should it become uncomfortable.**

Thank you for volunteering to take part in this pilot study. In the event of any unforeseen problems occurring after the procedure please contact Belinda Hornby on (01772) 893737, or alternatively seek medical advice from your GP.

Signed……. Date;……

## Diet restriction Instructions

Please refrain from tea coffee and tobacco products for at least 12 hours prior to the study if possible it would be preferable to avoid them for a couple of days before hand.

Drink plenty of water.

Try to avoid any scented teas or fruit drinks too.

The day before the study please could you refrain from juices, fruits, sweets, curries, anything with a strong flavour. Likewise processed foods such as sausages, burgers also contain flavouring additives such as those found in EOs.

Also, for two days please could you refrain from the use of perfumes and deodorants and use only simple unscented soap products. If at all possible try to avoid the use of cosmetics.

It would be helpful if you could keep a food and perfume/ shampoo/ soap exposure diary for the next two days.

Do not worry if something on the list is unavoidable, just record it and let me know that you have been exposed to it.

The most important time to refrain from these things is for the last twelve hours prior to the study.

For the night before the study please could you fast after your supper and not eat breakfast until after the study.

I will provide tea, coffee and sandwiches once I have taken the blood.
Let me know your preferences for food and drink.

# APPENDIX 6

# PUBLICATIONS

## Papers

**Bradley, B. F.,** Starkey, N. J., Brown, S. L., & Lea, R. W. 2007, "Anxiolytic effects of Lavandula angustifolia odour on the Mongolian gerbil elevated plus-maze", *Journal of Ethnopharmacology*, vol. 111, no. 3, pp. 517-525.
**Bradley, B. F.,** Starkey, N. J., Brown, S. L., & Lea, R. W. 2007, "The effects of prolonged rose odor inhalation in two animal models of anxiety", *Physiol Behav* vol 92,pp 931-938.

**Bradley, B. F.,** Bridges, N. J., Starkey, N. J., Brown, S. L., & Lea, R. W. 2008 "Differential drug effects on male and female gerbils in the black- white box", *Physiol Pharm Bioch .(Under review)*

**Bradley, B.F, S**.L.Brown, S.Chu, R.W. Lea, 2008 "The effects of orally-administered lavender on responses to anxiety-provoking film-clips" Neuropsychopharmacology (under review).

## Abstracts

**Bradley, B. F**.; Brown, S. L.; Chu, S.; Lea, R. W. The effects of orally-administered lavender in response to mood eliciting film-clips on male and female participants Planta Medica; 09, 2008 (Topic A: Pharmacology, toxicology and clinical studies of natural products and herbal drugs)
H.L. Mudie, A.M. Morley, **B.F. Hornby**, M.P. Roy, 2008. Cortisol and Physiological Crash in a Marathon Runner: A Case Study of Monozygotic Twins
**Bradley, B. F**., Starkey, N. J, Brown, S. L, Lea, R. W. (2007). The effects of inhalation of EO odour from Rosa damascena Mill. on gerbils in two models of anxiety. Planta Medica; 09, 2007 (6. Pharmacology, toxicology and clinical studies of natural products).
**Hornby, B. F**., Starkey, N. J., Brown, S. L., Lea, R. W., 2006. Anxiolytic effects of Lavender (Lavandula angustifolia) odour on the Mongolian gerbil *(Meriones unguiculatus)* elevated plus-maze Planta Medica; 11, (6. Other related topic).
**Hornby, B.F**., Starkey, N.J., Brown, S.L., Lea, R.W. 2005. The effects of prolonged exposure to Lavandula angustifolia odour in an experimental model of anxiety, the gerbil elevated plus-maze. UCLAN Faculty of Science Abstracts.
**Hornby, B.F**., Starkey, N.J., Lea, R.W. 2004. The effects of Lavandula angustifolia odour on two tests of anxiety pilot study. UCLAN Faculty of Science Abstracts.
**Hornby, B.F.,** Starkey N.J., 2003. The effects of rose (*Rosa centifolia*) and lavender (*Lavandula angustifolia*) oil odours in two test of anxiety, a pilot study. 54.02 P126 British neuroscience association Harrogate, Abstracts 2003.
Bridges N.J. **Hornby, B.F.,** Starkey N.J. 2003. Influence of closed arm transparency on anxiety-like behaviour in the gerbil elevated plus- maze. 53.04 P125 British neuroscience association Harrogate, Abstracts 2003.

## Conference presentations

**British Neuroscience Association Harrogate 2003,** poster "The Effects of Rose (Rosa Centifolia) and Lavender (Lavendula angustifolia) Oil Odours in Two Tests of Anxiety."
**UCLAN Faculty of Science annual research day conference 2004,** poster "The Effects of Lavender (*Lavendula Angustifolia*) Oil Odours in Two Tests of anxiety."
Department of Psychology annual post graduate student research day 2004 talk "The effects of lavender on the elevated plus-maze, an animal model of anxiety."
**UCLAN Faculty of Science annual research day conference** 2005, poster "The effects of prolonged exposure to *lavandula angustifolia* on the elevated plus-maze, model of anxiety."
**UCLAN Faculty of Science annual research day conference** 2006, talk "The effects of Rose (*Rosa Damascena*) EO odour in the Mongolian gerbil black/white box, a model of anxiety".**Awarded best overall presentation**
**GA post graduate symposium,** Helsinki 2006, talk; Anxiolytic effects of Lavender (*Lavandula angustifolia*) odour on the Mongolian gerbil (*Meriones unguiculatus*) elevated plus-maze.
**GA annual meeting** Helsinki 2006, poster: Anxiolytic effects of Lavender (*Lavandula angustifolia*) odour on the Mongolian gerbil (*Meriones unguiculatus*) elevated plus-maze.
**Faculty of science** annual research day 2007 and Department of Psychology annual research day 2007: "The effects of *Lavandula angustifolia* on laboratory induced anxiety in human participants."
**British Psychological Society**, Psychobiology meeting, Windermere, September 2008.
The effects of oral lavender on responses to anxiety eliciting film-clips.
**British Psychological Society**, Annual Conference Brighton April 2009. The effects of oral lavender on responses to anxiety eliciting film-clips. Paper accepted for oral presentation.